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ORIGINAL ARTICLE

Hematological changes before and after treatment in dairy cows with clinical and subclinical endometritis

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Abstract The aim of this study was to investigate the treatment-related changes in hematological parameters in dairy cows affected by clinical endometritis (CE) and subclinical endometritis (SE). One hundred seventy postpartum Holstein dairy cows were selected from a large commercial dairy farm. Cows of the SE group presented a significant decrease in PCV ($P<0.05$) and red blood cell (RBC) ($P<0.05$) values, when compared to healthy group, while the CE group presented a significant decrease ($P<0.01$) in PCV and RBC count, when compared to healthy and SE animals. Significant increases in white blood cell (WBC) ($P<0.05$), neutrophil ($P<0.001$), and lymphocyte ($P<0.001$) counts, in the CE and SE groups, were observed when compared to healthy cows. After treatment for CE, PCV and RBC count dropped significantly, whereas WBC, neutrophil, lymphocyte, and monocyte counts decreased significantly ($P<0.05$). In the SE group, WBC, neutrophil, lymphocyte, and monocyte counts decreased significantly after treatment ($P<0.05$). The results obtained in this study confirm endometritis-induced changes in hematological parameter which improve after treatment.

Keywords Hematology clinical endometritis · Subclinical endometritis · Treatment

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Introduction

Bacteria contaminate the uterus of >90 % of dairy cattle in the first 2 weeks after parturition. Although most cows eliminate this uterine bacterial contamination during the subsequent 5 weeks, bacterial contamination causes the uterine disease endometritis in >10 % of animals (Williams et al. 2005). Endometritis is defined as inflammation of the endometrium without systemic signs and is associated with delayed uterine involution. Endometritis has a negative effect on the reproductive performance as it increases services per conception, the calving to first service interval and the calving to conception interval, reduces the risk of pregnancy, and decreases the conception rate (Kasimanickama et al. 2004). Endometritis has been subdivided into clinical and subclinical categories (Sheldon et al. 2006). Clinical endometritis is characterized by the presence of a purulent uterine discharge after 21 days in milk or a mucopurulent discharge after 26 days in milk (Sheldon et al. 2006). Subclinical endometritis is defined as the presence of >18 % polymorphonuclear cells (PMN) in uterine cytology samples collected 21–33 days postpartum or >10 % PMNs in samples collected at days 34–47 (Sheldon et al. 2006). Cows with subclinical endometritis do not have uterine discharge; however, the severity of the disease is still considered sufficient to impair reproductive performance (Sheldon et al. 2006).

During the peripartum period, physiological changes occur that depress the defense mechanisms of the cow and render her more prone to uterine and mammary infections (Mateus et al. 2002). The prepartum leukocytosis is mediated by the antepartum cortisol rise (Preisler et al. 2000), and the decrease in the first week postpartum is associated with migration towards the uterine lumen and the mammary gland (Guidry et al. 1976).

Saad et al. (1989) found that the number of blood lymphocytes decreased before and at parturition, returning to a higher level during the second week postpartum. Similar findings were made by the other investigators in cows. Regarding the mammary gland, extensive influx of neutrophils into the colostrums and milk occurs around calving and during the first week after calving (Guidry et al. 1976). Although a few studies have investigated the changes of hematological parameters in postpartum period, however, no study has compared these parameters between clinical and subclinical endometritis and has evaluated the changes of these parameters after treatment. Therefore, the objectives of this study were: (a) to evaluate the hematological changes of cows with clinical and subclinical endometritis and to compare them with those of healthy animals and (b) to compare these parameters before and after treatment.

Materials and methods

Cows

The study was conducted from April to September 2010 in a large commercial dairy herd (Mashhad, North-east Iran), using multiparous Holstein cows. Cows were housed in free-stall barns. Artificial insemination was used exclusively after a voluntary waiting period of approximately 45 days. During scheduled weekly visits, all healthy cows between 21 and 33 days in milk were identified and were enrolled into the study. If cows had history of systemic or intrauterine antibiotic therapy within 7 days prior to enrollment, reproductive hormone administration in the current lactation prior to enrollment or abnormal genitalia including adhesions, laceration, and pyometra, they were excluded from the study. During the study period, all animals were kept under identical conditions.

Clinical endometritis

The presence of clinical endometritis (CE) was determined by finding pus in the lumen of the vagina by withdrawing the contents of the vagina by hand. First, the perineum and vulva were cleansed with paper towel, and a clean, lubricated, gloved hand was inserted through the vulva. The vagina was assessed for any signs of damage or injury by palpation of the lateral, dorsal, and ventral walls. Animals with palpable vaginal injury were excluded from the study. The mucus content of the vagina was withdrawn manually for examination. The vaginal mucus was characterized using an endometritis scoring system (Williams et al. 2005). Character score was assigned as follows: unaffected animals (0) clear or translucent mucus, (1) mucus containing flecks of white or off-white pus, (2) mucopurulent exudates containing ≤50 % white or off-white mucopurulent material, and (3) exudate containing >50 % purulent material, usually white or yellow, but occasionally sanguinous.

Subclinical endometritis

Cows with no abnormal discharge were used in this group. Endometrial cytology samples were collected using the modified cytobrush technique as described by Kasimanickama et al. (2004). Smears were prepared by rolling the brush on a microscopic slide, dried and fixed in pure methanol immediately after collection. Samples were stained (Giemsa at a dilution of 5 % in buffer solution) and evaluated by×400 magnification (Zeiss, Germany). A total of 300 cells were counted under the microscope to determine the proportion of PMNs. The endometrial cytology slides were assessed twice by a clinician.

Treatment

Treatment of the cows with either CE and/or subclinical endometritis (SE) was done by the farm veterinarians with standard protocols. At the conclusion of the first examination, the SE cows received one of the two treatments: 500 mg of cloprostenol im (Estroplan, Parnell, Australia) on days 1 and 14 or 500 mg of benzathine cephapirin in 19.6 g of ointment base iu (Metricure®, Intervet, Canada) and then 500 mg of cloprostenol im 7 days after benzathine cephapirin injection.

Treatment protocols for CE cows were as follows: 500 mg of cloprostenol im (Estroplan, Parnell, Australia) on days 1 and 14 or Na-ceftiofur im (1 mg/kg, Excenell®, Pfizer, Animal health S.A., Kalamazoo, MI, USA) in three consecutive days and then 500 mg of cloprostenol im 7 days after the last ceftiofur injection.

Sampling

Blood samples were taken from all cows with CE and SE at the time of diagnosis (before treatment) and on day 7 after treatment. Blood samples were transferred to tubes containing EDTA for hematological analysis. Anticoagulated blood was analyzed shortly after collection of the following: number of red blood cell (RBC), hemoglobin (Hb), PCV, white blood cell (WBC) count,

platelet (Plt), MCH, MCV, and MCHC by an automatic veterinary hematology cell counter (Nihon Kohden, Celltac α, Tokyo, Japan). Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique (Jain 1986). Blood samples were also taken from the control group (healthy cows with no clinical or subclinical endometritis, n=30) on the day of the first examination.

Statistical procedures

Values are reported in the text and tables as means ± standard deviations. Analyses of variance were used to compare means among the different groups (healthy control (n=30), CE (n=80), and SE cows (n=60)). Following analysis of variance, significant between-group differences were detected by Tukey's honestly significant difference test. We also used Student's t test to compare means before and after treatment in cows with CE and SE. All analyses were performed with the statistics package SPSS (release 16, SPSS Inc., Chicago, IL, USA). Statistical significance was taken to be indicated by P<0.05.

Results

In 80 cows, CE was defined by the presence of >50 % purulent uterine discharge (score 3) detectable in the vagina at 21 days or more postpartum or of mucopurulent discharge (score 2 or 3) detectable in the vagina after 26 days postpartum (Sheldon et al. 2006). Totally, 60 cows with >18 % PMNs were diagnosed as SE cows.

The mean and SD of the hematological parameters in CE, SE, and healthy cows are presented in Table 1. Cows of the SE group presented a significant decrease in PCV (P<0.05) and RBC (P<0.05) values, when compared to healthy group, while the CE group presented a significant decrease (P<0.01) in PCV and RBC count, when compared to healthy and SE animals (Table 1). Significant increases in WBC (P<0.05), neutrophil (P<0.001), and lymphocyte (P<0.001) counts, in the CE and SE groups, were observed when compared to healthy cows (Table 1).

After treatment for clinical endometritis, PCV and RBC count increased significantly (P<0.001), whereas WBC, neutrophil, lymphocyte, and monocyte counts decreased significantly (P<0.05, Table 2). In the subclinical endometritis group, WBC, neutrophil, lymphocyte, and monocyte counts decreased significantly after treatment (P<0.05, Table 3).

Discussion

Dairy cows undergo substantial physiological and hematological adaptations during the transition from pregnancy to lactation that render her more prone to uterine and mammary infections (Mateus et al. 2002). In the present study, the PCV and RBC count in SE and CE cows were significantly lower than in the healthy cows (P< 0.05). These changes in the erythrocyte parameters were probably due to the inflammatory cytokines produced in cow with endometritis. Cytokines play a crucial role in the modulation of local and systemic inflammatory responses and are of importance for the resolution of the induced inflammation (Henderson and Wilson 1996). Previous studies in cattle and ewes have suggested that acute phase proteins are produced systemically in animals with clinical endometritis (Regassa and Noakes 1999; Sheldon et al. 2001). The acute phase response is induced primarily by cytokines IL-1, IL-6, and TNFα

Table 1 Mean ± SD of hematological parameters in cows with CE and SE at the time of diagnosis

Value	Clinical (n=80)	Subclinical (n=60)	Health (n=30)
PCV (L/L)	0.23±0.04 a	0.25±0.02 b	0.27±0.03 c
Hb (g/L)	85.7±7.3	85.0±8.5	93.1±11.3
RBC ($\times 10^{12}$/L)	5.06±0.93 a	5.57±1.11 b	6.06±1.07 c
MCV (fL)	47.17±6.02	47.69±4.91	48.28±3.25
MCH (pg)	15.76±1.35	15.7±1.61	16.29±1.31
MCHC (g/dL)	33.16±1.36	33.16±0.54	33.61±1.44
Plt (10^9/L)	373±220	409±165	340±183
WBC ($\times 10^9$/L)	16.36±17.49 a	14.44±4.37 a	7.88±1.31 b
Neutrophil ($\times 10^9$/L)	8.16±2.66 a	8.45±3.36 a	3.49±8.64 b
Lymphocyte ($\times 10^9$/L)	5.56±1.65 a	5.58±1.32 a	4.21±7.51 b
Monocyte ($\times 10^9$/L)	0.50±0.37	0.33±0.24	0.12±0.14
Eosinophil ($\times 10^9$/L)	0.13±0.18	0.08±0.12	0.05±0.08

In each row, means with different lowercase letters show significant differences (P<0.05)

Table 2 Comparison of the level of hematological parameters in CE cows at the time of diagnosis (before treatment) and after treatment

Value	Before treatment	After treatment
PCV (L/L)[a]	0.22±0.04	0.26±0.03
Hb (g/L)	87.5±8.43	86.8±10.5
RBC ($\times10^{12}$/L)[a]	4.96±0.95	5.60±0.77
MCV (fL)	47.66±3.43	46.54±4.29
MCH (pg)	15.85±1.25	15.55±1.13
MCHC (g/dL)	33.29±1.22	33.18±0.61
Plt (10^9/L)	400±223	370±208
WBC ($\times10^9$/L)[a]	17.180±2.14	7.36±2.41
Neutrophil ($\times10^9$/L)[a]	8.32±2.97	3.61±1.12
Lymphocyte ($\times10^9$/L)[a]	5.72±1.83	3.88±0.99
Monocyte ($\times10^9$/L)[a]	0.48±0.36	0.07±0.11
Eosinophil ($\times10^9$/L)	0.08±0.13	0.02±0.04

[a] Significant difference between different times (before and after treatment)

(Jensen and Whitehead 1998). These cytokines play a central role in increasing hepcidin which inhibits both absorption of dietary iron from intestinal epithelium and export of iron from macrophages and hepatocytes (Nemeth et al. 2004). Therefore, affected animals have some laboratory features of iron deficiency (hypoferremia and decreased total iron-binding capacity, but increased iron stores in the mononuclear phagocyte system (Fry 2010). Inflammation can contribute to anemia via mechanisms other than hepcidin-mediated disruption of normal iron handling. Inflammatory cytokines act as inhibitors of erythropoiesis via direct toxic effects on erythroid precursors, decreased expression of hematopoietic factors including erythropoietin and stem cell factor, and decreased expression of erythropoietin receptors. Inflammation has also been shown to cause decreased erythrocyte survival (Fry 2010).

Table 3 Comparison of the level of hematological parameters in SE cows at the time of diagnosis (before treatment) and after treatment

Value	Before treatment	After treatment
PCV (L/L)	0.25±0.02	0.26±0.03
Hb (g/L)	84.5±8.2	86.2±10.4
RBC ($\times10^{12}$/L)	5.46±0.89	5.60±1.17
MCV (fL)	48.50±4.36	47.55±4.88
MCH (pg)	15.90±1.43	15.62±1.56
MCHC (g/dL)	33.08±0.54	32.66±0.76
Plt (10^9/L)	427±160	380±171
WBC ($\times10^9$/L)[a]	15.46±4.98	7.02±1.33
Neutrophil ($\times10^9$/L)[a]	9.17±3.87	2.73±0.75
Lymphocyte ($\times10^9$/L)[a]	5.85±1.42	4.18±1.12
Monocyte ($\times10^9$/L)[a]	0.35±0.24	0.12±0.14
Eosinophil ($\times10^9$/L)	0.09±0.13	0.05±0.08

[a] Significant difference between different times (before and after treatment)

In cows with CE and SE, the number of WBC, neutrophils, and lymphocytes were significantly higher than in the healthy cows ($P<0.05$). Similarly, Nazifi et al. (2008) observed significant differences in segmented neutrophils in the clinically healthy and affected cows by the subclinical endometritis in 25–30 days after parturition, and the percentage and absolute number of segmented neutrophils were significantly higher in the cows with subclinical endometritis than in the clinically healthy cows ($P<0.05$). Neutrophilia in ruminants is frequent in mild or moderate inflammation and following the acute stage of more severe inflammation. Inflammatory neutrophilia has been reported in infections in the mammary gland, liver, central nervous system, heart, gastrointestinal tract, respiratory tract, and urinary tract. Although lymphocytosis is not common in ruminants, increased lymphocyte count may occur in chronic inflammatory conditions (Fry 2010). During the periparturient period, dairy cows are subjected to physiological changes that may induce immunosuppression and an increased susceptibility of the animal to bacterial infections. An impairment of the blood PMN oxidative burst activity was observed in dairy cows with endometritis (Mateus et al. 2002). This decrease could be, at least partially, due to the impairment of the myeloperoxidase activity and the hydrogen peroxide production (Mateus et al. 2002). Cai et al. (1994) observed that the killing capacity of PMN is more impaired in cows with an abnormal puerperium than in those with a normal puerperium. These data suggest that the decrease in the killing capacity of blood PMN might favor the establishment of the uterine infection.

After treatment for CE and SE, the observed changes in hematological parameters were improved and PCV and RBC count increased significantly, whereas WBC, neutrophil, lymphocyte, and monocyte counts dropped significantly ($P<0.05$). These findings confirm the endometritis-induced changes in hematological parameters that improve after treatment. It has been reported that the severity of uterine bacterial contamination was correlated with the peripheral circulating concentrations of acute phase proteins (Sheldon et al. 2001). It seems that the acute phase proteins and subsequent inflammatory response were declined after treatment in cows with CE and SE.

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Conflict of interest None of the authors have any conflict of interest to declare.

References

Cai TQ, Weston PG, Lund LA, Brodie B, McKenna DJ, Wagner WC (1994) Association between neutrophil functions and periparturient disorders in cows. Am J Vet Res 55:934–943

Fry MM (2010) Anemia of inflammatory, neoplastic, renal and endocrine diseases. In: Weiss DJ, Wardrop KJ (eds) Schalms veterinary hematology, 6th edn. Blackwell, Iowa, pp 246–250

Guidry AJ, Paape MJ, Pearson RE (1976) Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. Am J Vet Res 37:1195–1200

Henderson B, Wilson M (1996) Cytokine induction by bacteria: beyond lipopolysaccharide. Cytokine 8:269–282

Jain NC (1986) Schalms veterinary hematology. Lea and Febiger, Philadelphia

Jensen LE, Whitehead AS (1998) Regulation of serum amyloid A protein expression during the acute-phase response. Biochem J 334:489–503

Kasimanickama R, Duffielda TF, Fosterb RA, Gartleya CJ, Lesliea KE, Waltonc JS, Johnsona WH (2004) Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62:9–23

Mateus L, Lopes da Costa L, Carvalho H, Serra P, Robalo Silva J (2002) Blood and intrauterine leukocyte profile and function in dairy cows that spontaneously recovered from postpartum endometritis. Reprod Dom Anim 37:176–180

Nazifi S, Ahmadi MR, Gheisari HR (2008) Hematological changes of dairy cows in postpartum period and early pregnancy. Comp Clin Pathol 17:157–163

Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 113:1271–1276

Preisler MT, Weber PS, Tempelman RJ, Erskine RJ, Hunt H, Burton JL (2000) Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. Am J Vet Res 61:14–19

Regassa F, Noakes DE (1999) Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. Vet Rec 144:502–506

Saad AM, Concha C, Astrom G (1989) Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. J Vet Med B 36:337–345

Sheldon IM, Noakes DE, Rycroft A, Dobson H (2001) Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet Rec 148:172–175

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006) Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530

Williams EJ, Fischer DP, England GCW, Dobson H, Pfeiffer DU, Sheldon IM (2005) Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle. Theriogenology 63:102–117