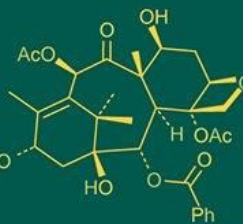
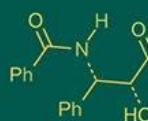


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Alterations in the haematological profile of cows affected with postpartum Metritis

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Abstract

The present investigation was undertaken to analysis the haematological changes in cows affected with postpartum metritis. In this study, eight healthy cows and eight cows with postpartum metritis were selected. Animals which were not having enlarged uterus and fetid uterine discharge within first 21 days after parturition were classified as healthy and included in the control group. Animals with an enlarged uterus and fetid uterine discharge within first 21 days after parturition were classified as postpartum metritis and included in the experimental group. Haematological parameters were assessed in both control and experimental animals including red blood cell count (RBC; $\times 10^6 / \text{mm}^3$), hemoglobin (Hb; g/dl) concentration and packed cell volume (PCV; %), total leukocyte count (TLC; $\times 10^3 / \text{mm}^3$) and differential leukocyte count (DLC; %) using an automatic haematology analyser (Mindray - BC - 2800 Vet). Blood smears were prepared, stained with Leishman stain and DLC was determined. The RBC count, Hb level and PCV value on day 0 were significantly reduced in cows affected with postpartum metritis than healthy cows. The TLC and DLC on day 0 were significantly elevated in cows affected with postpartum metritis than healthy cows. The present study reveals that hematological parameters were altered in cows affected with postpartum metritis on day 0 and progressively restored to normal levels in day 21 by suitable treatment.

Keywords: Postpartum metritis, cows, erythrocyte and leukocyte

Introduction

The postpartum phase in dairy cattle represents a vital physiological period marked by extensive metabolic, hormonal and immune system adaptations that facilitate calving, milk synthesis and uterine recovery. However, this transitional period is associated with heightened vulnerability to a range of reproductive and metabolic disturbances. Among these, postpartum metritis remains one of the most prevalent and economically impactful conditions affecting dairy herd productivity (Rafa *et al.*, 2024) ^[11].

The peak pro-inflammatory state may suppress erythropoiesis due to the direct effects of cytokines on bone marrow. This process reduces the erythropoietic response to erythropoietin, which becomes insufficient to compensate for the destruction of erythrocytes and results in decreased haematological values and the onset of anemia (Nemeth and Ganz, 2014) ^[9]. Pro-inflammatory cytokines are produced in greater quantities during the inflammatory process which inhibit erythropoiesis through the action on erythroid precursors (Chikazawa and Dunning, 2016) ^[13].

Joshi *et al.* (2018) ^[6] reported that significant increase in TLC during the postpartum phase compared to the prepartum period. This elevation was accompanied by higher lymphocyte and neutrophil counts indicating enhanced immune activation following calving. Such leukocytic responses are considered as part of the normal physiological adaptation to the stress and inflammatory challenges of parturition.

Cows suffering from metritis exhibit notable haematological disturbances during the transition period. A decline in Hb levels and an increase in leukocyte counts reflect systemic inflammation and immune activation (Ramadan *et al.*, 2020) ^[12]. Maintaining an adequate nutritional status is essential for optimal reproductive performance which often reflected through changes in haematological markers found in plasma or serum (Stengarde *et al.*, 2008) ^[13]. Periods of metabolic stress can lead to significant fluctuations in various blood constituents including red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV),

total leukocyte count (TLC) and differential leukocyte count (DLC) which are instrumental in diagnosing diseases in cattle.

Materials and Methods

In this study, eight healthy cows and eight cows with postpartum metritis were selected based on their history, breeding records, vaginal discharge, per vaginal and per rectal examinations of the reproductive tract. Previous breeding history, calving history, feeding habit, rectal temperature, date of calving and milk yield were collected. Animals which were not having enlarged uterus and fetid uterine discharge within first 21 days after parturition were classified as healthy and included in the control group (Group I). Animals with an enlarged uterus and fetid uterine discharge within first 21 days after parturition were classified as postpartum metritis and included in the experimental group (Group II), which were presented to Large Animal Obstetrics Outpatient Unit, Teaching Veterinary Hospital, Madras Veterinary College, Chennai-600 007. Cows affected with postpartum metritis were administered with oxytetracycline hydrochloride (Axyvet, Vetsteps pharmaceuticals) at a dose rate of 22 mg per kg body weight diluted in normal saline for five consecutive days.

Blood drawn from the jugular vein of healthy cows on day 0 (the first 21 days following parturition are considered to be healthy animals), day 5, and day 21 of the initial sampling procedure and blood drawn from postpartum metritis affected cows on day 0 (the first day of treatment), day 5 and day 21. Haematological parameters were assessed in both control and experimental animals including red blood cell count (RBC; $\times 10^6/\text{mm}^3$), hemoglobin (Hb; g/dl) concentration and packed cell volume (PCV; %), total leukocyte count (TLC; $\times 10^3/\text{mm}^3$) and differential leukocyte count (DLC; %) using an automatic haematology analyser (Mindray-BC-2800 Vet). Blood smears were prepared, stained with Leishman stain and DLC was determined.

Results and Discussion

The mean ($\pm$ SE) values of RBC ($\times 10^6/\text{mm}^3$), Hb (g/dL), PCV (%), TLC ($\times 10^3/\text{mm}^3$) and DLC (%) of eight healthy cows and eight cows with postpartum metritis on day 0, day 5, day 21 are presented on the Table 1 and 2. Significantly ($p < 0.05$) lower erythrocyte counts were observed in Group II than Group I on day 0 and day 5, but RBC counts on day 21 between the groups did not differ significantly. Significantly ($p < 0.05$) lower Hb level was noticed in Group II than Group I on day 0. The PCV value was significantly ($p < 0.05$) low in Group II than Group I on day 0. Although Hb and PCV values on day 5 and day 21 between the groups were non-significant, an increasing trend was noted in Group II as the day's progress following treatment.

Results of our study are in accordance with earlier studies. Weiss and Wardrop (2010) [16] reported that the elevation of RBC count and Hb concentration seen immediately post-calving may be due to erythrocytosis associated with uterine involution and endocrine changes. Barragan *et al.* (2018) [2] observed that circulating RBC concentration was significantly reduced in cows diagnosed with clinical metritis. Joshi *et al.* (2018) [6] reported a notable decline in Hb concentration in dairy cows with levels decreasing from the day of calving to 30 days postpartum. This reduction

may be linked to blood loss during parturition, increased metabolic demands and potential suppression of erythropoiesis due to inflammatory mediators during the early postpartum period. Cui *et al.* (2019) [4] compared the hematological profiles between healthy and cows diagnosed with metritis. Their findings revealed that metritic cows had significantly reduced RBC counts, Hb levels and PCV value on days 7 and 30 of postpartum. Cows with postpartum disease had considerably lower Hb contents and erythrocyte contents than normal cows (Baimishev *et al.*, 2020) [1]. Cows affected by puerperal metritis exhibited significant decline in PCV within the initial two weeks following parturition. It is closely associated with the development of anemia and the intensity of uterine infection (Ramadan *et al.*, 2020) [12]. These hematological impairments were attributed to systemic inflammation and disrupted erythropoiesis, potentially driven by elevated levels of pro-inflammatory cytokines, particularly IL-6, ongoing metabolic disturbances such as negative energy balance and associated conditions like hypocalcemia which collectively impair RBC production and overall hematological status (Cui *et al.*, 2019) [4].

The WBC count in Group II was elevated as compared to Group I and it was highly significant ($p < 0.01$) on day 0, significant ($p < 0.05$) on day 5 and insignificant on day 21. The WBC count in Group II showed a gradual reduction from day 0 to day 21 following treatment, indicating progressive improvement towards normal levels. Significantly ($p < 0.01$), higher percentage of neutrophils was observed on day 0 in Group II as compared to Group I. Though there was no significant variation in neutrophil percentage between the groups on day 5 and day 21, higher percentage of neutrophils were observed in Group II as compared to Group I. The neutrophil percentage showed a progressive decline from day 0 to day 21 in Group II. Significantly ($p < 0.01$), lower percentage of lymphocytes was noticed on day 0 in Group II as compared to Group I. Though there was no significant variation in lymphocyte percentages between the groups on day 5 and day 21, comparatively lower percentage of lymphocyte was observed in Group II when compared to Group I. No significant variation in monocyte percentages was found between the groups and between days within the group during the experiment period.

Previous studies have reported the similar findings as that of our study. Kornmatitsuk *et al.* (2001) [8] reported that TLC and polymorphonuclear cell concentrations peaked on the first day of postpartum, whereas mononuclear cell levels exhibited a delayed elevation reaching their maximum approximately three days after calving. Daramola *et al.* (2004) [5] stated that TLC along with average lymphocyte and neutrophil levels were significantly elevated in the postpartum period compared to the prepartum period. Joshi *et al.* (2018) [6] reported that significant increase in TLC during the postpartum phase compared to the prepartum period. This elevation was accompanied by higher lymphocyte and neutrophil counts indicating enhanced immune activation following calving. Such leukocytic responses are considered as part of the normal physiological adaptation to the stress and inflammatory challenges of parturition. Cui *et al.* (2019) [4] observed that neutrophil counts exhibit a temporary elevation at the time of calving in clinically healthy postpartum cows and it is primarily attributed to the physiological stress associated with

parturition. They also found that monocyte counts remain stable in healthy postpartum cows but are consistently higher in metritic cows at both early (0 days) and later (30 days) stages of postpartum. This elevation is indicative of systemic immune activation in response to persistent uterine infection. Ramadan *et al.* (2020) [12] revealed that white blood cell (WBC) counts were markedly elevated in cows diagnosed with metritis during the first and second weeks of postpartum.

Neutrophils are the first cells in the uterus to become active in response to uterine infection in initiating phagocytosis (Klucinski *et al.*, 1990) [7]. Mast cells and eosinophils also contribute for immune response. Immunoglobulin E (IgE)-high affinity receptors are present on the surface of these cells. Attachment to the antibody causes mast cells to degranulate which in turn causes the generation of interleukins and chemotactic factors for other immune cells

and other inflammatory mediators such as prostaglandins, histamine and TNF- α (Tizard, 1996 and Trowbridge and Emling, 1997) [14, 15]. Along with mast cells, eosinophils release chemicals that can kill bacteria including inflammatory mediators and reactive oxygen species (ROS). Specific immune mechanism against uterine infections includes cell-mediated immunity and humoral immunity. Lymphocyte populations present in the bovine endometrium, which are primarily located in the stromal tissues. These lymphocytes are a component of the acquired immune system, which can generate antibodies that kill bacteria and build immunological memory (Pantaleo *et al.*, 2014) [10].

From this study, it is evident that hematological parameters were altered in cows affected with postpartum metritis on day 0 and progressively restored to normal levels in day 21 by suitable treatment.

Table 1: Haematological parameters (RBC, Hb and PCV) in bovine postpartum metritis before and after treatment in Mean ($\pm$ SE)

Haematological parameters	Groups	Day 0	Day 5	Day 21	F-value
Red blood cells ($\times 10^6/\text{mm}^3$)	Group I	6.34 $\pm$ 0.32	6.46 $\pm$ 0.30	6.42 $\pm$ 0.23	0.046 ^{NS}
	Group II	5.26 $\pm$ 0.30	5.44 $\pm$ 0.30	5.75 $\pm$ 0.22	0.808 ^{NS}
	p-value	0.027	0.030	0.053	
Haemoglobin (g/dL)	Group I	10.93 $\pm$ 0.49	10.82 $\pm$ 0.45	10.95 $\pm$ 0.31	0.025 ^{NS}
	Group II	9.05 $\pm$ 0.41	9.71 $\pm$ 0.41	9.88 $\pm$ 0.41	1.121 ^{NS}
	p-value	0.012	0.094	0.062	
Packed Cell Volume (%)	Group I	30.58 $\pm$ 1.43	29.56 $\pm$ 1.52	30.85 $\pm$ 2.09	0.158 ^{NS}
	Group II	26.43 $\pm$ 0.84	27.14 $\pm$ 0.48	27.58 $\pm$ 0.65	0.745 ^{NS}
	p-value	0.026	0.151	0.158	

Values having different superscripts (a, b, c) within row differ significantly * ($p < 0.05$), ** ($p < 0.01$) and ^{NS}Non-significant ($p > 0.05$)

Table 2: Haematological parameters (TLC and DLC) in bovine postpartum metritis before and after treatment in Mean ($\pm$ SE)

Haematological parameters	Groups	Day 0	Day 5	Day 21	F-value
White blood cells ($\times 10^3/\text{mm}^3$)	Group I	8.70 $\pm$ 0.70	8.43 $\pm$ 0.84	7.76 $\pm$ 0.27	0.542 ^{NS}
	Group II	13.80 $\pm$ 1.24 ^a	10.81 $\pm$ 0.64 ^{ab}	8.75 $\pm$ 0.42 ^b	9.072 ^{**}
	p-value	0.003	0.043	0.068	
Neutrophil (%)	Group I	45.62 $\pm$ 1.30	43.37 $\pm$ 3.32	41.62 $\pm$ 1.2	0.847 ^{NS}
	Group II	61.88 $\pm$ 1.92 ^a	50.88 $\pm$ 2.57 ^a	42.25 $\pm$ 2.75 ^b	16.267 ^{**}
	p-value	0.000	0.096	0.838	
Lymphocyte (%)	Group I	47.12 $\pm$ 1.64	49.12 $\pm$ 2.80	51.87 $\pm$ 1.10	1.446 ^{NS}
	Group II	30.62 $\pm$ 2.41 ^a	42.37 $\pm$ 2.59 ^{ab}	50.62 $\pm$ 2.75 ^b	4.730 ^{**}
	p-value	0.000	0.099	0.680	
Monocyte (%)	Group I	6.87 $\pm$ 0.61	6.75 $\pm$ 0.81	6.62 $\pm$ 0.70	0.030 ^{NS}
	Group II	7.25 $\pm$ 0.72	6.50 $\pm$ 0.86	6.25 $\pm$ 0.88	0.396 ^{NS}
	p-value	0.699	0.837	0.745	

Values having different superscripts (a, b, c) within row differ significantly * ($p < 0.05$), ** ($p < 0.01$) and ^{NS}Non-significant ($p > 0.05$)

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