

Comparative efficacy of *Escherichia coli* lipopolysaccharide, oyster glycogen and enrofloxacin on uterine defense mechanism and fertility in crossbred cows with endometritis*

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ABSTRACT

Cows (48) tested positive for endometritis on the basis of appearance of cervical mucus and white side test were randomly divided into 4 groups with 12 cows in each group. In group A, 100 µg of *Escherichia coli* LPS once, in group B, 500 mg OG once, in group C, enrofloxacin was infused intrauterine for 3 consecutive days and in group D, 20 ml PBS was infused once which served as control group. Endometrial biopsy was collected at 0 h and 72 h from all the cows for histopathological study. Uterine flushing was collected at estrus prior to infusion of drug (0 h) and at 24, 48 and 72 h post treatment for evaluation of changes in total protein concentration, TLC, PMNs%, and the total immunoglobulin concentration.

The *E. coli* LPS and OG treatment significantly increased ($P < 0.05$ or $P < 0.01$) the total protein concentration, TLC, PMNs% and the concentration of total immunoglobulin at 24, 48 and 72 h post treatment. However, intrauterine enrofloxacin therapy reduced ($P < 0.01$) the total protein concentration and PMNs% at 72 h post treatment than pre-treatment levels.

It is concluded that *E. coli* LPS and OG may show better therapeutic efficacy to stimulate non-specific uterine defense mechanisms in crossbred cows suffering from endometritis. Further, *E. coli* LPS and OG may show better therapeutic efficacy than intrauterine enrofloxacin in curing the endometritis in crossbred cows.

Key words: Conception, Cows, Endometritis, Immunomodulators, Uterine defense

Endometritis, often considered to be the major cause of repeat breeding condition in bovine, occurs most commonly during post-partum period (Roberts 1971, Arthur *et al.* 1989). The incidence of endometritis varies between 8.5 to 56.6% in repeat breeding cows (Rahumathulla *et al.* 1986, Singh and Sinha 1991, Mohanty *et al.* 1992).

The therapeutic strategies have been reported to have adverse effect on cellular immune response of uterus (Bouters and Vandeplasseche 1977). Antibacterial drugs interfere the activity of leukocytes thereby decrease the uterine defense mechanism (Ziv *et al.* 1982). Indiscriminate use of antibacterial drugs may lead to development of partial or complete microbial resistance (Korudzhiiski *et al.* 1988). Hormonal therapy revealed variable fertility rates (Vukovic *et al.* 1989). The above approaches become uneconomical due to after effects (Hissain 1989). Attention is on alternative therapies, which stimulate the natural uterine defense

mechanisms through immunomodulation (Gilbert 1992).

Some biologically active immunomodulators like *E. coli* lipopolysaccharide (*E. coli* LPS), oyster glycogen (OG), leukotrine B₄, hyper immune serum etc. were used to treat the endometritis which stimulate the various uterine defense mechanisms (Saini *et al.* 1999, Singh *et al.* 2001, Singh *et al.* 2003). The present study was conducted with the idea for better understanding about the comparative efficacy of *E. coli* LPS, OG and enrofloxacin on non-specific uterine defense mechanism and conception rate in crossbred cows suffering from endometritis.

MATERIALS AND METHODS

Crossbred cows (48) with endometritis were selected on the basis of history of repeat breeding, thorough per-rectal examination and physico-chemical characteristic of cervical mucus. Cows with purulent or muco-purulent estrual discharge or containing white flakes and positive reaction to white side test were considered positive for endometritis. Besides, animals with history of repeat breeding and clear discharge but positive reaction to white side test were also

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included in the study. Cows were divided randomly into 4 groups. In group A cows, *E. coli* LPS (100 mg) was infused intrauterine once, in group B cows, oyster glycogen (500 mg) was infused intrauterine once, in group C cows, sensitive antibiotic i. e. enrofloxacin (1000 mg in 30 ml distilled water) on the basis of *in-vitro* antibiogram was infused intrauterine for 3 days and group D cows were served as untreated control in which PBS (20 ml) was infused intrauterine once. Endometrial biopsies were collected from all the cows of each group before the start of treatment (at 0 h) and at 72 h post treatment.

Collection of uterine flushing: All animals under study were flushed at 8–12 h after they showed first signs of heat, and was considered as 0 h. Next uterine flushings were collected at 24 h, 48 h and 72 h from LPS, OG and control group cows. Uterine flushings from antibiotic group were collected only at 0 h and 72 h.

The collection technique involved aspiration of uterine flushing by a 2-way Foley catheter as per method of Deori (2002) with some modification. The catheter was fixed in the body of uterus near internal os by inflating the balloon with 10–15 ml air. 50 ml of PBS was infused into the uterus with the help of a syringe. PBS was left in uterus for 2 minutes, uterus was massaged gently per rectum and flush was aspirated out through a syringe fitted in the Foley catheter. Uterine flushing was collected in a 50 ml glass cylinder with a stopper and brought to laboratory on ice packs in a thermocol box for further processing.

Out of total volume aspirated, 10 ml of uterine flushing was centrifuged at 3000 rpm for 15 min. The supernatant was used for estimation of total protein and total immunoglobulin concentration. The fresh cellular sediment was used for TLC and PMNs% determination. The final volume of the sediment was fixed 1.0 ml in PBS.

Total protein estimation: The total protein concentration in the cell free uterine flushing was estimated with Folin phenol reagent method using BSA as a standard (Lowry *et al.* 1951).

Uterine defense mechanism (UDM): The UDM was studied by estimating the total leukocyte count (TLC), polymorphonuclear neutrophil granulocytes (PMNs) per cent and the concentration of total immunoglobulin in the uterine flushing.

The TLC in the uterine flushing was determined by using a hemocytometer. For differential cell count, smears were prepared from the cell suspension stained with Giemsa stain, and percentage of PMNs out of total cells counted was determined.

The total concentration of immunoglobulin (Ig) in the uterine flushing was estimated as per McEwan *et al.* (1970) with modification.

The clinical recovery was assessed by clear appearance of discharge, reduction in pH 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 and pregnancy was confirmed per rectally 45–60 days after insemination. The data were analysed by two-way ANOVA and Chi-square test to evaluate the significant differences (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

All the cows taken in the study were positive to white side test and the mean pH of cervical mucus of all cows was more than 8.00. Based on endometrial biopsy, the incidence of acute/mild, sub-acute and chronic endometritis was 58.33, 14.58 and 27.08%, respectively. Acute/ mild type of endometritis was significantly higher ($P < 0.01$) than sub-acute and chronic endometritis.

Antibiotic sensitivity test conducted on uterine flushings from the cows suffering from endometritis revealed that maximum 82.05% isolates were sensitive to enrofloxacin followed by ciprofloxacin (76.92%) and gentamicin (74.36%).

Total protein concentration: Total protein concentration in cell free uterine flushing varied between 156.8 ± 5.19 and 161.8 ± 5.23 mg/dl among the groups prior to the treatment. It increased ($P < 0.01$) markedly at 24 and 48 h after *E. COLI* LPS and OG treatment compared to control (Table 1). Our results are in general agreement with the previous findings reported in mare (Strezeminski and Kenney 1984, Marti *et al.* 1988) and cow (Singh 1996, Deori 2002). The increased protein concentration of this study might be attributed to the inflammatory response with the influx of serum-derived proteins in the uterus after LPS administration (Martin *et al.* 1988). However, the protein concentration was decreased at 48 and 72 h after LPS and OG treatment as well as 72 h of

Table 1. Mean total protein concentration (mg/dl) in uterine flushings of endometritis cows treated with *E. coli* LPS, OG and enrofloxacin

Groups	No. of cows	Before treatment (0 h)	After treatment (h)		
			24	48	72
Group A (LPS)	12	160.50 ± 4.97^a	236.50 ± 4.34^{Ab}	210.25 ± 4.78^{Ac}	184.83 ± 4.51^d
Group B (OG)	12	156.75 ± 5.19^a	236.00 ± 3.66^{Ab}	207.42 ± 2.51^{Ac}	187.08 ± 3.63^d
Group C (antibiotic)	12	160.42 ± 5.39^a	—	—	120.92 ± 3.33^{Bb}
Group D (control)	12	161.83 ± 5.23^a	166.53 ± 5.49^{aB}	168.42 ± 5.11^{aB}	173.25 ± 6.69^a

Means with different superscripts within group (a, b, c, d) and between groups (A, B) vary significantly ($P < 0.01$).

enrofloxacin treatment. A declined inflammatory response over the time possibly is responsible for decreased protein concentration in our study (Table 1).

Uterine defense mechanism: The TLC and PMNs% in uterine flushing increased ($P<0.01$) significantly at 24, 48 and 72 h after LPS and OG administration compared to control (Tables 2, 3) and are similar to those reported earlier (Hussain and Daniel 1992, Singh *et al.* 2001, Deori 2002). The LPS acts as a chemo-attractant, stimulates infiltration of monocytes and macrophages, which produce pro-inflammatory cytokines causing acute inflammatory response. Thus LPS activates the infiltration of PMNs into the uterine lumen (Tizard 1998). Besides, the vasodilation (Sayer *et al.* 1988) and a direct chemotactic effect of endotoxin (Maestrelli *et al.* 1988) were also probably involved in the infiltration of PMNs that resulted into many fold increase in the TLC in this study. In a similar study, Raju and Rao (2006) reported a 100 fold increase in TLC

and 80% in PMNs after LPS (100–200 μg) and only 80% PMNs after OG administration in cows with endometritis. Although an influx of leucocytes and PMN cells occur even at natural bacterial infections. But the dramatic rise of such cells within 24 h following LPS and OG infusion (Figs 1, 2) indicated that the UDM is stimulated at a higher degree by the above said immunomodulators. Compared to 24 h, the number of leucocytes and PMNs% was significantly low at 48 and 72 h. This might be due to the shorter life span and death of leucocytes during the process of phagocytosis (Tizard, 1998).

The PMNs% was decreased ($P<0.01$) significantly after 72 h of enrofloxacin treatment in this study (Table 2). The TLC also showed a tendency to decline after 72 h of antibiotic treatment. At present we do not have any suitable explanation for such decline. Although in a previous report, it has been shown that repeated infusion of antibacterial drugs inhibit/destroy the phagocytic activity of uterine derived

Table 2. Mean total leukocyte count ($10^6/\text{ml}$) in uterine flushings of endometritis cows treated with *E. coli* LPS, OG and enrofloxacin

Groups	No. of cows	Before treatment (0 h)	After treatment (h)		
			24	48	72
Group A (LPS)	12	0.68 $\pm$ 0.05 ^a	16.1 $\pm$ 0.69 ^{Ab}	13.1 $\pm$ 0.62 ^{Ac}	8.97 $\pm$ 0.58 ^{Ad}
Group B (OG)	12	0.70 $\pm$ 0.05 ^a	15.8 $\pm$ 0.57 ^{Ab}	12.4 $\pm$ 0.52 ^{Ac}	8.56 $\pm$ 0.51 ^{Ad}
Group C (antibiotic)	12	0.66 $\pm$ 0.06 ^a	-	-	0.04 $\pm$ 0.08 ^{Ba}
Group D (control)	12	0.65 $\pm$ 0.04 ^a	0.67 $\pm$ 0.05 ^{aB}	0.67 $\pm$ 0.05 ^{aB}	0.66 $\pm$ 0.04 ^{aB}

Means with different superscripts within group (a, b) and between groups (A, B) vary significantly ($P<0.01$).

Table 3. Mean polymorphonuclear neutrophil granulocytes% in uterine flushings of endometritis cows treated with *E. coli* LPS, OG and enrofloxacin

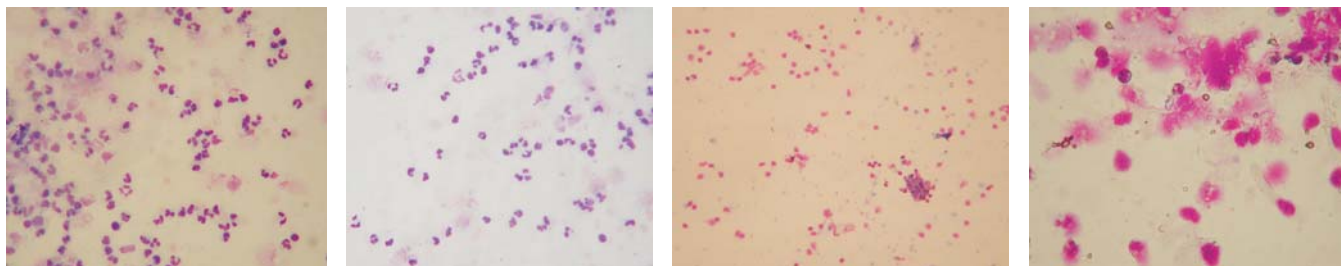
Groups	No. of cows	Before treatment (0 h)	After treatment (h)		
			24	48	72
Group A (LPS)	12	28.5 $\pm$ 0.78 ^a	79.17 $\pm$ 1.55 ^{Ab}	70.25 $\pm$ 1.82 ^{Ac}	61.25 $\pm$ 2.12 ^{Ad}
Group B (OG)	12	29.33 $\pm$ 1.43 ^a	78.75 $\pm$ 1.28 ^{Ab}	69.91 $\pm$ 1.22 ^{Ac}	55.08 $\pm$ 2.60 ^{Ad}
Group C (antibiotic)	12	29.17 $\pm$ 1.04 ^a	-	-	21.08 $\pm$ 1.49 ^{BbC}
Group D (control)	12	29.17 $\pm$ 1.34 ^a	31.41 $\pm$ 1.29 ^{aB}	29.58 $\pm$ 1.34 ^{aB}	28.83 $\pm$ 2.05 ^{aB}

Means with different superscripts within group (a, b) and between groups (A, B, C) vary significantly ($P<0.01$).

Table 4. Mean total immunoglobulin concentration (mg/dl) in uterine flushings of endometritis cows treated with *E. coli* LPS, OG and enrofloxacin

Groups	No. of cows	Before treatment (0 h)	After treatment (h)		
			24	48	72
Group A (LPS)	12	79.52 $\pm$ 3.98 ^{aA}	96.75 $\pm$ 5.15 ^b	99.42 $\pm$ 5.76 ^{aB}	104.17 $\pm$ 6.79 ^{aB}
Group B (OG)	12	77.92 $\pm$ 7.68 ^{aA}	101.42 $\pm$ 6.11 ^b	109.23 $\pm$ 8.24 ^b	110.42 $\pm$ 7.76 ^{aB}
Group C (antibiotic)	12	73.08 $\pm$ 7.31 ^a	-	-	88.87 $\pm$ 8.50 ^a
Group D (control)	12	76.33 $\pm$ 8.30 ^a	94.88 $\pm$ 5.67 ^a	98.28 $\pm$ 11.91 ^a	96.95 $\pm$ 10.26 ^a

Means with different superscripts within group (a, b and A, B) vary significantly (a, b; $P<0.05$ and A, B; $P<0.01$).



Figs 1–4. **1.** Infiltration of PMN cells 24 h after LPS treatment, Giemsa stained smear. 400 ×. **2.** Infiltration of PMN cells 24 h after OG treatment, Giemsa stained smear. 400 ×. **3.** degenerated endometrial cells 72 h after antibiotic treatment, Giemsa stained smear. 100 ×. **4.** Degenerated endometrial cells after 24 h in control group, Giemsa stained smear. 1000 ×.

polymorphonuclear granulocytes and lower the uterine defense (Paisley *et al.* 1986). Such type of inhibitory effect of enrofloxacin on PMNs% and TLC cannot be ignored in this study and warrants detailed investigations. Degenerated endothelial cells were also present in abundance after enrofloxacin treatment in this study (Fig.3). In control group, infusion of PBS, however, did not alter the TLC and PMNs% although an increased number of degenerated endothelial cells were present in uterine flushing (Fig. 4).

The total Ig concentration varied from 73.1 ± 7.31 to 79.5 ± 3.98 mg/ dl in all the cows prior to the treatment. It increased significantly at 24, 48 and 72 h post LPS and OG infusion compared to that remained before treatment in this study (Table 4). This increased Ig in uterine flushing indicated that the process of uterine inflammation initiated after LPS administration and served as the criterion for the clinical recovery (Aknazarov 1988). Enrofloxacin treatment, however, had no marked effect on Ig levels in this study. Cows in control group did not show any distinct trend in Ig level over the period of treatment (Table 4).

Recovery and conception rate: The recovery rate was higher ($P < 0.01$) both in LPS and OG groups (83.3%) than the control group (25%). But in antibiotic group, 75% of the treated cows showed ($P < 0.05$) clinical recovery from endometritis as against the control.

The conception rate was significantly higher with LPS and OG ($P < 0.01$) (75%, 9/12) as well as with enrofloxacin ($P < 0.05$) (58.3%, 7/12) treatment compared to the control (16.7%, 2/12). These results strengthen the previous findings on bovine endometritis treated with LPS (Saini *et al.* 1999, Singh *et al.* 2000, Deori 2002) and antibiotic (Singh 1996, Saini *et al.* 1999, Shukia and Sharma 2005). However, in contrast to the report of Rane *et al.* (2003), the conception rate obtained after enrofloxacin treatment was bit in lower side in our study.

In conclusion, intrauterine administration of *E. coli* LPS and oyster glycogen may be better therapy to stimulate non-specific uterine defense mechanisms in crossbred cows suffering from endometritis. Further, *E. coli* LPS and OG may show better therapeutic efficacy than intrauterine enrofloxacin in curing the endometritis in crossbred cows.

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