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 were divided randomly into three groups. Group-I (LPS) cows were administered 100 µg of *E. coli* LPS in 30 ml sterile phosphate buffered saline (PBS) intrauterine once at estrus. Group-II (LI) cows were administered 1% Lugol's iodine in 30 ml of distilled water. Group-III (Control) cows were administered 30 ml sterile PBS intrauterine once at estrus. AI was done in the subsequent estrus in all the cows. Haematological parameters such as Hb, TLC and DLC were studied before initiation of treatment and on the subsequent estrus. Pregnancy verification was done on 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 percent for LPS, LI and control groups, respectively.

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

INTRODUCTION

Subclinical endometritis (SE) refers to inflammation of endometrium in the absence of clinical signs of the disease (Sheldon *et al*., 2006). Treatment of cows with SE using antibiotics resulted in inconsistent recovery rate, emergence of microbial resistance and reduced innate uterine defense 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 defense mechanisms gains importance. Immunomodulator such as non-pathogenic *Escherichia coli* LPS, could be an effective 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 SE (Dash *et al*., 2019). *E. coli* LPS administration is an approach to treat SE which would eliminate infection 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). Hence the work was designed to study the effect of LPS in cows affected with SE.

MATERIALS AND METHODS

Repeat breeder cows which were positive for SE (n = 45) by white side test were divided randomly into three groups. Group-I (LPS): Animals were administered with 100 µg of *E. coli* LPS (E. coli O26:B6, Sigma-Aldrich, USA) dispensed in 30 ml sterile phosphate buffered saline (PBS) intrauterine once at estrus. Group-II (LI): Cows were administered with Lugol's iodine (1%) in 30 ml of distilled water intrauterine. Group-III (Control): Cows were administered with 30 ml sterile PBS intrauterine once at estrus. Haematological parameters such as haemoglobin (Hb), total leucocyte count (TLC) and differential leucocyte count (DLC) were studied before initiation of treatment and on the subsequent estrus. AI was done in the subsequent estrus in all the treated cows. Pregnancy verification was done on 45th day post AI. The first service conception rate was evaluated in each group, statistically analysed using Fisher's Exact test 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) decrease in the mean TLC count was observed in LPS and LI groups in the succeeding estrus. This could be due to the effect of E.

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coli LPS and Lugol's iodine, which have 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 those lymphocytes 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 favoring 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 count in 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. There was significant (P<0.05) difference in the conception rate between cows treated with *E. coli* LPS and control group,but no significant differences were observed between LI and control groups.

The first service conception rate obtained in LPS group was in agreement with Raju *et al*. (2009) who reported a similar conception rate of 53.33 % in endometritic buffaloes treated with *E. coli* LPS. However higher conception rate of 83.33% and 77.78% were reported by Palanisamy (2012) and Arjunrao (2017) respectively. 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 how????? ?
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	* how?????
	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 e" 0.05); * Significant (p < 0.05); ** Highly significant (p< 0.01)

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