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Research Article

Escherichia coli lipopolysaccharide; a better alternative therapy of endometritis in crossbred cows*

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ABSTRACT

Intrauterine therapy with 100 µg *E. coli* LPS was evaluated for its therapeutic efficacy in crossbred cows with endometritis and was compared with conventional antibiotic therapy based on *in-vitro* antibiogram. At pre-treatment and post-treatment estrus, estrual cervical mucus was studied for change in appearance, white side test, pH and bacterial load. The conception rate was high in LPS group (71.43%) followed by antibiotic group (57.14%) with low conception rate in control group cows (28.52%). Hence, it is concluded that intrauterine infusion of 100 µg *E.coli* LPS is a better alternative to conventional antibiotic treatment in cows with endometritis.

Key words: *E. coli* LPS, *in-vitro* antibiogram, estrual cervical mucus, white side test, pH, conception rate

A normal uterus and particularly a normal endometrium is one of the important components of fertility (Kenney, 1978). Endometritis, mainly of bacterial origin constitutes a major cause of repeat breeding, especially in crossbred cattle (Maurya *et al.*, 1992). In present times, treatment of endometritis is mainly done by antibiotics and antiseptics, and less commonly by hormones. The inconsistent results, high cost of treatment and compulsory milk disposal after antibiotic treatment made it uneconomical (Hussain, 1989). Antiseptics are generally irritating in nature and hamper natural defense mechanism of uterus (Vandeplassche, 1981). $PGF_2\alpha$ has been used for treating endometritis but it requires a corpus luteum to be present for its effect and uniformly successful results are not always obtained (Whittier *et al.*, 1989). Higher doses of estrogen could lead to the development of cystic ovaries (Parkinson, 2001). Increasing recognition of the disappointing efficacy of intrauterine antimicrobial therapy in most instances has rightly focused attention on alternative therapies which stimulate the natural uterine defense mechanisms through immuno-modulation (Gilbert, 1992). The uterine immuno-modulation by chemotaxis of polymorphonuclear neutrophil granulocytes (PMNs) to uterine lumen has been reported to play an important role in the pathogenesis and resolution of endometritis (Cheung *et al.*, 1985; Asbury and Hansen, 1987; Williamson *et al.*, 1987; Hussain and Daniel, 1992). Several agents viz., *E. coli* lipopolysaccharide, bacteria free filtrate, plasma, serum or hyperimmune serum, oyster glycogen, leukotriene B_4, granulocyte-macrophage colony stimulating factor etc. possess the ability to attract PMNs. In this study, *E. coli* LPS was used to treat endometritis in crossbred cows and its therapeutic efficacy was compared with conventional antibiotic therapy.

MATERIALS AND METHODS

Twenty one crossbred cows with purulent or muco-purulent estrual discharge or containing white flakes and positive reaction to white side test were considered positive for endometritis. All the animals were maintained under similar feeding and management conditions at Instructional Dairy Farm, G.B.P.U.A.&T., Pantnagar. All the cows were randomly divided into three groups of seven animals each. Cows of group A served as control in which 20 ml PBS was infused intrauterine

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on the day of estrus. A single treatment of 100 µg of *E. coli* LPS dissolved in 20 ml of phosphate buffer saline (PBS) was infused intrauterine on the day of estrus in group B cows. Group C cows were treated with intrauterine infusion of most sensitive antibiotic for four consecutive days selected on the basis of *in-vitro* antibiotic sensitivity test. Estrual cervical mucus was collected by recto-vaginal technique as per Dabas and Maurya (1988). The estrual cervical mucus was also collected at subsequent estrus following the treatment before AI. Estrual cervical mucus was studied for its appearance, white side test, pH and bacterial load.

White side test: Estrual cervical mucus was subjected to white side test as described by Popov (1969) and considered positive to endometritis if the colour of the mucus turned yellow after boiling with equal volume of 5% sodium hydroxide solution. The pH of the estrual cervical mucus was assessed by a digital pH meter.

Bacterial load: The estrual cervical mucus was mixed with equal volume of PBS and mucolysed using a magnetic stirrer for 5 minutes. 1 ml of this sample was serially diluted with PBS in 10 fold dilutions and inoculated by standard "Pour Plate Technique" on nutrient agar plates (Cruick Shank *et al.,* 1975). Triplicate plates for each sample were incubated at 37°C for 24 hours.

Bacterial count per ml = Average no. of colonies counted × dilution factor × 2

The clinical recovery was assessed by clear appearance of discharge, reduction in pH and bacterial load and no colour reaction of estrual cervical mucus to white side test at subsequent estrus. Cows during subsequent standing estrus were inseminated twice, 12 hours apart using French mini semen straws. Cows which returned to heat after first AI were again inseminated at second subsequent estrus after treatment. Pregnancy was confirmed per rectally 45-60 days after insemination. Two-way analysis of variance was used to test the significant differences within and between the groups (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Most of the cows with endometritis gave purulent and muco-purulent discharge prior to treatment. After treatment with LPS, maximum cows (85.71%) showed clear discharge followed by antibiotic group (71.43%) at subsequent estrus. In untreated control group, only 28.57% cows gave clear discharge (Table 1). Results of prewsent study are lower than Deori (2002) who reported 100% clear estrual mucus following treatment with *E. coli* LPS. The clear estrual cervical mucus after LPS treatment may be due to increase in phagocytosis and elimination of infection with stimulation of uterine defense mechanisms. Percentage of antibiotic group cows with clear discharge after treatment is similar to findings of Saini *et al.* (1999) and Rane *et al.* (2003). This may be attributed to the use of most sensitive antibiotic after *in-vitro* antibiogram.

White Side Test: 100% of cows in all the groups were positive to white side test prior to treatment. At subsequent estrus after treatment, 85.71% in LPS group followed by 71.43% in antibiotic group and only 14.29% cows in untreated control group became negative to white side test (Table 1). Positive reaction to white side test could be explained on the basis of number of leukocytes present in the uterine discharge (Popov, 1969). The normal discharge has less number of leukocytes to cause any change of colour whereas in clinical and subclinical cases of endometritis, discharge contains increased number of leukocytes causing a colour reaction (Pateria and Rawal, 1990). The absence of colour development to white side test in higher number of cows treated with LPS and antibiotic revealed their efficacy for combating infection.

pH: The pH of estrual cervical mucus of all the groups was above 8 prior to treatment. This increase in pH might be caused due to metabolites of bacteria and inflammatory exudates in estrual cervical mucus (Salphale *et al.*, 1993) and once the infection is eliminated, the pH of cervical mucus returns towards the neutral side. After treatment pH declined significantly in LPS and antibiotic group as well as in untreated control group cows at subsequent estrus (Table 1). Deori (2002) also found significant decline in pH of estrual cervical mucus at estrus following to LPS treatment. This shows effectiveness of LPS and antibiotic treatment in controlling infection. Reduction in pH in control group

Table 1: Physico-chemical parameters of estrual cervical mucus of control, LPS and antibiotic group cows with endometritis before and after treatment

Parameters		Group A (Control) n = 7		Group B (LPS) n = 7		Group C (Antibiotic) n=7	
		Pre- treatment estrus	Post-treatment estrus	Pre- treatment estrus	Post-treatment estrus	Pre- treatment estrus	Post-treatment estrus
Appearance of CVM							
% animals (No. of animals)							
1	Purulent	42.86(3)	28.57(2)	42.86 (3)	0 (0)	42.86 (3)	0 (0)
2	Muco-purulent	42.86(3)	42.86 (3)	57.14 (4)	14.29 (1)	42.86 (3)	28.57 (2)
3	Clear	14.29 (1)	28.57 (2)	0 (0)	85.71 (6)	14.29 (1)	71.43 (5)
White side test							
% animals (No. of animals)							
1	Positive	100 (7)	85.71 (6)	100 (7)	14.29 (1)	100 (7)	28.57 (2)
2	Negative	0 (0)	14.29 (1)	0 (0)	85.71 (6)	0 (0)	71.43 (5)
pH (Mean ± SE)		8.17^{a}± 0.09	7.69^{b}±0.12	8.19^{a}± 0.07	7.20^{c}± 0.05	8.24^{a}± 0.08	7.31^{c}±0.11
Bacterial load (Mean ± SE) ($\times 10^{6}$ /ml)		308.0^{a} ±25.64	109.43^{b} ±28.77	310.86^{a} ±29.56	0.47^{c} ±0.42	293.43^{a} ±23.76	2.95^{c} ±1.96

Means with different superscripts (a, b, c) in same row within group and between groups vary significantly (P<0.01)

might be due to of natural uterine defense mechanisms.

Bacterial load: Higher bacterial load was recorded prior to treatment which ranged from 293.43 ± 23.76 to 310.86 ± 29.56 $\times 10^{4}$ per ml of cervical mucus. At subsequent estrus after treatment, there was a significant decline in bacterial load in the cervical mucus of all the groups (Table 1). Reduction was highest in LPS group followed by antibiotic group. Deori (2002) also reported similar reduction in bacterial load in cervical mucus after *E. coli* LPS infusion. Intrauterine infusion of *E. coli* LPS efficiently increases the influx of PMNs into the uterine lumen (Klucinski *et al.*, 1990; Hussain and Daniel, 1992). With increased neutrophil count, rate of phagocytosis might have increased resulting in reduction in viable bacterial count. Infusion of suitable antibiotic after *in-vitro* antibiogram worked effectively against the bacteria present in the uterus and resulted in significant reduction of bacterial count after treatment. Reduction in bacterial load in control group may be due to of natural uterine defense mechanisms.

***In-vitro* antibiotic sensitivity test:** Based on *in-vitro* antibiogram, the most sensitive antibiotic enrofloxacin at total dose of 1000 mg was infused intrauterine for 4 consecutive days in antibiotic group cows.

Recovery and conception rate : At subsequent estrus after treatment in LPS group 85.71% (6 out of 7) cows and in antibiotic group 71.43% (5 out of 7) cows showed recovery from endometritis in comparison to 14.29% (1 out of 7) cows in untreated control group. Maximum conception rate of 71.43% (5 out of 7) was achieved in LPS group followed by 57.14% (4 out of 7) in cows with antibiotic treatment. Only 28.52% (2 out of 7) cows conceived in untreated control group (Table 2). A single *E. coli* LPS infusion which had caused increased influx of PMNs and serum proteins in uterine lumen resulted in rapid elimination of bacteria through phagocytosis and brought endometritis under control (Anderson *et al.*, 1985; Hussain and Daniel, 1992). Saini *et al.* (1999), Singh *et al.* (2000) and Deori (2002)

Table 2: Recovery rate and conception rate in control, LPS and antibiotic group cows with endometritis after treatment

Groups	No. of cows	Recovery rate % of animals (No. of animals)	Conception rate % of animals (No. of animals)
Group A (Control)	7	14.29 (1)	28.52 (2)
Group B (LPS)	7	85.71 (6)	71.43 (5)
Group C (Antibiotic)	7	71.43 (5)	57.14 (4)

reported similar conception rate following LPS infusion in cows with endometritis. Better conception rate was observed in antibiotic group in comparison to untreated control cows. Antibiotic infused on the basis of *in-vitro* antibiogram effectively eliminate the infection. Hence, good conception rate was observed in antibiotic group also. Similar conception rate was reported after antibiotic treatment based on *in-vitro* antibiogram by Singh (1996), Saini *et al.* (1999) and Shukla and Sharma (2005) while Rane *et al.* (2003) reported higher conception rate after antibiotic treatment. The conception in untreated cows might be due to spontaneous elimination of infection by natural uterine defense mechanisms.

Several workers have demonstrated a marked increase in protein concentration and PMNs infiltration in uterine flushings following uterine contamination (Hawk *et al.*, 1963; Strezemienski and Kenney, 1984). It is reasonable to assume that this response may play an important role in controlling bacterial infection introduced at coitus or at parturition. LPS infusion appeared to increase this inflammatory response many folds resulting in clear appearance of estrual cervical mucus, reduction in pH and bacterial load and no colour reaction to white side test. Antibiotics have altogether different mechanism of action by directly killing the bacteria. Antibiotic therapy could be effective if sensitive antibiotic is infused on the basis of *in-vitro* antibiogram. But it is well documented that antibiotics are secreted in milk after its intrauterine infusion resulting in development of resistant bacterial strains in human population. Moreover, cost of antibiotic treatment is high and it is very difficult to go for antibiotic sensitivity tests under field conditions. Hence, it is concluded that intrauterine infusion of 100 µg *E.coli* LPS is a better alternative to conventional antibiotic treatment in cows with endometritis.

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