RESEARCH ARTICLE

Effect of Immunomodulators and Antibiotic on Haematological Profile of Gir Cows with Subclinical Endometritis

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Abstract

The present study was aimed to assess the influence of immunomodulators and antibiotic on haematological profile in subclinical endometritis (SCE) affected Gir cows. Forty-eight apparently healthy Gir cows at 40-60 days postpartum, 4 to 8 years old, were randomly selected from an organized dairy farm. All the postpartum cows with (n=40) or without (n=8) subclinical endometritis were screened using cytobrush technique. Subclinical endometritis affected cows were randomly divided into 5 groups (8 animals/group). In Group A, *Escherichia coli* LPS (100 µg in 30 mL PBS once i/u), in Group B, oyster glycogen (500 mg in 30 mL PBS once i/u), in Group C, levofloxacin (30 mL for three consecutive days i/u), in Group D, garlic extract (5 mL in 30 mL distilled water for three consecutive days i/u) was used, while Group E (positive control) did not receive any treatment and was allowed for self-cure. A group of normal healthy cows (n=8) served as negative control (Group F). Blood samples were collected at estrus before treatment (0 h) and after treatment (72 h and at subsequent estrus) and compared for changes in haematological parameters. The results revealed significantly ($P < 0.05$) decreased haemoglobin, packed cell volume, lymphocyte, eosinophil, and increased total leukocyte count and neutrophil counts in SCE affected cows as compared to healthy control group. After 72 h of treatment and at subsequent estrus, the values of TLC decreased significantly ($P < 0.05$), whereas the Hb and PCV increased significantly ($P < 0.05$) in all the SCE groups. The conception rates at subsequent estrus were relatively higher with *E. coli* LPS (50.00%) and oyster glycogen (37.50%), at par with a control group (37.50%), than antibiotic and garlic treated groups (25.00% each), and nil in the positive control group. The immunomodulators were thus the drugs of choice for treatment of postpartum subclinical endometritis with improved haematological profile and conception rate in Gir cows.

Keywords: Gir cows, Haematological profile, Subclinical endometritis, Therapeutic regimens.

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Introduction

The sustainability and profitability of the dairy industry mainly depend upon proper and optimal reproductive rhythm of each animal in a herd. Any deviation may cause economic losses due to infertility in terms of widening of dry period, reduced calving and lactation yield during the lifespan of an animal. High yielding dairy cows experience metabolic stress during early postpartum period and, subsequently, are more susceptible to metabolism-related diseases, such as ketosis, metritis and endometritis (Nazhat *et al.,* 2018). Subclinical endometritis (SCE) is one of the emerging fertility constraints in dairy bovine (Bajaj *et al.*, 2016). It is the most prevalent of all uterine diseases as it affects 30 % of lactating dairy cows, with the prevalence ranging from 11 to >70 % in some herds (Galvao *et al.,* 2011) and has been associated with decreased pregnancy per insemination, extended calving intervals and increased culling rate (Gilbert *et al.,* 2005).

Inspite of various diagnostic techniques, the diagnosis of SCE is still a challenging task, compounded with unavailability of universally accepted disease condition (Wagener *et al.,* 2017). Following accurate diagnosis, one must use appropriate therapy for management of this condition, for which antibiotics and chemotherapeutic agents are traditionally used (Kasimanickam *et al.,* 2005). In the present scenario, various immunomodulators are considered as an alternative therapy for uterine infections (Bajaj *et al.,* 2016). When infused into the uterus, these substances activate

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uterine defence mechanism and initiate local immune system in SCE affected cows (Krishnan *et al.,* 2015). The antibacterial and immunomodulatory effects of garlic (*Allium sativum*) on SCE affected dairy bovine have been reported recently by some scientists (Kumar *et al.,* 2018; Lawange *et al.,* 2019). The information regarding SCE in indigenous zebu cattle like Gir is still patchy. Therefore, investigations to ascertain relationships between SCE and the haematological parameters are warranted to establish their utility for the further diagnostic purposes. Hence, this study was planned to assess the haematological profile in subclinical endometritis affected Gir cows before and after various therapies.

Materials and Methods

The study was carried out following approval (No. JAU/JVC/IAEC/LA/57/2019) of experimental protocol by the Institutional Animal Ethics Committee of the College. A total of forty-eight (n=48) apparently healthy postpartum Gir cows, 4 to 8 years old and around 40-60 days in milk (DIM), were selected from the Gir herd maintained at the Cattle Breeding Farm, JAU, Junagadh, India. These animals were maintained as per the standard feeding schedule followed at the farm. Rectal palpation of the genital tract of each cow was performed to exclude any palpable defects. The subclinical endometritis was confirmed based on clear cervicovaginal discharge and >5 % PMNs in endometrial cytology (Gilbert *et al.,* 2005). Subclinical endometritis affected Gir cows (n=40) were randomly divided into five groups (8 animals/group). In Group A, *E. coli* LPS (100 µg in 30 mL PBS once i/u), in Group B, oyster glycogen (500 mg in 30 mL PBS once i/u), in Group C, levofloxacin (30 mL for three consecutive days i/u), in Group D, garlic extract (5 mL in 30 mL distilled water for three consecutive days i/u) was used, while Group E (positive control) did not receive any treatment and was allowed for self-cure. A group of cows (n=8) found with <3 % PMNs served as normal healthy negative control (Group F). Animals were inseminated at subsequent estrus and pregnancy was confirmed by per rectal palpation 60 days post-AI.

From each animal, blood samples were collected at estrus pre-treatment (0 h), post-treatment (72 h) and at subsequent estrus in heparinized vials for estimation of haematological parameters like, haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), and percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils. The estimation was performed immediately after collecting blood samples with the help of automatic haematology analyzer (Abacus Vets - Diatron, Budapest, Hungary). The data was analyzed for Mean ± SEs by descriptive statistics. Two-way analysis of variance (ANOVA) was used to see the treatment and period effect on the haematological profile (Snedecor and Cochran, 1998). The Duncan's MRT post hoc test was used to compare pair-wise mean differences between the groups at $p < 0.05$.

Results and Discussion

The initial haematological profile of subclinical endometritic cows (n=40) revealed significant drop in Hb (8.58 ± 0.09 vs. 11.96 ± 0.14 gm/dL), PCV (29.38 ± 0.35 vs. 35.38 ± 0.92 %) and eosinophils (4.69 ± 0.18 vs. 6.17 ± 1.70 %), and increase in TLC (10.25 ± 0.15 vs. 8.73 ± 0.59 x 10^3/µL) and neutrophils (35.03 ± 0.24 vs. 32.50 ± 1.77 %) as compared to healthy cows (n=8). However, the lymphocytes, monocytes and basophils did not vary much between healthy and SCE affected cows. The lowered Hb and PCV and higher TLC recorded in SCE affected cows might be due to anaemic changes resulting from mild uterine infection (Kekan *et al.,* 2005). Thrall (2004) reported that in inflammatory disease, erythropoietin is diminished because of inflammatory cytokines leading to lowered erythropoiesis and ultimately lowered Hb, PCV and TEC in blood. Higher TLC in SCE affected cows might also be due to the increased cortisol (immuno-suppressive) level as the MDA levels in these cows are higher than in the normal, unaffected cows (Perumal *et al.,* 2020).

The group-wise findings on various haematological attributes before and after treatment and at subsequent estrus are presented in Table 1. The mean values of both Hb and PCV were significantly ($p < 0.05$) lower, and TLC higher, in all SCE affected groups than the healthy negative control group F at both 0 h and 72 h post-treatment. However, TLC increased significantly within 72 h of treatment in Groups A, B and D, and deceased in group B over 0 h values due to specific drug effect. At subsequent estrus, the differences in values of haemoglobin, PCV and TLC among group A, B, C and D were statistically similar, and significantly ($p < 0.05$) improved over pre-treatment values to normal ones of healthy group, except in the positive control group E, wherein they remained unaltered. The 1st service conception rate at subsequent estrus for groups A, B, C, D, E and F was 50.00, 37.50, 25.00, 25.00, 0.00, and 37.50 %, respectively. It can be thus concluded that immunomodulators *E. coli* LPS, oyster glycogen and sensitive antibiotic (levofloxacin) and garlic extract treatment cured the SCE condition effectively and increased haemoglobin and PCV concentration with reduction of TLC to a normal level as compared to other treatment groups at subsequent estrus (Table 1) concurrent with improved conception rates. Improved conception rates with immunomodulators, levafloxacin and garlic extract have also been reported in endometritic and repeat breeder bovines by earlier workers (Sarkar *et al*., 2006; Prasad *et al*., 2009; Singh, 2014, 2017, Bhardwaz *et al*., 2018; Lawange *et al*., 2019).

Prior to the treatment in all five SCE groups, the per cent neutrophils and basophils were non-significantly higher, and eosinophils and monocytes were lower than in negative control group F, while lymphocytes were almost same in all groups. A non-significant ($p > 0.05$) increase in neutrophil count of treatment groups A, B and D, and decrease in

Table 1: Haematological changes before and after treatment in healthy and SCE affected Gir cows (Mean ± SE)

Sr. No.	Treatment groups (n=8 each)	Before treatment 0 h	After treatment 72 h	Next estrus
Haemoglobin Concentration (gm/dL)				
1.	Gr A (*E. coli* LPS)	8.54 ± 0.28^{Aa}	8.44 ± 0.29^{Aa}	11.49 ± 0.24^{BCb}
2.	Gr B (Oyster glycogen)	8.69 ± 0.26^{Aa}	8.67 ± 0.31^{Aa}	11.28 ± 0.18^{BCb}
3.	Gr C (Sensitive antibiotic)	8.85 ± 0.26^{Aa}	8.86 ± 0.18^{Aa}	11.42 ± 0.22^{BCb}
4.	Gr D (Garlic extract)	8.57 ± 0.25^{Aa}	8.52 ± 0.34^{Aa}	11.14 ± 0.17^{Bb}
5.	Gr E (Positive control)	8.26 ± 0.25^{Aa}	8.28 ± 0.33^{Aa}	8.21 ± 0.28^{Aa}
6.	Gr F (Negative control)	11.96 ± 0.14^{Ba}	11.54 ± 0.18^{Ba}	11.95 ± 0.26^{Ca}
Packed cell volume (%)				
1.	Gr A (*E. coli* LPS)	28.03 ± 0.59^{Aa}	30.14 ± 0.59^{Ab}	35.88 ± 0.61^{Cc}
2.	Gr B (Oyster glycogen)	29.62 ± 0.79^{Aa}	29.78 ± 1.07^{Aa}	35.12 ± 0.46^{BCb}
3.	Gr C (Sensitive antibiotic)	29.74 ± 0.52^{Aa}	29.76 ± 0.38^{Aa}	34.34 ± 0.73^{BCb}
4.	Gr D (Garlic extract)	29.97 ± 0.39^{Aa}	29.25 ± 0.52^{Aa}	33.61 ± 0.86^{Bb}
5.	Gr E (Positive control)	29.54 ± 0.78^{Aa}	29.48 ± 0.66^{Aa}	29.94 ± 0.72^{Aa}
6.	Gr F (Negative control)	35.38 ± 0.92^{Ba}	35.53 ± 0.89^{Ba}	36.19 ± 0.76^{Ca}
Total leukocyte count (x 10^3/µL)				
1.	Gr A (*E. coli* LPS)	10.26 ± 0.45^{Bb}	11.89 ± 0.08^{Dc}	8.58 ± 0.35^{Aa}
2.	Gr B (Oyster glycogen)	9.76 ± 0.41^{ABb}	10.93 ± 0.10^{Cc}	8.67 ± 0.19^{Aa}
3.	Gr C (Sensitive antibiotic)	10.66 ± 0.25^{Bc}	9.11 ± 0.21^{Ab}	8.27 ± 0.07^{Aa}
4.	Gr D (Garlic extract)	10.13 ± 0.26^{Bb}	10.89 ± 0.21^{Cc}	8.35 ± 0.09^{Aa}
5.	Gr E (Positive control)	10.45 ± 0.15^{Bc}	10.13 ± 0.14^{Bbc}	9.98 ± 0.13^{Ba}
6.	Gr F (Negative control)	8.73 ± 0.59^{Aa}	8.72 ± 0.38^{Aa}	8.64 ± 0.36^{Aa}
Neutrophil counts (%)				
1.	Gr A (*E. coli* LPS)	35.33 ± 0.88^{Ab}	37.00 ± 0.63^{Cb}	31.83 ± 0.60^{Aa}
2.	Gr B (Oyster glycogen)	35.33 ± 1.05^{Aab}	36.33 ± 1.05^{BCb}	32.50 ± 1.06^{Aa}
3.	Gr C (Sensitive antibiotic)	34.83 ± 1.25^{Aa}	32.83 ± 1.05^{Aa}	32.17 ± 1.01^{Aa}
4.	Gr D (Garlic extract)	35.50 ± 1.34^{Aab}	36.67 ± 1.23^{BCb}	32.00 ± 1.46^{Aa}
5.	Gr E (Positive control)	34.17 ± 1.05^{Aa}	33.50 ± 0.99^{ABa}	33.33 ± 1.05^{Aa}
6.	Gr F (Negative control)	32.50 ± 1.77^{Aa}	33.00 ± 1.32^{Aa}	32.83 ± 1.35^{Aa}
Lymphocyte counts (%)				
1.	Gr A (*E. coli* LPS)	57.17 ± 1.58^{Aa}	56.67 ± 1.58^{Aa}	58.83 ± 1.45^{Aa}
2.	Gr B (Oyster glycogen)	58.33 ± 1.84^{Aa}	57.33 ± 1.80^{Aa}	59.50 ± 2.06^{Aa}
3.	Gr C (Sensitive antibiotic)	58.50 ± 2.83^{Aa}	60.25 ± 2.73^{Aa}	59.83 ± 2.75^{Aa}
4.	Gr D (Garlic extract)	58.67 ± 1.43^{Aa}	56.17 ± 1.49^{Aa}	57.50 ± 1.38^{Aa}
5.	Gr E (Positive control)	58.37 ± 1.19^{Aa}	59.50 ± 2.67^{Aa}	59.67 ± 1.28^{Aa}
6.	Gr F (Negative control)	59.33 ± 3.08^{Aa}	59.87 ± 2.82^{Aa}	60.00 ± 3.02^{Aa}
Monocyte counts (%)				
1.	Gr A (*E. coli* LPS)	2.17 ± 0.65^{Aa}	2.50 ± 0.43^{Aa}	2.83 ± 0.48^{ABa}
2.	Gr B (Oyster glycogen)	1.83 ± 0.31^{Aa}	2.50 ± 0.22^{Aab}	3.00 ± 0.37^{Bb}
3.	Gr C (Sensitive antibiotic)	1.83 ± 0.31^{Aa}	2.00 ± 0.26^{Aab}	2.67 ± 0.21^{ABb}
4.	Gr D (Garlic extract)	1.67 ± 0.33^{Aa}	2.50 ± 0.22^{Aab}	2.67 ± 0.33^{ABb}
5.	Gr E (Positive control)	2.00 ± 0.37^{Aa}	2.17 ± 0.17^{Aa}	1.83 ± 0.17^{Aa}
6.	Gr F (Negative control)	2.33 ± 0.49^{Aa}	2.00 ± 0.26^{Aa}	2.17 ± 0.31^{ABa}
Eosinophil counts (%)				
1.	Gr A (*E. coli* LPS)	4.50 ± 0.96^{Aa}	4.33 ± 0.61^{Aa}	6.00 ± 0.93^{Aa}
2.	Gr B (Oyster glycogen)	4.33 ± 0.49^{Aa}	4.17 ± 0.31^{Aa}	5.83 ± 0.54^{Aa}

Sr. No.	Treatment groups (n=8 each)	Before treatment 0 h	After treatment 72 h	Next estrus
3.	Gr C (Sensitive antibiotic)	4.50 ± 0.50^{Aa}	4.67 ± 0.49^{Aa}	5.50 ± 0.56^{Aa}
4.	Gr D (Garlic extract)	4.83 ± 0.54^{Aa}	4.50 ± 0.50^{Aa}	5.83 ± 0.54^{Aa}
5.	Gr E (Positive control)	5.33 ± 0.99^{Aa}	5.00 ± 0.77^{Aa}	5.17 ± 0.70^{Aa}
6.	Gr F (Negative control)	6.17 ± 1.70^{Aa}	5.80 ± 1.28^{Aa}	6.00 ± 1.03^{Aa}
Basophil counts (%)				
1.	Gr A (*E. coli* LPS)	0.67 ± 0.33^{Aa}	0.50 ± 0.34^{Aa}	0.17 ± 0.17^{Aa}
2.	Gr B (Oyster glycogen)	0.50 ± 0.22^{Aa}	0.33 ± 0.21^{Aa}	0.33 ± 0.21^{Aa}
3.	Gr C (Sensitive antibiotic)	0.83 ± 0.31^{Aa}	0.92 ± 0.20^{Aa}	0.33 ± 0.33^{Aa}
4.	Gr D (Garlic extract)	0.50 ± 0.22^{Aa}	0.67 ± 0.21^{Aa}	0.33 ± 0.21^{Aa}
5.	Gr E (Positive control)	0.67 ± 0.33^{Aa}	0.17 ± 0.17^{Aa}	0.67 ± 0.33^{Aa}
6.	Gr F (Negative control)	0.33 ± 0.33^{Aa}	0.75 ± 0.25^{Aa}	0.33 ± 0.21^{Aa}

Means with different superscripts within group/row (a,b,c) and between groups/within the column (A,B,C) differ significantly ($p < 0.05$).

group C was observed at 72 h of treatment, which decreased significantly ($p < 0.05$) at subsequent estrus to near normal values in all these groups. The neutrophil count of *E. coli* LPS group showed highest reduction at subsequent estrus compared to other groups. The mean lymphocyte counts of SCE groups A, B and D declined and of group C increased non-significantly at 72 h of treatment, though statistically neither groups nor periods had significant influence on this parameter. The monocytes count increased non-significantly at 72 hours of treatment in groups A, B, C and D, but it was significantly increased ($p < 0.05$) at subsequent estrus over pretreatment values. However, no clear change or statistically significant alteration was seen in eosinophils and basophils counts between groups and between periods (Table 1).

A lower dose of *E. coli* LPS activates monocyte and macrophage to produce tumor necrosis factors and thereby helps in the secretion of IL-1, IL-6 and IL-8. This leads to an increased local inflammatory response. The secretions of cytokines/chemokines attract the PMN cells within the uterine lumen and cause resolution of infection (Prasad *et al.*, 2009). Oyster glycogen also acts to combat the infection (Prasad *et al.*, 2009; Singh, 2014) and improves haematological profile in SCE cows. Krishnan *et al.* (2015) reported a significant increase in TLC at 24 h after intrauterine infusion of oyster glycogen. The present findings therefore concurred well these reports and of Sarma (2007) and Singh (2017) using the same immunomodulators in endometritic cattle. Contrary to this, Sahoo *et al.* (2014) observed non-significant variation in Hb-PCV-TLC values of endometritic cows following treatment with *E. coli* LPS. In the present study, the reduced level of TLC was found at subsequent estrus in garlic extract group also.

Levofloxacin, a broad-spectrum antibiotic, might have killed bacteria effectively by inhibiting bacterial DNA gyrase and cell division, and thus improved the haematological profile and conception rate in this group. The present findings regarding effect of levofloxacin treatment on SCE corroborated well with the findings of Pandey *et al.* (2018) and Satish Kumar (2018). A similar trend was also observed by Heidarpour *et al.* (2014) and Singh (2017) with cloprostenol + benzathine cephapirin and ciprofloxacin treatment, respectively. Garlic has been observed to possess marked antibacterial, antiinflammatory and immunomodulatory properties against a wide variety of bacteria and inhibits the growth of pathogens responsible for endometritis in dairy cows (Sarkar *et al.*, 2006; Kumar *et al.*, 2018). The increase in Hb-PCV concentrations and decrease in TLC in garlic extract group at subsequent estrus observed might be due to the influx of PMN cells within the uterine lumen and reduction in inflammatory conditions. The treatment of garlic extract to SCE affected cows enhances the immune system by stimulating the release of cytokines to increase the natural killer activity and phagocytic activity of peritoneal macrophases (Sarkar *et al.*, 2006).

The acute phase proteins and subsequent inflammatory response were declined after treatment in cows with SCE. An impairement of blood neutrophil oxidative burst activity observed in dairy cows with endometritis might be due to an impairement of myeloperoxidase activity and hydrogen peroxide production (Mateus *et al.*, 2002). Singh (2014) reported non-significant variation in monocytes and eosinophils counts. The findings with levofloxacin treatment concurred with observations of Singh (2017) using ciprofloxacin. The present findings on differential leukocytes regarding the effect of *E. coli* LPS and oyster glycogen treatment were in accordance with the observations of Sarma (2007) and Singh (2017). Reddy *et al.* (2012) found a significant increase in lymphocytes, monocytes and eosinophils counts in repeat breeder cows following treatment with antibiotics, while Satish Kumar (2018) observed reduced lymphocyte, monocyte and eosinophil counts of endometritic cows following treatment with levofloxacin. In the present study, a reduced level of neutrophil and lymphocyte counts was found in garlic extract treatment group at subsequent estrus, however, the literature did not reveal any report regarding the effect of garlic extract on haematological profile of SCE affected cows to elaborate our findings, though the conception rates were

reported to be improved with this extract in repeat breeder crossbred cows (Bhardwaz *et al*., 2018).

Conclusion

From the study it can be concluded that subclinical endometritis postpartum significantly altered the haematological profile (Hb, PCV, TLC, and DLC), of Gir cows, and that intrauterine treatment with immunomodulators, particularly *E. coli* LPS and oyster glycogen normalized these changes at subsequent estrus with improved conception rate.

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