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Effect of immunomodulators on certain haematological parameters in ameliorating bovine Endometritis

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Abstract
In the present investigation seventy two endometritic cows selected from some parts of Odisha and West Bengal after meticulous screening and were allotted randomly into six equal groups (n=12). Group I, II and III animals were administered with 100µg of *E. coli* LPS (LPS), 500 mg of oyster glycogen (OG), and 1000 mg enrofloxacin through i.u route after reconstituted with 30 milliliter of Phosphate Buffer Saline (PBS). Oral herbal drugs were used in group IV animals. In group V, 50 ml of normal saline was infused i.u. Endometritis animals receiving no treatments were considered as control for the study. Blood sample was collected from experimental cows prior to institution of therapy, 24 hrs after treatment and at next Estrus for evaluation of different haematological parameters. Use of immunomodulators improved Hb concentration Significantly ($p < 0.05$) at post treatment succeeding estrous. A significantly lower ($p < 0.05$) mean TLC value was observed at succeeding estrus in group I and II than other treatment groups. mean Nutrophil value at 24 hrs of treatment increased significantly ($p < 0.05$) from pretreatment value and it decreased significantly at the next succeeding etrous.

Keywords: Endometritis, cows, immunomodulators, haematology

Introduction
Repeat breeding is one of the major problems affecting reproductive efficiency. The repeat breeding syndrome is a major source of economic waste and poor reproductive performance in dairy herds. The presence of clinical and Subclinical Endometritis (SE) is one of the etiological factors of repeat breeding syndrome. Endometritis is termed as the inflammation of endometrium and underlying glandular tissue without any systemic signs, occurring at least 21 days after calving (Sheldon *et al.*, 2006) [1]. Uterine infections are observed following twin births, dystocia and surgical interventions employed to aid in cases of dystocia, retention of fetal membrane, stillbirth, prolapse of uteri, artificial insemination, copulation, and the use of intrauterine irritants. The inflammatory and immune response to uterine bacterial infection compromises animal welfare as well as cause subfertility and infertility (Sheldon *et al.*, 2004) [2]. Dysfunction of cell immunity coexisting with subclinical Endometritis may be the main factor causing advanced inflammation of the uterus. There are substantial changes in subpopulations of immune cells in cows with subclinical Endometritis. Local mechanisms of uterine immunity vary from general immune mechanisms, the evidence of which are differences in the percentages of leukocyte subpopulations between uterus and peripheral blood of cows and possession of a lower phagocytic activity of uterine phagocytes than blood phagocytes. Knowledge of immunological mechanisms in the uterus of cows during subclinical Endometritis should enable more precise diagnosis of uterine inflammatory reactions and may be helpful in choosing proper adjuvant therapy with Immunomodulating agents. Immunomodulators are the new approach in treating Endometritis. They play their role to minimize infection by enhancing both systemic and local immunity through proliferating Certain body components lymphocytes, neutrophils, monocytes, humoral antibodies and PMN cells (Singh *et al*., 2001) [3]

Materials and Method
A total number of seventy two Endometritis affected cows were selected after screening and were allotted randomly into six equal groups having 12 animals in each group. Group I and II

animals were administered with 100µg of *E. coli* LPS and 500 mg of oyster glycogen respectively after reconstituted with 30 ml of PBS. 1000 mg of Enrofloxacin reconstituted with 30 ml PBS was infused Intrauterinely in Group III Endometritis animals. In Group IV, the cows were treated with 100 ml of oral ecbolic drug (UPLUS) daily for 5 days. Group V animals received 30 ml Normal Saline in the similar manner as I.U route. Group VI, Constituted of Endometritic animals at estrus presented for artificial insemination (AI) without institution of any kind of therapy taken for comparative study and were considered as negative control. Blood samples were collected from experimental cows prior to institution of therapy, 24 hrs after treatment and at next estrus for evaluation of different haematological parameters. Certain haematological parameters like Haemoglobin concentration, Total leukocyte count, Differential Leucocyte count (Nutrophil , lymphocyte, Eosinophil and Monocyte percentage) were estimated by standard protocol as described by Schalm, 1965 [4].

Results

Hemoglobin concentration (Hb)

The pre-treatment Hb concentration was recorded to be 10.30 ± 0.22, 10.15 ± 0.29, 9.97 ± 0.34, 10.28 ± 0.43, 9.90 ± 0.24 and 9.89 ± 0.25 for group I, II, III, IV, V and VI respectively (Table 1). The corresponding Hb concentration at succeeding estrus for group I, II, III, IV, a and VI were recorded to be 11.86 ± 0.29, 12.2 ± 0.23, 10.45 ± 0.33, 11.68 ± 0.29, 10.12 ± 0.25 and 9.97 ± 0.36 respectively. Statistically there was no significant difference between the groups prior to treatment and at 24 HRS after treatment. The mean Hb concentration at subsequent estrus varied non-significantly between group I, II and IV. Mean Hb concentration in all the above groups varied significantly ($p < 0.05$) from that of group III, V and VI. A significant ($p < 0.05$) increase was observed in mean Hb concentration at post-treatment succeeding estrus in group I, II and IV.

Table 1: Pre and post treatment mean haemoglobin concentration (g/dL) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	10.30 ± 0.22^{a}	10.44 ± 0.32^{a}	11.86 ± 0.29^{Bb}
Group II (n= 12)	10.15 ± 0.29^{a}	10.24 ± 0.27^{a}	12.2 ± 0.23^{Bb}
Group III (n= 12)	9.97 ± 0.34	9.84 ± 0.34	10.45 ± 0.33^{A}
Group IV (n= 12)	10.28 ± 0.43^{a}	10.07 ± 0.28^{a}	11.68 ± 0.29^{Bb}
Group V (n= 12)	9.90 ± 0.24	9.87 ± 0.31	10.12 ± 0.25^{A}
Group VI (n= 12)	9.89 ± 0.25	9.82 ± 0.29	9.97 ± 0.36^{A}

Means bearing different superscripts within group (a,b,c) and between groups (A, B,C,D) vary significantly (p< 0.5).

Total Leukocyte count (TLC)

Prior to treatment the mean TLC was found to be non-significant between all groups (Table 2). After 24 hrs of treatment the corresponding values were 13.07 ± 0.47, 13.10 ± 0.38, 11.39 ± 0.29, 11.38 ± 0.36, 11.08 ± 0.26 and 11.96 ± 0.39 in Gr I, II, III, IV, V and VI respectively. In group I and II the value of mean TLC differed significantly at 24 hrs and next estrous of treatment from the pretreatment value. No significant difference was observed within days among group III, IV, V and VI. At 24 hrs significantly higher ($p < 0.05$) values were found in group I and II from other treatment groups. A significantly lower ($p < 0.05$) value was observed in mean TLC at succeeding estrus in group I and II followed by V, III, IV and VI.

Table 2: Pre and post treatment mean Total Leucocyte Count (TLC) ($\times 10^3/mm^3$) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	11.23 ± 0.24^{b}	13.07 ± 0.47^{Bc}	9.60 ± 0.30^{Aa}
Group II (n= 12)	11.55 ± 0.37^{b}	13.10 ± 0.38^{Bc}	9.64 ± 0.30^{Ab}
Group III (n= 12)	11.47 ± 0.36	11.39 ± 0.29^{A}	10.35 ± 0.34^{AB}
Group IV (n= 12)	11.94 ± 0.44	11.38 ± 0.36^{A}	11.26 ± 0.39^{BC}
Group V (n= 12)	11.45 ± 0.30^{b}	11.08 ± 0.26^{Ab}	10.21 ± 0.22^{Aba}
Group VI (n= 12)	11.36 ± 0.38	11.96 ± 0.39^{A}	11.78 ± 0.38^{C}

Means bearing different superscripts within group (a,b,c) and between groups (A, B, C, D) vary significantly (p< 0.5)

Differential leucocyte count

Neutrophil count

Prior to treatment the mean neutrophil count was found to be 37.64 ± 0.81, 37.81 ± 0.88, 38.62 ± 1.33, 38.76 ± 0.61, 38.91 ± 0.96 and 38.44 ± 0.83 in Gr I, II, III, IV, V and VI respectively (Table 3). There was no significant difference between the groups prior to treatment was seen. At 24 hrs after treatment the mean neutrophil count of group I and II was significantly higher ($p < 0.05$) than other treatment groups. At succeeding post treatment estrous group VI value was significantly higher than group I, II, IV V and non-significant with group III.

In group I and II the mean value at 24 hrs of treatment increased significantly from pretreatment value and it decreased significantly at the next succeeding etrous. In group III, IV and V the mean neutrophil percentage was non-significant between pretreatment and 24 hrs of post treatment but was significant at next estrous. In group VI no significant difference was seen in any days of sampling.

Table 3: Pre and post treatment mean nutrophil concentration (%) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	37.64 ± 0.81^{b}	42.92 ± 0.77^{Bc}	33.34 ± 0.64^{Aa}
Group II (n= 12)	37.81 ± 0.88^{b}	41.46 ± 0.91^{Bc}	34.13 ± 0.80^{Aa}
Group III (n= 12)	38.62 ± 1.33^{b}	36.19 ± 1.30^{Aab}	35.12 ± 0.90^{Aba}
Group IV (n= 12)	38.76 ± 0.61^{c}	36.45 ± 0.80^{Abc}	33.73 ± 0.61^{Aa}
Group V (n= 12)	38.91 ± 0.96^{b}	36.72 ± 0.71^{Aab}	34.32 ± 0.59^{Aa}
Group VI (n= 12)	38.44 ± 0.83	38.12 ± 1.10^{A}	37.74 ± 0.26^{B}

Means bearing different superscripts within group (a,b,c) and between groups (A, B, C, D) vary significantly (p< 0.5)

Lymphocyte count

No significant difference was observed between the groups prior to treatment. At 24 hrs of treatment the mean lymphocyte count of group I and II was significantly lower ($p < 0.05$) than other treatment groups (Table 4). No significant difference seen between group III, IV, V and VI. At subsequent estrus only group VI varied significantly from other groups.

In group I and II Mean lymphocyte count differed significantly ($p < 0.05$) within different days of sampling which was highest in succeeding estrous and lowest in 24 hrs of treatment. In group III no significant difference was seen within pretreatment and 24 hrs post treatment and within 24 hrs and next estrous was found. But the value in succeeding estrous was significantly higher than pretreatment sampling. No significant difference was observed in group IV, V and VI within different collection samples with respect to mean lymphocyte count.

Table 4: Pre and post treatment mean lymphocyte concentration (%) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	53.28 ± 0.79^{b}	48.51 ± 0.81^{Aa}	58.10 ± 0.88^{Bc}
Group II (n= 12)	53.52 ± 0.86^{b}	49.42 ± 0.87^{Aa}	56.67 ± 0.83^{Bc}
Group III (n= 12)	54.22 ± 0.61^{a}	55.38 ± 0.74^{Bab}	57.78 ± 0.36^{Bb}
Group IV (n= 12)	52.70 ± 1.13	54.47 ± 1.03^{B}	56.26 ± 1.01^{B}
Group V (n= 12)	53.18 ± 0.81^{a}	53.97 ± 0.67^{Bab}	56.36 ± 1.12^{Bb}
Group VI (n= 12)	53.92 ± 1.12	53.60 ± 1.13 ^{B}	53.22 ± 0.61^{A}

Means bearing different superscripts within group (a,b,c) and between groups (A, B, C, D) vary significantly (p< 0.5).

Eosinophil count

The mean eosinophil count was found to be 5.40 ± 0.22, 5.90 ± 0.48, 5.50 ± 0.34, 5.50 ± 0.40, 5.46 ± 0.43 and 5.60 ± 0.55 in Gr I, II, III, IV, V and VI respectively at the pretreatment stage. At succeeding estrus the mean eosinophil count was found to be 3.85 ± 0.36, 4.25 ± 0.39, 5.30 ± 0.45, 5.80 ± 0.33, 4.40 ± 0.46 and 5.45 ± 0.50 in Gr I, II, III, IV, V and VI respectively. Statistically there was no significant difference between the treatment groups prior to treatment, after 24 hrs of treatment. At post treatment succeeding estrus group I and II values differed significantly between other groups and within other sampling days (Table 5).

Table 5: Pre and post treatment mean eosinophil count (%) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	5.40 ± 0.22^{b}	5.72 ± 0.24^{b}	3.85 ± 0.36^{Aa}
Group II (n= 12)	5.90 ± 0.48^{b}	5.35 ± 0.53^{b}	4.25 ± 0.39^{Aa}
Group III (n= 12)	5.50 ± 0.34	5.86 ± 0.34	5.30 ± 0.45^{B}
Group IV (n= 12)	5.50 ± 0.40	5.80 ± 0.83	5.80 ± 0.33^{B}
Group V (n= 12)	5.46 ± 0.43	5.10 ± 0.53	4.40 ± 0.46^{B}
Group VI (n= 12)	5.60 ± 0.55	5.85 ± 0.83	5.45 ± 0.50^{B}

Means bearing different superscripts within group (a, b, c) and between groups (A, B, C, D) vary significantly (p< 0.5).

Monocyte count

Statistically there was no significant difference between the groups prior to treatment and after 24 hrs of treatment (Table 6). The mean monocyte count at subsequent estrus varied non-significantly among group I, II and III. Mean monocyte count in all the above groups varied significantly ($p < 0.05$) from that of group IV, V and VI. A significant increase ($p < 0.05$) was observed in mean monocyte count at succeeding estrus in group I, II and III. No significant difference was observed between group IV, V and VI with respect to mean monocyte count.

Table 6: Pre and post treatment mean Monocyte count (%) in various experimental groups

Intervals / Groups	Pre-treatment	Post treatment	
		After 24 hrs	Succeeding estrus
Group I (n= 12)	2.70 ± 0.30^{a}	2.60 ± 0.37^{a}	3.90 ± 1.29^{Bb}
Group II (n= 12)	2.60 ± 0.27^{a}	2.50 ± 0.37^{a}	3.70 ± 1.16^{Bb}
Group III (n= 12)	2.80 ± 0.25^{a}	2.66 ± 0.37^{a}	3.90 ± 1.20 ^{Bb}
Group IV (n= 12)	2.50 ± 0.34	2.40 ± 0.31	2.30 ± 0.95^{A}
Group V (n= 12)	2.90 ± 0.46	2.80 ± 0.36	2.50 ± 0.97^{A}
Group VI (n= 12)	2.86 ± 0.65	2.80 ± 0.50	2.76 ± 0.42

Means bearing different superscripts within group (a,b,c) and between groups (A, B, C, D) vary significantly (p< 0.5)

Discussion

Hemoglobin concentration

Hemoglobin is regarded as a major constituent of erythrocyte that is responsible for maintaining homeostasis and physiological equilibrium. In certain conditions like anaemia, haemorrhages, toxaemia, shock and poisoning, the level of Hb decreases. The Hb concentration in the blood also fluctuates due to differences in genotype, pregnancy and other stress factors (Schalm, 1965) [4]. The discrepancy in Hb concentration recorded in the treatment groups might be attributed to bacterial invasion of endometrium which might have altered the normal uterine environment in all experimental cows. John and Lisa (1997) [5] in their study on the effect of endotoxin on human hematopoietic cell precursors *in vitro* suggested that, LPS induced release of hematopoietic growth factor which altered proliferative response of hematopoietic precursors. The increased level of Hb in LPS and OG treated cows might be the indication of greater efficacy of treatment protocols in maintaining the normal levels of haematological parameters. These therapies might have higher efficacy in controlling bacterial infection of the cows. Sarma *et al.* (2012) [6] and Sahoo *et al*. (2014) [7] in their study on Endometritis reported that at post-treatment estrus all the hematological parameters returned significantly ($P<0.05$) towards normal in all the treatment groups treated with single intrauterine infusion of LPS or single dose of OG. The low level of Hb, in the endometritic cows of the control groups might be due to destruction of blood cells by the uterine bacteria through the process of haemolysis (Thrall, 2004) [8].

Total Leukocyte count (TLC)

The TLC of different treatment groups in pre and post treatment period were consistent with the finding of Coles (1968) [9], Blood *et al.* (1989) [10] and Chauhan (1995) [11] and ranged between 4 to 12 ($\times 10^3$/cmm). Both LPS and OG has the ability for proliferation of leucocytes, mostly PMN cells and macrophages. They produce different types of amines like interleukin (IL) that might have augmented more production of leucocytes resulting in elevation of WBC count in the systemic circulation (Sheldon *et al.* 2009a) [12]. The decrease in leucocyte value might be attributed to temporary transition to potentiate local innate immunity in tubular genital tract.

Differential Count (DC)

In the present study, the neutrophil value in pre and post treatment period irrespective of drug treatment was well within physiological range of 30.00 to 45.00 per cent (Runnells *et al.*, 1965 and Chauhan, 1995) [13, 11]. Similarly, Reddy *et al.* (2012b) [14] reported a pre and post treatment values of 34.00 ± 1.42 and 27.67 ± 1.42, 33.67 ± 2.70 and 30.50 ± 2.70, 34.50 ± 2.29 and 26.50 ± 2.29, 30.50 ± 1.58 and 31.17 ± 1.58 respectively for endometritic cows treated with either antibiotics lavage, normal saline lavage, their combination and for untreated control. However, they recorded a non-significantly lower value of neutrophil following normal saline lavage. Neutrophil is a potent mobile cell generated by bone marrow and the cells are dedicated for provision of innate immune system which is rightly known as first line of defence. Their decrease in post treatment period at 24 hours and in succeeding estrus corroborated with the findings of Ray *et al.* (2004) [15]. Similarly, both local and parenteral immunomodulators have been shown to normalize the haematological parameters resulting in recovery from uterine infection. Neutrophil is a major defence cell to elicit initial immunomodulation either locally or systemically. Hogarth (1982) [16] reported that neutrophillia is observed following OG treatment which might be due to the effect of

the drug to elicit local proliferation of PMN cells with production of various substances which have leucocyte chemotactic function. This phenomenon might be responsible for elevation in per cent of neutrophil in systemic circulation.

A significant ($p<0.05$) increase was observed in mean lymphocyte count at post-treatment succeeding oestrus in LPS, OG and enrofloxacin treated groups. Perusal of table 4 suggested that with the fall of neutrophil value, the count lymphocyte in blood have synchronously increased in the post treatment period irrespective of the drug treatment. The lymphocyte count observed in the present study is within range of 45 to 70 and is comparable to the report of Chauhan (1995) [11] and also is in agreement with the findings of Ahmad *et al.* (2003) [17] (60.76 ± 2.92) in cyclic cows and Singh and Singh (2006a) [18] (55.56 ± 4.44) in cows having normal estrus. Lymphocytes are unique cell population which show constant fluctuation in their count depending upon their necessity towards both systemic and local immunomodulation.

The normal physiological value of eosinophil varies between 1-15 percent (Runnells *et al*., 1965 [14] and Chauhan, 1995) [11]. The eosinophil values in the present study are within normal range both at pre and post treatment regimen. In the present study the eosinophil count in the post treatment period corroborates with Swain (2009) [19] where these workers recorded lower value in eosinophil count in the post treatment sampling irrespective of drug used. The treatment with sexual rest might have suppressed mast cells in preventing histamine like substances responsible for allergy. Moreover a comprehensive critical comparison was not possible due to absence of relevant reports. Eosinophils are generally activated during allergy hypersensitivity but they do not have active role in phagocytosis. Elevation of eosinophil count is marked in parasitic infestations and in allergic reactions. The discrepancy of this eosinophil count in pre and post treatment period may be due to some intrinsic and extrinsic factors.

The monocytes are blood scavengers and in the tissue they are known as macrophages. It is the most powerful cell of innate immune system. Their appearance at the site of inflammation induces immunomodulation by the action of both humoral and cellular immunity. In the present study the monocyte count (%) of blood is comparable to physiological norms which indicated that the monocyte value in the normal bovine blood varies between 1-5 per cent.

Competing Interests

The authors declare that they have no competing interests.

References

1. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining post-partum uterine disease in cattle, Theriogenology. 2006; 65:1516-1530.
2. Sheldon IM, Noakes DE, Rycroft A, Dobson H. Effect of intrauterine administration of estradiol on postpartum uterine bacterial infection in cattle, Animal Reproduction Science. 2004; 8:13-23.
3. Singh J, Dhaliwal GS, Nanda AS, Arora AK, Pangaonkar GR Dynamics of uterine micro flora in cows suffering from Endometritis following intrauterine administration of Escherichia coli lipopolysaccharide, Indian Journal of Animal Sciences. 2001; 71:1156-58.
4. Schalm OW. Veterinary Haematology. 2nd Edn, 1965.
5. John JR, Lisa K. Effects of endotoxin on proliferation of human hematopoietic cell precursors. Cytotechnology. 1997; 24(2):153-159.
6. Sarma DK, Singh B, Singh MP, Tiwary BK, Sinha MP, Therapeutic use of immunomodulatory in endometritic cows and their effect on WBC and RBC indices, Intas Polivet. 2012; 13:26-28.
7. Sahoo S, Mohanty DN. Effect of Certain Immunomodulators on Serum Haptoglobulin Concentration in Cyclic Non-Breeding Cows. Indian Vet. J. 2014; 92(9):56-57.
8. Coles EH. Veterinary Clinical Pathology, W.B. Saunders, 1968.
9. Blood DC, Rodostits CM, Handerson JA. Veterinary Medicine, 1989, 7.
10. Chauhan RS. Text Book of Veterinary Clinical and Laboratory Diagnosis, 1, 1995.
11. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod. 2009; 81:1025-1032.
12. Runnells RA, Monlux WS, Monlux AW. Principles of Veterinary Pathology. 7th Edn.
13. Reddy NCS, Bramhaiah KV, Naidu KS, Kumar RVS. Effect of uterine lavage on bacterial count and conception rate in repeat breeder crossbred cows, Indian Journal of Animal reproduction. 2012b; 33(1):59-61.
14. Ray SKH, Dash HD, Mohanty DN, Das S, Bisoi PC, Barik AK. Studies on certain haematological and biochemical constituents in cows with retained fetal membranes, Indian Journal of Animal reproduction. 2004; 25: 128-130.
15. Hogarth PJ. Immunological Aspects of Mammalian Reproduction, 1982.
16. Ahmad I, Gohar A, Ahmad N, Ahmad M. Haematological profile in cyclic, non-cyclic and endometritic crossbred cattle, International Journal of Agriculture and Biology. 2003; 5:332-334.
17. Singh AS, Singh ON. Haematological studies on crossbred heifers with anestrus, Indian Journal of Animal Research. 2006a; 40(2):170-172.
18. Swain PK. Comparative efficacy of certain immunomodulators on uterine microbial picture in repeat breeding cows. M.V.SC. thesis submitted to Odisha University of Agriculture and Technology, Bhubaneswar.