EFFECT OF *E. COLI* LPS AND LUGOL'S IODINE ON CONCEPTION RATE IN REPEAT BREEDER COWS WITH SUBCLINICAL ENDOMETRITIS

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ABSTRACT

Forty-five repeat breeder cows positive for subclinical endometritis by white side test were divided randomly into three groups. Group-I cows (LPS) were administered 100 µg of *E. coli* LPS in 30 ml sterile phosphate buffered saline intrauterine once at estrus. Group-II cows (LI) were administered 1% Lugol's iodine in 30 ml of distilled water (LI). Group-III cows (Control) were administered 30 ml sterile PBS intrauterine once at estrus. Haematological parameters such as Hb, TLC and DLC were studied before initiation of treatment and on the subsequent estrus also. AI was done in the subsequent estrus in all the cows. Pregnancy verification was done 45th day post AI. The mean Hb level significantly increased in LPS and LI groups in subsequent estrus. The TLC count significantly decreased in LPS and LI groups in subsequent estrus. There was significant increase in the mean lymphocyte count and significant decrease in neutrophil count in LPS group in subsequent estrus. There was significant decrease in monocyte count in LPS, control group and significant decrease in the eosinophil count in LI group in the subsequent estrus. The first service conception rate was 53.33%, 20.00 % and zero per cent for LPS, LI and control groups, respectively.

Keywords: Repeat breeder cows, *E. coli* LPS, Lugol's iodine, Haematology, Conception rate.

Introduction

Subclinical endometritis refers to inflammation of endometrium in the absence of clinical signs of the disease (Sheldon *et al*., 2006). Treatment of cows with subclinical endometritis using antibiotics resulted in inconsistent recovery rate, emergence of microbial resistance and reduced innate uterine defence mechanism by poor phagocytic activity of PMN cells (Sarkar *et al*., 2006). Hence, an alternative therapy by using natural substances as a means of activation of natural uterine defence mechanisms gains importance. Immunomodulator such as non-pathogenic *E. coli* LPS could be effective and an alternative approach to antibiotics which can act as a potent chemo-attractant to clear uterine infections in repeat breeder cows (Singh *et al*., 2000). Dysfunction of cell immunity coexisting with SE may be the main factor causing uterine inflammation resulting in substantial changes in subpopulation of immune cells in cows with subclinical endometritis (Dash *et al*., 2019). *E. coli* LPS are the new approach to treat subclinical endometritis which will eliminateinfection by enhancing both humoral and cellular immunity through proliferating body components such as lymphocytes, neutrophils, monocytes, humoral antibodies and PMN cells (Chauhan and Singh, 2001).

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MATERIALS AND METHODS

Forty-five repeat breeder cows positive for subclinical endometritis by white side test were divided randomly into three groups. Group-I cows (LPS) were administered 100 µg of *E. coli* LPS (E. coli O26:B6, Sigma-Aldrich, USA) dispensed in 30 ml sterile phosphate buffered saline intrauterine once at estrus. Group-II cows (LI) were administered 1% Lugol's iodine in 30 ml of distilled water. Group-III cows (Control) were administered 30 ml sterile Phosphate Buffered Saline (PBS) intrauterine once at estrus. Haematological parameters such as Hb, TLC and DLC were studied before initiation of treatment and on the subsequent estrus and compared. AI was done in the subsequent estrus in all the treated cows. Pregnancy verification was done 45th day post AI. The first service conception rate was evaluated in each group, statistically analysed and compared (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The mean values for various haematological parameters in the three groups are presented in Table 1. Significant ($P<0.01$) increase in the Hb levels after treatment was observed in LPS and LI group. In normal healthy cows, Hb concentration ranges between 8-15 g/dl (Chauhan, 1995). Similar to our findings, Dash *et al*.

(2019) reported significant increase in the mean Hb levels after treatment with *E. coli* LPS in endometritic cows. The improved Hb levels could be due to the effect of LPS which might have induced release of hematopoietic growth factor resulted in altered proliferative response of hematopoietic precursors (John and Lisa, 1997).

Highly significant (P<0.01) decreasein the mean TLC count was observed in LPS and LI groups in the succeeding estrus. This could be due to the effect of *E. coli* LPS and Lugol's iodine, which has the ability to induce leucocytosis, mostly PMN cells and macrophages. The increased phagocytic activity of PMN cells and macrophages would have resulted in clearance of infection and subsequent reduction in the TLC count in the succeeding estrus.

There was significant (P<0.05) increase in the mean lymphocyte count in LPS group. Similarly, Dash *et al*. (2019) reported significant increase in the mean lymphocyte count at succeeding estrus in endometritic cows treated with *E. coli* LPS. They opined that lymphocyte are unique cell population which showed constant fluctuation in their count depending upon their necessity towards systemic and local immunomodulation.

There was significant (P<0.05) decrease in the mean neutrophil count in LPS group in the succeeding estrus which could be due to the effect of immunomodulation resulting in normalizing and favouring recovery from uterine infection. Dash *et al*. (2019) also observed significant decrease in the neutrophil count in cows treated with LPS in the subsequent estrus.

There was significant (P<0.01) decrease in the mean monocyte countin LPS and control groups after treatment in the succeeding estrus. Monocytes are referred to as macrophages as they have blood scavenging action in the tissues that act both on humoral and cellular immunity and induce immunomodulation at the site of inflammation (Dash *et al*. 2019). Significant reduction in the mean monocyte count in LPS group reflected the recovery from uterine infection.

In the present study, there was significant (P<0.05) decrease in the mean eosinophil count in LI group after treatment in the subsequent estrus. Eosinophils are generally activated during allergy but do not have any phagocytic activity (Dash *et al*., 2019). The treatment of repeat breeder cows with *E. coli* LPS and Lugol's iodine along with sexual rest might have suppressed mast cells in preventing the histamine like substances responsible for allergy and resulted in decreased eosinophilic count.

A total of 8 cows among 15 repeat breeder cows became pregnant to first service in LPS group (53.33%) whereas, only 3 cows conceived to first service in LI group (20%). None of the repeat breeder cows became pregnant in control group (Table 2). There was significant (P<0.05) difference in the conception rate between cows treated with *E. coli* LPS and control group. No significant differences were observed in the conception rate obtained between LPS and LI, LI and control group.

The first service conception rate obtained in LPS group is in agreement with Raju *et al*. (2009) who reported a similar conception rate of 53.33 % in endometritic buffaloes treated with *E. coli* LPS. The conception rate obtained in the present study is much lower than the conception rate of 83.33% (Palanisamy, 2012) and 77.78% (Arjunrao, 2017). The low conception rate obtained in the present study could be due to less number of cows utilized in the study. Moreover, the repeat breeder cows selected in this study were maintained in an uncontrolled environment scattered in and around different villages reared by different landless livestock farmers. Contrary to our findings, higher conception rate of 55.55% (Arjunrao, 2017) was obtained in endometritic cows treated with Lugol's iodine.

SUMMARY

The effect of *E. coli* LPS and Lugol's iodine on certain haematological parameters and conception rate was studied. The mean Hb level and lymphocyte count increased; TLC count, neutrophil count and monocyte count decreased in LPS group after treatment in the subsequent estrus. The mean monocyte count decreased in control group, eosinophil count decreased in LI group after treatment in the subsequent estrus. The first service conception rate was 53.33% in LPS, and 20% in LI groups. None of the repeat breeder cows conceived in control group.

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Table1. Haematological values (Mean ± SE) in repeat breeder cows before and after treatment

Parameters	Groups	Before treatment	After treatment	p-value	Significance
Hb (g/dl)	LPS	7.90 ± 0.20[a]	9.73 ± 0.38[b]	0.009	**
	LI	7.82 ± 0.45[a]	8.48 ± 0.56[b]	0.000	**
	CONTROL	8.38 ± 0.42	5.62 ± 0.14	0.065	ns
TLC (x 10^3 /µl)	LPS	14.58 ± 1.23[a]	9.98 ± 0.71[b]	0.000	**
	LI	14.75 ± 1.27[a]	12.98 ± 0.73[b]	0.003	**
	CONTROL	10.34 ± 0.69	19.83 ± 0.81	0.085	ns
Lymphocyte (%)	LPS	63.66 ± 1.91[a]	64.26 ± 1.32[b]	0.048	*
	LI	66.53 ± 1.09	66.26 ± 0.70	0.057	ns
	CONTROL	67.86 ± 0.94	67.06 ± 0.81	0.014	ns
Neutrophil (%)	LPS	35.80 ± 1.76[a]	35.66 ± 1.29[b]	0.045	*
	LI	31.66 ± 1.17	33.80 ± 0.68	0.585	ns
	CONTROL	31.13 ± 0.91	32.26 ± 0.79	0.067	ns
Monocyte (%)	LPS	0.26 ± 0.15[a]	0.06 ± 0.06[b]	0.000	**
	LI	0.73 ± 0.20	0.07 ± 0.06	0.743	ns
	CONTROL	0.66 ± 0.27[a]	0.33 ± 0.18[b]	0.000	**
Eosinophil (%)	LPS	0.26 ± 0.11	0.00 ± 0.00	-	ns
	LI	1.07 ± 0.34[a]	0.86 ± 0.21[b]	0.046	*
	CONTROL	0.40 ± 0.19	0.13 ± 0.09	0.229	ns

Mean values with different superscripts within each row differ significantly
ns = non-significant (p ≥ 0.05); * Significant (p < 0.05); ** Highly significant (p< 0.01)

Table 2. Conception rate in various treatment groups

Groups	Pregnant cows (n)	Non-pregnant cows (n)	Conception rate (%)
LPS (n=15)	8	7	53.33[a]
LI (n=15)	3	12	20[ab]
Control (n=15)	0	15	0[b]

Conception rate bearing different superscripts differ significantly (p < 0.05)

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