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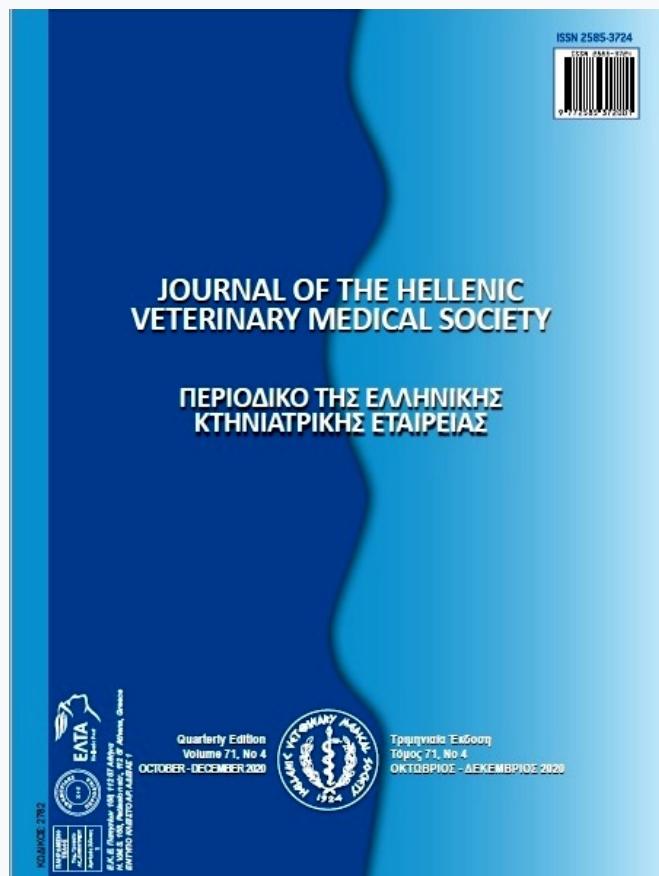
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Effect of Cefazolin on endometrial cytology and reagent test strips parameters in bovine endometritis

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Βιβλιογραφική αναφορά:

Effect of Cefazolin on endometrial cytology and reagent test strips parameters in bovine endometritis

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ABSTRACT: Endometritis is one of uterine disorders in dairy cows causing low fertility. This study explored effects of intrauterine (IU) infusion of Cefazolin on endometrial cytology and reagent test strips parameters (leukocyte esterase [LE] and pH) in bovine subclinical endometritis. A total of 90 cows at 49-56 days in milk (DIM) diagnosed for subclinical endometritis were divided randomly into three groups, group I (n = 35), treated with IU infusion of 2 grams Cefazolin (Zinol ®, Pharco) diluted with 50 ml saline; group II (n = 28), treated with IU infusion of 2 gramsCephalexin (Ceporex®, GlaxoSmithKline) diluted with 50 ml saline; group III (n = 27), cows kept as untreated control. Cytological examination and reagent test strips were performed in uterine material derived with the cotton swab technique before treatment program and repeated two times later, in 10 days interval. The cows were artificially inseminated at first oestrous after the end of treatment program and conception rates were evaluated. After the end of treatment program, polymorphonuclear cells (PMN) decreased significantly (P <0.05) in Cefazolin and Cephalexin groups (3.23% and 4.39% respectively) compared tocontrol (24.89%) group, also means of LE in these groups became significantly (P<0.05) lower. The pH value decreased after treatment, this reduction was significant after the first dose of Cefazolin, while in Cephalexin reduction of pH became significant after second dose. Cefazolin had conception rate (77.14%) significantly (P<0.05) higher than cephalexin and control groups (57.14% and 25.93% respectively). Cefazolin and Cephalexin decrease uterine PMN, LE concentration. The pH value after treatment by Cefazolin was lower than in Cephalexin. Moreover, treatment with Cefazolin resulted in a significantly higher conception rate. Thus, Cefazolin is recommended for the treatment of subclinical endometritis in dairy cows.

Keywords: Polymorphonuclear cells; Leukocyte esterase; Conception rate; Cows

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INTRODUCTION

Endometritis is a common uterine disorder in dairy cows that is strongly associated with reproductive and economic losses (Giuliodori et al., 2013). Various diagnostic methods are needed in order to treat the condition quickly and effectively to reduce these losses. These methods include vaginal examination, ultrasound, cytology, uterine biopsy and reagent test strips (Cheong et al., 2012; Casarin et al., 2018). Cytological examination of the reproductive tract is one of these methods, often used to assess possible reproductive lesions in humans and animals (Kasimanickam et al., 2005). In this method of examination, it has been reported that the neutrophil ratio to endometrial cells becomes an indicator of the inflammatory process (Couto et al., 2013).

To properly evaluate this inflammatory process, it is necessary to know the threshold values for polymorphonuclear cells (PMN) through which the presence of inflammation is established. Several studies have discussed this point including a study reporting 5% PMN as a threshold value; another study reported that this value varies depending on the examination stage. It was ≥8%PMN at the period equal or less than 33 days postpartum and ≥6% PMN at 34 to 47 days postpartum.Moreover, it was ≥4% PMN at the period equal or more than 48 days postpartum (Madoz et al., 2014; Chaudhari et al., 2017). Reagent test strips results were strongly related with endometrial cytology for the diagnosis of endometritis(Cheong et al., 2012).Several studies have addressed the use of reagent test strips as a rapid test to diagnose the inflammatory process. These strips contain different parameters including leukocyte esterase (LE), protein concentration and pH value. It has been suggested that LE in vaginal discharge can be used as a method for screening subclinical endometritis in cattle (Hajibemani et al., 2016). Endometritis changes the physical and chemical properties of cervical mucus. Therefore, examination of this mucus for appearance, consistency and pH may be useful for its diagnosis (Kumar et al., 2017).After diagnosis of endometritis, the role of treatment is important, with a variety of intrauterine antibiotic, antiseptic chemicals, systemic antibiotics and hormones to have been used for this purpose (Jeremejeva et al., 2012; Singh et al., 2018). Cefazolin and Cephalexin are first generation cephalosporin antibacterialswhich have a wide range of activity against gram-positive and gram-negative bacteria (Moradi et al., 2020).Therefore, this study was designed to evaluate the effects of IU infusion of Cefazolin on endometrial cytology and reagent test strips parameters (LE and pH) in dairy cows affected withsubclinical endometritis.

MATERIALS AND METHODS
Animals

Ninety Holstein-Friesian cows aged from 3-5 years at 49–56 days in milk (DIM) affected by subclinical endometritis were used in the study during the period from March 2017 to February 2019. Cows were housed outdoors in open hygienic yards; they were fed grass silage, concentrate and hay. The cows had a good health status and they received ordinary vaccines against foot and mouth disease (FMD), Lumpy skindisease (LSD) and rift valley fever (RVF).Cows with arthritis, hoof problems, retained placenta , cesarean section, mastitis and other clinical diseases were excluded from the study. A clinical examination of the reproductive tract was performed by vaginal examination and transrectal ultrasonography. Cytological examination and reagent test strips were performed initiallyand repeated twice in 10 days interval. The treatment program started after the first examination.

Vaginal examination

The perineum and vulva were cleaned with a paper towel and a lubricated vaginal speculum was inserted deepinto the vagina to enable visualization of the cervix with the help of a light source (Barlund et al., 2008).Cows with abnormal vaginal discharge were excluded from the study.

Ultrasonography

Ultrasonography was performed using a portable real-time B-mode transrectal ultrasound scanner (SonoScape, Model: M12, SonoScape Medical Corp, Guangdong, China). Ultrasonography was used to determine ovarian activity by the presence of a CL, a follicle, or both. Cows with ovarian abnormalities, i.e., ovarian cysts were excluded from the study (Pothmann et al., 2015).

Samples collection

A few drops of sterile normal saline were used to make the cotton swab wet before obtaining the uterine sample (Salah et al., 2017).Samples of uterine swabs were derived using sterilized transcervical guarded swabs. The swab was placed in the body of the uterus. The sterile swab was then removed from its protective tube and pressed against the uterine mucosa. Consequently, the swab was pulled into the protective tube and pulled out of the reproductive system.

Cytological examination

The cotton swab was rolled on a clean glass slide and was left to dry. The slide was stained by Rapi-Diff II Stain (Diff-Quick) according to the manufacturer's instructions (Constantin et al., 2017). Cytological evaluations were performed by counting a minimum of 100 cells at 400X and 1000X magnification of a microscope to determine the percentage of PMN cells. To evaluate the inflammatory process 5% PMN was considered as the cut-off value (Kumar et al., 2013). Polymorphonuclear cells(%) was calculated just before initiation of the treatment program and was repeated twice in 10 days intervals.

Evaluation of reagent test strips results

The cotton swab was immersed in a 3 ml glass tube containing 1 ml of 0.9% saline and was gently shaken for 30 seconds. Reagent test strip (Medi-Test Combi 10® SGL) was then immersed in the glass tube. The strip was removed from the tube and the result was recorded after 1-2 minutes(Couto, 2009). The pH results were recorded in five categories: 5, 6, 7, 8 and 9. Results of LE were recorded in five categories: negative, 25, 75 and 500 leukocytes / µL. Leukocyte esterase and pH results were evaluated in all stages of examination.

Treatment protocols

After first examination, subclinical endometritis cows were randomly divided into three groups: group I (n = 35), treated with IU infusion of 2grams Cefazolin (Zinol ®, Pharco) diluted with 50 ml saline;group II (n = 28), treated with IU infusion of 2grams Cephalexin (Ceporex®, GlaxoSmithKline) diluted with 50 ml saline.Both treatments were repeated after 10 days; group III (n = 27), cows kept as untreated control.

Evaluation of conception rate

The cows were artificially inseminated at first oestrous after theend of the treatment program (2nd dose of treatment). Conception rate was calculated as the total number of pregnant cows divided by the total number of inseminated cows multiplied by 100 (Schefers et al., 2010).

Statistical analysis

The results were statistically analyzed using the Statistical Package for Social Sciences version 22.0 (SPSS for Windows 22.0, Inc., Chicago, IL, USA). ANOVA test was performed for comparing mean values. Data are represented asmeans ± SE. Conception rates were analyzed by Chi-square test. Comparisons showing$P<0.05$ were considered to be significant.

RESULTS

We have assessed parity and milk yield. There were no significant differences (P>0.05) between all groups for these parameters.Parity ranged from 3.29 to 3.36 and milk yield from 35.60 to 36.70 kg/day (Table 1).

Table 1: Means (± SE) of parity and milk yield at first examination in the different treatment groups

Variable	Treatment groups			P-value
	Cefazolin	Cephalexin	Control	
Parity	3.29±0.15	3.36±0.16	3.33±0.18	0.77
Milk yield (kg/day)	35.60±0.36	36.25±0.51	36.70±0.48	0.10

After the end of the treatment, PMN%, LE concentration and pH value decreased significantly (P<0.05) compared to first examination for Cefazolin and Cephalexin groups, whereas this decrease was not significant for the control group. The PMN% at first examination ranged from 26.21% to 28.91%. At third examination, PMN% in Cefazolin and Cephalexin (3.23%and 4.39%,respectively) groups became significantly (P<0.05) lower than in the control (24.89%) group. Also, means of LE concentration ranged from 374.07 to 408.93Leukocytes /µL before treatment.At third examination, LE concentration in Cefazolin and Cephalexin groups became significantly (P<0.05) lower than in the control group. pH values before treatment ranged from 8.46 to 8.56.At second examination, the pH value in Cefazolin group (7.34) became significantly (P<0.05) lower than in Cephalexin and control groups (8.25 and 8.41, respectively). Moreover, at third examination the values in treated groups became significantly (P<0.05) lower than in the control group (Table 2).

Days Openin Cefazolin and Cephalexin (87.07and 97.75 days, respectively) groups were significantly (P<0.05) lower compared to the control(122.29 days) group. At first service, conception rate in Cefazolin group was significantly (P<0.05) higher than Cephalexingroup while there was no conception in con-

trol group. At second service, conception rates were 50.00%, 27.27% and 7.41% in Cefazolin, Cephalexin and control groups but this difference was not significant. Moreover, there was no significance difference between all groups at third service. Total conception rate in Cefazolin (77.14%) group was significantly higher than in Cephalexin and control groups (57.14 and 25.93%, P<0.05, respectively, Table 3).

Table 2: Means (± SE) of uterine PMNs %, LE concentration (Leukocytes /μL) and pH values in the different treatment groups

Variable	Stage of examination	Treatment groups		
		Cefazolin (N=35)	Cephalexin (N=28)	Control(N=27)
PMN %	Ex.1	28.91±2.37 [aA]	26.21±2.06[aA]	27.74±2.73[aA]
	Ex.2	12.57±2.37[aB]	14.14±2.01[aB]	27.30±3.09[bA]
	Ex.3	3.23±0.38[aC]	4.39±0.87[aC]	24.89±3.14[bA]
LE concentration (Leukocytes /μL± SE)	Ex.1	390.71±31.86[aA]	408.93±33.56[aA]	374.07±38.06[aA]
	Ex.2	148.57±30.33[aB]	166.07±33.56[aB]	358.33±39.29[bA]
	Ex.3	47.86±4.27[aC]	68.75±16.65[aC]	340.74±40.76[bA]
pH values	Ex.1	8.51±0.51[aA]	8.46±0.10[aA]	8.56±0.10[aA]
	Ex.2	7.34±0.48[aB]	8.25±0.10[bA]	8.41±0.10[cA]
	Ex.3	7.29±0.08[aB]	7.57±0.10[bB]	8.37±0.09[cA]

Ex.1:1st examination. Ex.2: 2nd examination, Ex.3:3rd examination
Different superscripts (small letters) indicate significant differences (P<0.05) between different groups within rows
Different superscript (capital letters) indicate significant differences (P<0.05) between different times of examination within each group and variable

Table 3: Days Open(Means ± SE) and conception rate (%) in the different treatment groups

Groups	N	Days Open	Conception rate			
			1st service*	2nd service	3rd service	Overall*
Cefazolin	35	87.07±2.84[a]	48.57% (17/35)	50.00% (9/18)	11.11% (1/9)	77.14% (27/35)
Cephalexin	28	97.75±11.34[a]	21.43% (6/28)	27.27% (6/22)	25.00% (4/16)	57.14% (16/28)
Control	27	122.29±7.71[b]	0.00% (0/27)	7.41% (2/27)	20.00% (5/25)	25.93% (7/27)

Different superscripts(small letters) indicate significant differences(P<0.05) between different groups
*Denotes significant difference (P<0.05) within column for conception rate
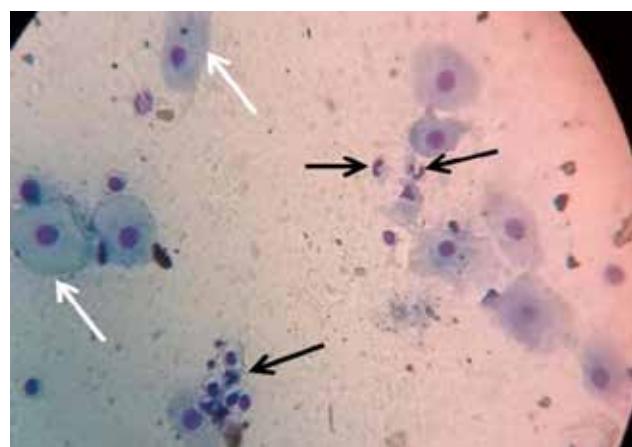
Figure 1: Cytology smear obtained from cow's uterine content at first examination, stained with Rapi-Diff II Stain. Endometrial epithelial cells (white arrow) and PMN (black arrow).
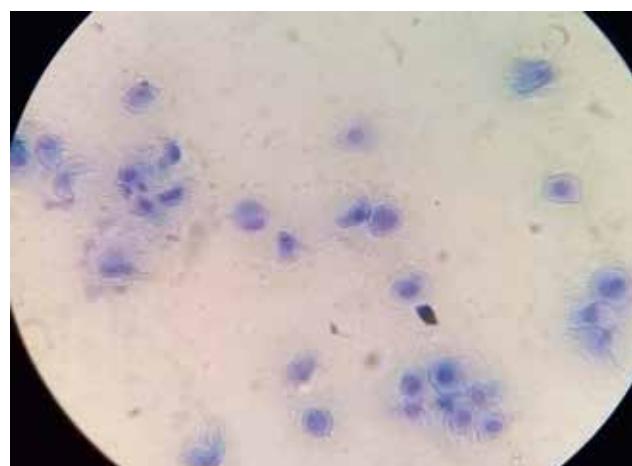

Figure 2: Cytology smear obtained from cow's uterine content at third examination, stained with Rapi-Diff II Stain. Endometrial epithelial cells.

DISCUSSION

The study design followed those of previous published studies that had collected similar information (Kumar et al., 2017; Tomar et al., 2017).The retrospective power analysis indicated that it is important to conduct further studies with a larger sample size. However, statistically significant differences between treatment groups with P-valuesof <0.05 have been detected.

Endometrial cytology has beenthe most reliable method for diagnoses of endometritis in cattle (Barlund et al., 2008). The presence of neutrophils within the uterine cavity indicates an inflammatory process (Brick, 2011). These neutrophils form the first line of defense against invasive pathogens, resulting in an increase in the number of PMN within the uterine cavity (Kasimanickam et al., 2004). Antibiotics reduce uterine bacterial load and inflammation of the endometrium (Lefebvre and Stock, 2012).Regarding our results, thefirst dose of treatment withCefazolin or Cephalexin significantly decreased PMNs. However,this decrease was not significant regarding the untreated group.At the third examination, which was10 days after the second dose of treatment, Cefazolin andCephalexin further reduced the percentage of PMNsfrom 28.91%and 26.21% to 3.23%and 4.39%, respectively. This reduction is in agreement with a previous study which reported a significance decrease in neutrophils after 14 days from an intrauterine infusion of antibiotic at 50-60 days after parturition. However, no significance difference was found at 20-30 after parturition(Ahmadi et al., 2005). An explanation may be that after antibiotic administration, the elimination of pathogenic organisms occurs and inflammation subsides; neutrophils become confined to the fluid in the uterine cavity and are expelled by uterine contractions (Couto et al., 2013).

Uterine LE activity was associated with neutrophil ratio as determined by endometrial cytology. Therefore, it is an alternative method for the evaluation of inflammatory cells in the uterine cavity (Couto et al., 2013). A previous study reported that LE activity in the genital discharge was significantly higher inendometritis than that in healthy cows anda significant difference in percentage of LE activity was observed after intrauterine infusion of penicillin and streptomycin (Hajibemani et al., 2016).In the present study LE concentration wassignificantly decreased from 390.71 and 408.93 Leukocytes /µLto 47.86 and 68.75Leukocytes /µL. after Cefazolin and Cephalexin treatment, respectively.

The pH value of genital discharge could be used for the diagnosis of endometritis in cattle (Palanisamy et al., 2014).In the present study pH value ranged from 8.53 to 8.57 before treatment. The change in pH of uterine fluid to the alkaline side is usually due to the presence of metabolites of bacteria and inflammatory exudates associated with uterine infection. This increase of pH is not suitable for the survival of sperm and fetus in the uterus (Palanisamy et al., 2014; Kumar et al., 2014). In this study, the pH value decreased after treatment, and this reduction was significant after the first dose of Cefazolin, while Cephalexin needed two doses to be effective. The reduction of the pH value in our results agrees with several studies reporting a decrease in cervical and uterine pH after antibiotic therapy, including those that reported a decrease in value from 7.83 to 7.26(Tomar et al., 2017). Another study reported a decrease in value from 8.10 to 7.05 after treatment with levofloxacin (Kumar et al., 2017).

Reproductive performance was monitored after treatment. Previous studies have discussed conception rate after Cephalexin treatment, including a study that achieved 40.00% and 33.33% conception rate respectively at first and second insemination and 60% total conception rate (Resum and Singh, 2016). Moreover, Parikh et al. (2017) achieved high conception rate (83%) after treatment by Cephalexin. In the present study, total conception rate in Cefazolin group was significantly (P<0.05) higher than in Cephalexin and control groups. This may be due to the decrease of high pH value after theend of treatment noticed in our study for theCefazolin group. This decrease of pH value can be critical for the survival of sperm and fetus in the uterus (Palanisamy et al., 2014; Kumar et al., 2014).

CONCLUSION

Intrauterine infusion of Cefazolin and Cephalexin leads to a decrease in uterine PMN and LE concentration. The pH value in Cefazolin group was significantly lower than in the Cephalexin group. Moreover treatment with Cefazolin resulted in a significantly higher conception rate. Thus, Cefazolin is recommended for the treatment of subclinical endometritis in dairy cows.

CONFLICT OF INTEREST

None declared.

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