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Serum IL-6 and C-Reactive Protein Concentration in Uterine Inflammatory Diseases Affected Crossbred Cows

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ABSTRACT

Background: Diagnostic methods such as visual examination of cervico-vaginal mucus discharge, white side test, uterine endometrial cytology, uterine endometrial biopsy and ultra sonography imaging, has been described by various researchers for diagnosing uterine inflammatory diseases viz. metritis, clinical endometritis and subclinical endometritis,. Estimation of serum IL-6 and C - reactive protein provides biochemical means to diagnosethese uterine inflammatory diseases.The objective of this study was to compare the concentrations of inflammatory cytokine (IL -6) and C-reactive protein in healthy (control) postpartum crossbred cows and cows suffering from uterine inflammatory diseases.

Methods: On the basis visual examination of vaginal discharges and white side test 96 cows with abnormal puerperium were categorized in three uterine inflammatory groups *viz*. metritis (M), clinical endometritis (CE) and subclinical endometritis (SCE) each inflammatory group containing 30 animals.A group of 6 cowsnegative for white side test were categorized as control group. The blood samples were collected and serum was separated for the estimation of IL-6 and C-reactive protein in different inflammatory groups and control group cows.

Result: The mean serum concentration of IL 6 in metritis, clinical endometritis, subclinical endometritis and control group cows were 3130±64.13, 3220±58.11, 2650±68.91 and 1820±6 1.46 pg/ml respectively. The mean concentration of serum C- reactive protein in Metritis (Group A), CE (Group B), SCE (Group C) and control (Group D) cows were 115.43±0.61, 86.24±0.61, 56.60±0.24 and 47.82±0.69 µg/ml respectively. This study showed that IL-6 concentrations in uterine inflammatory disease affected cows were higher than those in healthy control group cows. Similarly, serum concentration of CRP in metritis group was approximately three times higher than in the control group.

Key words: C-reactive protein, Clinical endometritis, Crossbred cows, IL-6, Metritis, Subclinical endometritis.

INTRODUCTION

Reproductive performance is one of the major determinants for the economic improvement of a dairy farm (Dutta *et al.,* 2025). Endometritis is an inflammation of the endometrial lining of uterus without systemic signs, which is associated with chronic postpartum infection of the uterus with pathogenic bacteria. Prevalence rate of endometritis in India ranges from 3 to 25% in cows (Parmar, 2021). Postpartum metritis is one of the most important disorders in cows causing high economic losses due to prolonged open days and inter-calving intervals, resulting in involuntary culling of animal (Kumar *et al.,* 2022). Uterine inflammatory diseases associated with abnormal puerperium such as metritis, clinical endometritis and subclinical endometritis is considered as major cause of infertility in cows. The incidence of uterine inflammatory diseases varies between 8 to 90%. Subclinical endometritis it ranges from 20 to 90%, depending on the threshold used and the postpartum examination timing (Yusuf *et al*., 2010). Genital infection is a major cause of infertility with an incidence up to 56.6% in crossbred cows (Maurya *et al.,* 1992). Uterine infection, mainly of bacterial origin attributes as major cause of uterine inflammatory disease especially in dairy animals in developing countries like India (Saini, 1993). In normal

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circumstances majority of the infections occurred during parturition and early puerperial period are eliminated itself by complex involution process however, in approximately 10 to 17% of postpartum cows, conditions favouring bacterial growth persist and eventually cause endometritis (Sheldon *et al.*, 2009).

Failure of reproduction in dairy animals results in progressive economic losses to the farmers. Jeyakumari *et al.* (2003) revealed that the annual losses incurred as a result of endometritis / metritis ranged between Rs. 2902.32 to Rs. 3101.70 per animal under Indian conditions.

Various diagnostic methods such as analysis of discharge, white side test (Dutta *et al.,* 2025) , endometrial cytology, endometrial biopsy and ultra sonography imaging has been described by various researchers to diagnose uterine inflammatory diseases like metritis, clinical endometritis and subclinical endometritis however estimation of IL-6 and C - reactive protein provides biochemical markers to diagnosis these uterine inflammatory diseases.

C-reactive protein (CRP) was identified in 1930 and was subsequently considered to be an ″acute phase protein,″ an early indicator of infectious or inflammatory conditions. Since its discovery, CRP has been studied as a screening device for inflammation, a marker for disease activity and as a diagnostic adjunct.

MATERIALS AND METHODS

The present work was carried out in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Mhow (M.P.) from October 2016 to June 2020.

Experimental design

Ninety six cows with abnormal puerperium were categorized into three uterine inflammatory group containing 30 animals in each group viz. metritis (M), clinical endometritis (CE), subclinical endometritis (SCE) and 6 cows were categorized as control. The animals showing abnormal discharge having pus plaques 30 days postpartum were included in the metritis group. The animals showing whitish vaginal discharge were included in the clinical endometritis (CE) group and the animals after 30 days postpartum showing apparently clear vaginal discharge but positive to white side test were included in the subclinical endometritis (SCE) group animals. The animals showing apparently clear vaginal discharge and negative to white side test were served as healthy control group. The blood samples were collected on the day of examination and serum was separated for the estimation of IL-6 and C-reactive protein in different inflammatory groups and control group cows.

Procedure

White side test

Equal volume of cervico-vaginal mucus and 5% NaOH were mixed in a test tube and heated up to boil. Development of yellow colour was considered as positive white side test.

Estimation of interleukin -6 (IL-6)

The concentration of IL-6 in serum samples were determined using bovine IL - 6 ELISA kit (Fine test).

Estimation of C –Reactive protein (CRP)

The concentration of CRP was measured using CRP - TURBI (SPINREACT) diagnostic reagent kit.

Data analysis

The data was analysed by using the standard statistical procedures described by Snedecor and Cochran (1994) and WASP -1 (Web Agri Stat Package) online software.

RESULTS AND DISCUSSION

Concentration of serum IL-6

The mean serum concentration of IL 6 in metritis, clinical endometritis, subclinical endometritis and control group cows were 3130±64.13, 3220±58.11, 2650±68.91 and 1820±6 1.46pg /ml respectively (Table 1).

The mean concentration of IL-6 in inflammatory group with metritis and clinical endometritis did not differ significantly from each other. The mean concentration of IL-6 in cows affected with subclinical endometritis were significantly lower ($p<0.01$) from cows affected with metritis and clinical endometritis however it was significantly higher ($p<0.01$) from healthy animals. The concentration of IL-6 in all three affected groups was significantly higher than the healthy cows.

In this study, cows diagnosed with metritis, clinical endometritis and subclinical endometritis had higher serum concentrations of IL-6 compared to normal cows. Similar findings were also observed by Kasimanickam *et al.* (2004). They also observed serum concentration of IL 6 as 3.23±0.16, 3.34±0.17, 2.58±0.13 and 1.72±0.09 (ng/ml) in

Table 1: Concentration of serum IL-6 in uterine inflammatory group cows.

Uterine inflammatory groups	No. of samples	IL-6 pg/ml (Mean±SE)
Group “A” (Metritis)	30	3130±64.13[a]
Group “B” (CE)	30	3220±58.11[a]
Group “C” (SCE)	30	2650±68.91[b]
Group “D” (Normal)	06	1820±61.46[c]

Different superscripts differ significantly ($P<0.05$).

Table 2: Concentration of serum C - reactive protein in uterine inflammatory group cows.

Uterine inflammatory groups	No. of samples	CRP µg/ml (Mean±SE)
Group “A” (Metritis)	30	115.43±0.61[a]
Group “B” (CE)	30	86.48 ±0.24[b]
Group “C” (SCE)	30	56.60±0.24[c]
Group “D” (Control)	06	47.82±0.69[d]

Different superscripts differ significantly ($p<0.01$).

metritis, clinical endometritis, subclinical endometritis and in normal cows, respectively.

Concentration of serum CRP

The mean concentration of serum C- reactive protein in metritis (Group A), CE (Group B), SCE (Group C) and control (Group D) were 115.43±0.61, 86.24±0.61, 56.60±0.24 and 47.82±0.69 µg/ml respectively.

The statistical analysis of variance revealed significant difference ($p<0.01$) among all inflammatory groups including healthy animals for concentration of C- reactive protein. It was highest in the animals affected with metritis followed by clinical endometirtis, group 'C' (subclinical endometritis) and the control group 'D' in that order (Table 2).

The mean serum CRP concentration (47.82±0.69) in healthy cows (control group) was in close approximation to the finding of Morimatsu (1989) who found the mean CRP level as 39.8±47.5 µg/ml in healthy cows.

According to one study, lower concentrations of tumor necrosis factor (TNF)-α, IL-1β, IL-6 and IL-8 before calving in cows that developed retained fetal membranes after parturition (Gajewski *et al.,* 1999) while elevated serum and tissue concentrations of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, were observed in cows with metritis, endometritis, or subclinical endometritis (Boro *et al.,* 2015).

The findings of present research are in agreement with (Kim *et al.,* 2014) who found higher ($P<0.05$) IL-6 concentration in cows with clinical endometritis than in cows with subclinical endometritis and healthy controls. According to (Brodzki *et al.,* 2015b) significantly higher levels of IL-6 in the serum of subclinical endometritis affected cows (68.73±12.15 pg/ml) were observed as compared to control group cows (53.59±3.19 pg/ml) ($p<0.001$). However, the study by (Ishikawa *et al*., 2004) showed no difference in the concentration of these cytokines in the serum of cows with endometritis and healthy animals .

This study showed that IL-6 concentrations in uterine inflammatory disease affected cows were higher than those in healthy control group cows. IL-6 is known to be a cytokine produced by T cells. T cells are classified roughly into Th1 cells and Th2 cells by the method of cytokine production. The Th1 cell induces cell mediated immunity by producing mainly IL-2, TNF-α and IFN-γ and the Th2 cell induce antibody production by producing mainly IL-4, IL-5, IL-6, IL-10 and IL-13 (Mosmann *et al.*, 1986).

The CRP value found in the present study is in partial agreement with the study of Lee *et al.* (2003) who reported significantly higher serum CRP level in cows with clinical endometritis on day 28 postpartum (262.47±8.69 µg/ml) than in healthy cows (1.39±0.04 µg/ml) in their study. Consistent with their findings, our study also found that serum CRP levels were higher with uterine inflammatory disease group cows than in control group cows (47.82±0.69 µg/ml). Kaya *et al.* (2016) also reported 48.88± 3.92 µg/ml mean serum CRP level in control group cows, 82.86±4.28 µg/ml in mild endometritis cows and 122.34± 12.72 µg/ml in severe endometritis cows.

It is known that the potential for diseases is higher under poor body conditions. In this respect, it is thought that there is a relation between health conditions in dairy farming and CRP levels (Lee *et al*., 2003). C - reactive protein (CRP) is one of the major acute phase proteins (APP) and is widely distributed in nearly all vertebrates (Sarikaputi *et al*., 1991). During infections and under stressful conditions, human and animal mononuclear series cells, including monocytes and macrophages, secrete cytokines; such as, IL-1; IL-6; tumor necrosis factor- α and interferon, which stimulate the liver to rapidly synthesize large amounts of CRP (Godson *et al*., 1996). According to Horadagoda *et al*. (1999) C-reactive protein is the serum APP that responds most quickly to infections. C-reactive protein can regulate the immune system during the early stage of an infection. C-reactive protein plays a role in destroying infectious agents, minimizing tissue damage and facilitating tissue repair and regeneration. Metritis (M), clinical endometritis (CE) and subclinical endometritis (SCE) are infectious and inflammatory diseases of the uterus that significantly contribute to stress in the animal, resulting in increased levels of IL-6 and CRP.

CONCLUSION

IL-6 concentrations in uterine inflammatory disease affected cows were higher than those in healthy control group cows. Serum concentration of CRP in metritis group was approximately three times higher than in the control group. This difference in CRP levels suggests that serum CRP levels are sensitive to uterine infections and can be a significant indicator in the diagnosis of inflammatory conditions of uterus.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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