Efficacy of Immunomodulators, Lugol’s Iodine and PGF<sub>2α</sub> on the Bacterial Load in the Endometritis Affected Cows

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Abstract

The present study was conducted to assess the efficiency of immunomodulators in controlling the microbial load in endometritis cows in comparison with Lugol’s Iodine (LI) and Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>). A total of 72 crossbred cows divided equally in to six groups viz. Group I - treated with 30 ml of 2 per cent Lugol’s iodine for 3 days, Group II, III and IV - single intrauterine dose of 30 ml PBS containing 100 µg of E. coli LPS, 2 mg of LYZs and 500 mg of OG, respectively, Group V - 25 mg of PGF<sub>2α</sub> and Group VI - control cows given 30 ml of PBS intrauterine. The bacterial colony counts recorded were significantly (P≤0.01) reduced after treatment. The elimination of bacterial load was better in the immunomodulator treated groups than other groups. E.Coli LPS was found to be most effective in controlling uterine infections followed by LYZ and OG.

Key words: endometritis, immunomodulators, bacterial load

Infectious agents affect fertility by altering the uterine environment resulting in impairment of sperm transport, sperm death and hostile environment to the subsequent development and maintenance of the conceptus leading to their death (Sheldon et al., 2009). There are many approaches for preventing and controlling postpartum endometritis. The utilization immunomodulators is attracting attention of researchers in recent times because, in public and political opinion, the use of antibiotics ana hormones in food animals is increasingly under critical discussion (Raju et al., 2009). Hence, the present study was conducted to assess the efficacy of intra uterine immunomodulators in reducing uterine infection in endometritis affected cows.

Materials and Methods

The postpartum lactating cows brought for artificial insemination (AI) to the AI unit of Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal and Veterinary Dispensaries of Namakkal District, Tamil Nadu were selected based on the breeding history, physicochemical characteristics of oestral mucus and white side test. In all, 72 crossbred cows affected with endometritis were utilized for the study. The selected crossbred cows were randomly divided into five treatment (Group I to V) and one control (Group VI) group and were marked as Group I (Li group; 30 ml of 2% Lugol’s iodine solution through intrauterine route for three consecutive days from the day of oestrus), Group II (LPS group; single intra uterine infusion of E.coli LPS, 100 µg/cow in 30 ml of PBS), Group III (LYZ group; single intra uterine infusion of Lyzoxyme, 1 00 µg/cow in 30 ml of PBS), Group IV (OG group; single intra uterine infusion of Oyster Glycogen, 100 µg/cow in 30 ml of PBS), Group V (PGF<sub>2α</sub> group; 25 mg of Dinoprost Tromethamine, Lutalyse®, Pfizer India Private Limited on day 10 of the estrous cycle) and Group VI (control group; sterile intrauterine single dose of 30 ml PBS during oestrus).

Nutrient broth, thiglycollate broth, Robertson cooked meat medium, Nutrient agar, MacConkey agar, Blood agar base, perfringens agar and perfringens supplement I and II (M/s HiMedia Laboratories, Mumbai) was prepared as per the manufacturer’s instructions and
Blood agar was prepared with five per cent defibrinated sterile sheep blood.

Isolation of organisms in the cervical mucus was done as per Barrow and Feltham (1993). Following incubation, growth characteristics and colony morphology of the cultures were studied. The individual pure colonies obtained from primary isolation were stained by Gram's method of staining as per the procedures of Barrow and Feltham (loc cit) and further subcultured in different medias mentioned above for identification of bacteria. The ready-made dehydrated media were prepared as per the manufacturer's instruction (M/s. Hi-Media laboratories, Mumbai) viz. eosin methylene blue agar, mannitol salt agar, brilliant green agar, triple sugar iron agar, Simmons's citrate agar, milk medium with reducing agent, egg yolk agar (EYA) base, egg yolk emulsion, blood agar base, peptone water and Methyl Red-Voges Proskauer (MR-VP) medium. Blood agar was prepared with five per cent defibrinated sterile sheep blood and was also utilized for identification of organisms.

Materials for various biochemical tests were procured from HiMedia Laboratories, Mumbai. The positive colonies were subjected to biochemical test for identification such as Catalase, Coagulase, Oxidase, Indole, Methyl red, Voges-Proskauer, Citrate, TSI, Stormy clot fermentation, Lecithinase, CAMP tests and Sugar fermentation reaction as per Barrow and Feltham (loc cit).

The collected cervical mucus was subjected to bacterial colony count as per the standard plate count method before and after treatment (Raju et al., loc cit).

Results and Discussion

From the cervical mucus of endometritis-affected cows in this study, various bacteria were identified. They were Staphylococcus aureus, Escherichia coli, Arcanobacterium pyogenes, Pseudomonos aeruginosa, Proteus vulgaris, Klebsiella sp. and Clostridium perfringens and their number of isolates obtained before treatment were 19 (26.30), 18 (25.00), 9 (12.60), 8 (11.00), 7 (9.80), 6 (8.40) and 5 (6.90 per cent). The corresponding values after treatment were 4 (5.60), 5 (6.90), 4 (5.60), 3 (4.20), 3 (4.20), 4 (5.60) and 3 (4.20 per cent), respectively. The results clearly indicated that there was a reduction in each type of bacteria after treatment which concurred with the results of Abdullah et al. (2007) and Azawi et al. (2008).

The efficacy of various treatments on bacterial colony count of cervical mucus in cows affected with endometritis is given in Table I. Before treatment, mean (±SE) bacterial counts recorded in this investigation in cows affected with endometritis ranged from 72.84 ± 0.97 to 82.93 ± 0.91 x 10⁶/ml of cervical mucus. The bacterial colony counts recorded before treatment in cows affected with endometritis significantly (P≤0.01) reduced after treatment.

In this study, it was observed that the

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment groups</th>
<th>Total bacterial count (10⁶/ml of cervical mucus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>1</td>
<td>I (LI)</td>
<td>81.21±0.75</td>
</tr>
<tr>
<td>2</td>
<td>II (LPS)</td>
<td>72.84±0.97</td>
</tr>
<tr>
<td>3</td>
<td>III (LYZ)</td>
<td>82.93±0.91</td>
</tr>
<tr>
<td>4</td>
<td>IV (OG)</td>
<td>79.37±1.04</td>
</tr>
<tr>
<td>5</td>
<td>V (PGF₂α)</td>
<td>81.72±1.03</td>
</tr>
<tr>
<td>6</td>
<td>VI (Control)</td>
<td>77.81±1.55</td>
</tr>
</tbody>
</table>

Mean values bearing different superscripts between columns (x, y) and among various rows (a, b, c, d, e, f) differ significantly (P≤0.01)
LPS treatment was found to be most effective in controlling uterine infections by reducing bacterial count followed by LYZ and OG which concurred with the reports of Raju et al. (loc cit) and Sarma et al. (2010). In general, there was a reduction in the bacterial load and bacterial colony count in all the cows after the treatment but intrauterine immunomodulators were effective when compared to LI and PGF\(_2\alpha\). Singla et al. (2004) opined that significant increase in the influx of PMNL cells into the uterine lumen following infusion of immunomodulators could be followed by phagocytosis and killing of the invading microorganisms. Apart from this, the immunomodulators like E.coli LPS and OG might have stimulated the macrophages which in turn produced interleukin-1 and interleukin-8 that up regulated the production of Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) for rapid recruitment of PMN cells into uterus. The elimination and clearance of bacteria by phagocytosis and ultimate recovery observed in the present study was in concurrence with the findings of Sarma et al. (loc cit).

PGF\(_2\alpha\) and Lugol's iodine also cleared uterine infection in this study when compared to control group. Kasimanickam et al. (2006) reported that administration of PGF\(_2\alpha\) during the postpartum period induced luteolysis thereby stimulating the uterine defense mechanism by removing the suppressive effect of progesterone and enabling the stimulation effect of estrogen. Also, PGF\(_2\alpha\) induced estrus augmented the uterine clearance mechanism, thereby expelling debris and microorganisms contaminating the uterus (Lindell and Kindhull, 1983). The reduced efficiency of LI treatment in the current study could be due to the resistant bacteria to iodine therapy viz. Staphylococcus aureus, Streptococcus pyogenes and E. coli. The present findings are in accordance with Rao (1994) who reported that Lugol's iodine alone was not effective in controlling bacterial infection.

It was evident from the present findings that immunomodulators had an increased efficiency in controlling uterine infection. The variation in the elimination of the causative organisms from endometritis in the present investigation might be due to different etiological agents, severity of the disease, level of uterine defense mechanism and the treatment regimen.

Summary

The efficacy of immunomodulators, LI and PGF\(_2\alpha\) in eliminating bacterial load in endometritis affected cows is studied in 72 crossbred cows divided equally in to six groups which received I). 30 ml of 2 per cent Lugol's iodine (Group I), 30 ml PBS containing 2). 100 µg of E.coli LPS (Group II), 3). 2 mg of hydroxymethyl 4.500 mg of oyster glycogen (Group IV), 5) 25 mg PGR\(_2\alpha\) (Group V) and 6) 30 ml PBS intrauterine on the day of estrus (Group VI, control). The bacterial colony counts recorded were significantly (P<0.01) reduced after treatment in all the treated groups.

References


