EFFECT OF SYSTEMIC AND LOCAL IMMUNOMODULATION THERAPIES ON CONCEPTION RATE IN ENDOMETRITIC COWS

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ABSTRACT

A study was carried out to evaluate the efficacy of different immunomodulators by evaluating its effect on PMN cells, blood biochemical parameters and conception rate in 21 endometritic cows. The cows were randomly assigned into three groups (n=7) and treated with three different immunomodulators (Oyster glycogen 5 g i/ut once; Levamisole 10 ml s/c thrice, and $PGF_2\alpha$ 500 µg once i/m), while seven healthy cows at estrus were taken as control. Cervical mucus samples were collected from all the animals and subjected to PMN cell count. Besides, serum samples were analyzed for total protein, albumin, AST and ALT. Conception rate was assessed following different immunomodulation therapies. There were significant differences ($p<0.01$) in PMN cell counts and AST level between before and after treatments. However, no significant variations ($p<0.01$) were observed for protein, albumin and ALT level before and after treatment. The conception rate was 57.14, 42.84, 42.84 and 71.42 % for Oyster glycogen, Levamisole, $PGF_2\alpha$ and Control groups, respectively.

KEY WORDS – Immunomodulators, Endometritis, PMN cells, Conception rate.

INTRODUCTION

Endometritis is defined as inflammation of the endometrium extending not deeper than stratum spongiosum without showing any systemic signs. Contamination of uterus following calving is common. But most of the cows are able to eliminate the infection within first 2 to 3 weeks after calving (Bondurant, 1999). Those animals that fail to eliminate the infection subsequently develop endometritis (Dhaliwal *et al.,* 2001). As a result, there is increase in services per conception, calving to first service interval, calving to conception interval (Heuwieser *et al.,* 2000), and decrease in pregnancy rate (Leblanc *et al.,* 2002). Traditionally, antibiotics and chemotherapeutic agents are used to treat endometritis. But administering antibiotics through intrauterine route with no knowledge of either sensitivity or their utility in anaerobic pyemic environment or insensitive low dosage through parenteral route predisposes to growth of disease resistant strains. It also diminishes host resistance and poses threat to human health. The isolation of these superbugs has necessitated an alternative therapeutic approach for clinical recovery of uterine infection without compromising host immunity and health. So now a days immunomodulators and proinflammatory proteins have gained momentum to give a new dimension in therauptic procedures. Keeping the above facts in view, the present study was designed to evaluate the efficacy of both systemic and local immunomodulators in treating endometritic cows.

MATERIALS AND METHODS

The study was conducted in Department of Gynaecology and TVCC, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar from

September 2012 to July 2013. Twenty one endometritis cows were randomly assigned to three groups (n=7) and treated with three different immunomodulators while seven healthy cows served as control (Group IV) .

Group I (OG) : Oyster Glycogen 5 g (Hi Media Laboratories Pvt. Ltd.) was reconstituted with 50 ml of phosphate buffered saline and given as intrauterine infusion once.

Group II (PG) : $PGF_2\alpha$ 500 µg (Pragma, Intas Pharmaceuticals Ltd.) was administered once through intramuscular route.

Group III (LE) : Levamisole hydrochloride 10 ml (Kalmisol, KAPL) was administered by subcutaneous route thrice on alternate days.

Group IV(CON): Normal cyclic animals at oestrus presented for AI served as control without institution of any therapy.

Cervical mucus samples were collected aseptically before treatment, 24 hours after treatment and at succeeding estrus as per method described by Stiffens *et al.* (1984) for PMN cell count. A thin smear of cervical mucus was prepared on a grease free glass slide. It was air dried and subjected to Giemsa stain as per routine staining technique and cells were counted under microscope and expressed in percentage. Blood samples were collected prior to institution of therapy and during succeeding oestrus for analysis of total protein (g/dl), albumin (g/dl), AST/ SGOT (U/L) and ALT/ SGPT (U/L) by using diagnostic kit of Crest Biosystems, Goa, India. The data were analyzed statistically (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The percentage of PMN cell counts ranged between 9.57 and 14.42 in different experimental groups with no significant difference at pre-treatment stage. In the post-treatment samples at 24 hours and succeeding estrus, the values were 73.71±3.59 and 71.71±2.15 in group I; 42.71±1.48 and 40.29±1.43 in group II and 30.00±2.28 and 36.57±1.85 in group III, respectively. The comparison of PMN cell counts at 24 hours post-treatment revealed highly significant differences ($p<0.01$) between different treatments /drugs. At succeeding estrus, the oyster glycogen treated group I had a significantly ($p<0.01$) higher PMN cell count as compared to group II ($PGF_2\alpha$) and III (Levamisole), which were not different statistically. Comparison of PMN cell count at different intervals in group I showed significantly higher ($p<0.01$) count in post- treatment sampling than the pre-treatment value but it was not significant between 24 hrs post- treatment and succeeding estrus, which corroborated with the finding of Singh *et al.* (2003). The same statistical trend was also evident in group II and group III animals with respect to PMN cell count.

Normally, the PMN cells are sparse in the reproductive tract. During uterine infection chemotactic molecules are released by the bacteria which stimulate the influx of neutrophil from blood vessels into the uterus. Sometimes in cows receiving oyster glycogen, the neutrophil count may go up to 8 to 9 folds (Khadrawy *et al.,* 2011). In response to chemotactic response, opsonin bound micro-organisms also stimulate phagocytosis with increase in number of phagocytic cells like neutrophils and macrophages. Hence, the significant increase in PMN cells following 24 hrs of i/ut treatment and maintaining up to succeeding estrus is the effect of immunomodulation by oyster glycogen. The significant increase of PMN cells following systemic (i/m, s/c) immunomodulation therapies might have passively influenced the rise of PMN cells in the uterus (Sarma *et al*., 2010).

The pre- and post-treatment total protein concentrations (g/dl) were 7.24 ± 0.18, 8.04 ± 0.35; 7.41 ± 0.35, 7.93 ± 0.36 and 7.15 ± 0.31, 8.12 ± 0.41 for group I, II, III, respectively as against 8.34 ± 0.34 g/dl in control group IV. The comparison of pre-treatment values revealed significant difference ($p<0.05$) between group I and IV, while group II, III and IV had no significant difference.

Irrespective of drug/treatment, the comparison between pre- and post-estrus values did not differ. The present findings in pre-and post-treatment periods corroborated with the findings of Khan *et al.* (2010) and Virmani *et al.* (2011). However, higher protein value in normal cyclic cows has also been reported by Ahmad *et al.* (2003). Analysis of total blood protein does not necessarily indicate a very useful index for fertility assessment. However, very low protein level in the blood leads to subfertility. The differences in blood protein concentrations may be due to genetic variation, nutrition and management. The pre-treatment serum albumin concentrations (g/dl) were found to be 4.23 ± 0.10, 4.21 ± 0.17, 4.02 ± 0.16 and 4.81 ± 0.15, respectively, for group I, II, III and IV. The post-treatment values at succeeding estrus for 3 treatment groups were observed to be 3.86 ± 0.18, 4.10 ± 0.18 and 3.91 ± 0.18 g/dl, respectively. The pre- and post-treatment values did not reveal significant difference in any of the groups. The albumin value was nearly same as reported by Khan *et al*. (2010) and Virmani *et al.* (2011), suggesting no significant effect on albumin value following either systemic or local immunomodulation.

The serum AST values (U/L) in group I, II, III and IV were estimated to be 78.28 ± 2.66, 76.71 ± 2.36, 67.53 ± 1.71 and 39.87 ± 1.66, respectively just before treatment. The initial AST value of group I and II differed significantly ($p < 0.01$) from group III and IV. Similarly, group III had significantly higher value ($p < 0.01$) than the group IV. The post-treatment values in succeeding oestrus were 47.98 ± 2.95, 56.34 ± 2.55, 48.31 ± 1.98 and 39.87 ± 1.66 U/L, respectively for aforesaid groups and revealed highly significant differences ($p< 0.01$). The initial serum ALT values were 32.58 ± 2.26, 30.15 ± 2.62, 29.66 ± 1.79 and 19.21 ± 1.04 U/L, respectively. The normal cyclic group IV had significantly lower ($p < 0.01$) ALT level compared to remaining experimental groups. The ALT values in post-treatment sampling were found to be 28.34 ± 2.37, 22.92 ± 1.80 and 22.49 ± 1.80 U/L, respectively with significant differences ($p < 0.01$). In the post-treatment period, the oyster glycogen (group I) registered a significantly higher ($p < 0.01$) value compared to parenteral immunomodulatory drugs (group II and III). In the post-treatment period, the ALT values were significantly ($p < 0.05$) reduced, except in group I which was non-significant.

The present findings on serum AST and ALT were comparable to the findings of Sattler and Furll (2004) although they recorded higher values of the aforesaid enzymes. Normal cyclic cows recorded significantly lower value ($p<0.01$) than the rest of the experimental cows showing endometritis. AST activity increases in hepatic disorder and other acute inflammatory conditions of tissues specifically in muscular tissues. The present elevation observed in endometritis cases might be due to tissue damage (Benjamin, 2005). The significant reduction in both the enzymes might be attributed to their recovery following immunomodulation therapy against endometritis.

The conception rates were 57.14, 42.84, 42.84 and 71.42 per cent for Group I, II and III and normal cyclic cows (group IV), respectively. Oyster glycogen treated group achieved higher conception rate compared to other drugs. Higher conception rate of 66% (Singh *et al.*, 2003) and 60% (Sharma *et al.*, 2006) have been reported following oyster glycogen in endometritic cows. Similarly, $PGF_2\alpha$ treatment have shown very favorable conception rate of 60 to 80 % (Kumar *et al.,* 2004) in endometritis cases. The group III animals subjected to Levamisole treatment showed a pregnancy rate at par with $PGF_2\alpha$ treatment indicating potent systemic immunomodulation activity. So in conclusion the pregnancy rate was more in oyster glycogen treatment but Levamisole is cost effective.

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