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Short Communication

Effect of immunomodulators on biochemical attributes of cows with Endometritis*

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ABSTRACT

Concentration of certain serum biochemical constituents and pH of uterine flushings in 84 crossbred dairy cows were studied. Six groups with 12 cows in each group received either 1) LPS from E.coli, 100 µg, 2) Oyster glycogen, 500 mg, 3) Autologus plasma (AP), 30 ml, 4) LPS 100 µg plus AP 10 ml, 5) Antibiotic (based on ABST) or 6) Phosphate buffer saline 30 ml (Control) intrauterine. Mean total serum protein, albumin, globulin, albumin/globulin ratio, alkaline phosphotase and serum urea nitrogen did not vary significantly ($P \leq 0.05$) after 24 hours of treatment. The mean pH decreased significantly ($P \leq 0.05$) in all the treated groups compared to its initial level. It was concluded that estimation of these serum biochemical parameters may not be tool for diagnosis of endometritis but pH would certainly help.

Key words: Lipopolysaccharide(LPS), Oyster glycogen, Autologus plasma, pH, Endometritis, Biochemical attributes

Endometritis is a major postpartum reproductive disorder with an incidence varying from 7.5 to 61.6% (Gilbert, 2003) as well as one of the most important causes for conception failure, increase in the number of services per conception, calving to first service and decrease in milk production (Sudhakar *et al.*, 1986; Borsberry and Dobson, 1989). Abnormal parturition, puerperal complications, inseminations without aseptic precautions and infected semen will lead to uterine infection (Steffen *et al.*, 1984) resulting in development of endometritis (Roberts, 1986). The alkaline pH of uterine secretions indicates infection with possible alterations in serum biochemical constituents which invites attempts to rectify.

Seventy two crossbred dairy cows among those presented for artificial insemination, aged between 3.5 and 11.0 years and an average parity of 2.3 were utilized for the study. Based on the history, nature of estrual discharge, gynaecological examination and white side test, the cows were screened and confirmed for endometritis. The positive cases were divided into six equal groups and different treatment protocols viz., lipopolysaccharide from E. coil (serotype: 026: B6 with ≥ 10,000 endotoxin units per mg of LPS, Sigma-Aldrich, USA) 100 µg/animal in 30 ml PBS (Group 1), oyster glycogen (HiMedia Laboratories Ltd., Bombay) 500 mg/animal in 30 ml PBS (Group II), autologus plasma (AP) 30 ml (Group III), LPS (100µg in 20 ml PBS) plus 10 ml AP (Group IV), antibiotic selected on ABST (Group V) and control (30 ml PBS, Group VI) infused intrauterine. First four protocols were administered once on the day of estrus while latter two protocols were infused for three consecutive days. Further, twelve cows negative for endometritis were included for comparison of the biochemical attributes with that of cows having endometritis.

Uterine flushings were aspirated aseptically on the day of estrus following infusion of 30 ml sterile neutral phosphate buffer saline (pH7.0) using a sterile catheter and syringe after gentle massage of uterus per-rectally for 2-3 minutes (Singh *et al.*, 2000). The flushing samples were subjected for pH estimation using calibrated Systronic digital pH meter (Model- 335). The pH of uterine flushings aspirated during next estrus following various treatment regimens was estimated. Serum samples were collected before (on the day of

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estrus) and 24 hours after treatment and preserved at –20^{0}C for estimation of biochemical constituents, viz. total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphotase (ALP) and serum urea nitrogen (SUN) using BM Hitachi 704 Automatic Clinical Chemistry Autoanalyser (Boehringer Mannheim, Germany) with standard kits (Human Geselleschaft fur Biochemical and Diagnostica mbH, Max-Planck-Ring21-D-65205, Germany). One-way analysis of variance was performed to test the variation between the groups as per the methods described by Snedecor and Cochron (1980) and the group means were compared by least square significance difference test (LSDT) as per the technique of Steel and Torrie (1981).

The mean pH of uterine flushings of endometritis affected cows was significantly higher compared to that of healthy cows (Table 1) which is in conformity with Wani *et al.*, (1979), Boitor *et al.*, (1980), Rane *et al.*, (1992), Saini (1993) and Ravikumar (2004). Alteration in the pH of uterine fluid to alkaline side was attributed to inflammation due to infection of endometrium, metabolites of bacteria and inflammatory exudates which results in conception failure due to death of spermatozoa (Raghavan *et al.*, 1971; Singla *et al.*, 1991). Contrarily, Akhtar and Singh (1979) and Rangnekar *et al.*, (2002) reported significantly higher pH of estrual mucus in normal crossbred cows than in repeat breeding cows. However, nonsignificant difference between pH of estrual mucus of normal and repeat breeding cows and buffaloes has been reported (Gunther, 1982; Vadodaria and Prabhu, 1990; Salphale *et al.*, 1993).

The mean pH of uterine flushings decreased significantly ($P \leq 0.05$) in next immediate estrus after treatment with all the protocols followed with no change in the control group (Table 2). The metabolites of bacteria and inflammatory exudates that contributed for higher pH (Raghavan *et al.*, 1971; Singla *et al.*, 1991) were perhaps removed after treatment that resulted in significant decrease in the pH of uterine flushings. Normal cervical mucus is important for sperm transport (Rowson *et al.*, 1972) and its pH in normal cows ranged from 7.00 to 7.22 (Wani *et al.*, 1979; Rane *et al.*, 1992; Saini., 1993).

The serum biochemical parameters estimated did not vary significantly between healthy and cows effected with endometritis (Table 1). Further, there was no significant change in their levels 24 hrs after treatment in all the groups. Hence, estimation of serum biochemical constituents may not help in diagnosis of endometritis but estimation of pH of uterine flushings would help to associate the infection of the endometrium in cows.

Table 1: pH of uterine flushing and certain serum biochemical attributes in healthy and endometritis affected cows (Mean ±SE)

Parameter	Healthy cows (n=12)	Cows with endrometritis (n=72)
pH of uterine flushing	7.34±0.09	8.12±0.04*
Total serum protein (g/dl)	8.49±0.17	8.32±0.07^{ns}
Albumin (g/dl)	3.46±0.09	3.36±0.04 ^{ns}
Globulin (g/dl)	5.03±0.15	4.98±0.06 ^{ns}
A/G ratio	0.70:1±0.03	0.68:1±0.01 ^{ns}
Alkaline Phosphatase (U/L)	37.32±4.30	47.93±2.71 ^{ns}
Serum urea nitrogen (mg/dl)	12.28±1.28	12.33±0.53 ^{ns}

*P≤ 0.05, ^{ns}non significant

Table 2: Effect of treatment for endometritis on pH of uterine flushings in Crossbred cows (Mean ±SE)

Treatment group (n=12)	Before treatment	After treatment
Gr I	8.22±0.09	7.69±0.09*
Gr II	793±007	7.57±0.11*
Gr III	8.11±0.06	7.69±0.11*
Gr IV	8.30±0.11	7.77±0.18*
Gr V	8.15±0.07	7.69±0.09*
Gr VI	8.01±0.10	7.93 ± 0.10^{ns}

*$P \leq 0.05$ ^{ns} non-significantly

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