IMMUNOMODULATORY EFFECT OF OYSTER GLYCOGEN ON ENDOMETRITIC CROSSBRED COWS

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ABSTRACT

The purpose of the study was to evaluate the immunomodulatory effect of intrauterine infusion of oyster glycogen on endometritic cows. 500mg of oyster glycogen reconstituted in phosphate buffer saline (PBS) was given as intrauterine infusion to cows of group I (n=10) during estrus. In second group (n=10) normal estrus cows without any treatment formed as control. Cervical mucus sample was collected aseptically and sent to laboratory for bacterial culture and count.Polymorph nuclear (PMN) cell count from uterine fluid showed a significant elevation (13.14 ± 1.35vs.73.71 ± 3.59) before and after 24hrs. of treatment. Serum globulin, albumin: globulin ratio, aspartate amino transferase (AST), haptoglobin and serum amyloid A (SAA) values before treatment were significant ($p<0.01$) than in succeeding estrus after treatment.While serum total protein, albumin and ALT concentration doesn't vary significantly ($p<0.01$). The conception rate in case of endometritis cows was 60 per cent after first post treatment insemination as compared to 70 per cent in normal cows. So it is concluded that oyster glycogen can be used as an effective immunomodulator as an alternative to antibiotic therapy.

Keywords: Oyster glycogen, Immunomodulator, Endometritis, Acute phase proteins

INTRODUCTION

Endometritis implies superficial inflammation of endometrium extending not deeper than the stratum spongiosum. It not only affects the milk production but also adversely affects the reproductive efficiency by disrupting uterine and ovarian function (Markendeya and Desmukh, 1995).Economic impact of endometritis is quite alarming as a result of undue calving to conception interval and long term consequences can cause irreversible changes of genital tract leading to metritis and sterility (Sheldon *et al.*, 2009).

Various antibiotics are used for treatment of endometritis through intrauterine route. But time to time evaluation of efficacy of antibiotics is needed since new strains of bacteria can develop due to indiscriminate use of antibiotics. As an alternative now a days uses of probiotics, both systemic and local immunomodulators and proinflammatory proteins have gained momentum to give a new dimension on therapeutic procedures. Oyster glycogen enhances PMN cell migration in to uterine lumen,with a peak PMN concentration 12hrs. after intrauterine administration. These PMN cells enhance the phagocytic activity in uterine lumen and helps to combat infection which can be monitored by change in concentration of haptoglobin and serum amyloid A. Therefore the present study was planned with the objective to determine the efficacy of oyster glycogen for treatment of endometritis in cross bred cows and its subsequent effect on conception rate.

MATERIALS AND METHODS

The present study was carried on twenty crossbred jersey cows within 1st to 5th parity which are patients of Teaching Veterinary Clinical Complex (TVCC), O.V.C. Bhubaneswar in years 2012 -2013. In group I (treatment group) 10 endometritis cows were selected while group II (control group) consisted of 10 healthy dairy cows which were in various lactational phases and dry period. The nature of vaginal discharge pertaining to color, consistency and transparency were ascertained and subjected to qualitative examination

for accurate diagnosis of endometritis. Cervical mucus samples from cows with mucopurulent discharge were collected as per method described by Stiffens *et al.*, (1984) and immediately send to laboratory for bacterial isolation, identification and count. The predominant bacterial count was made by agar plate count method. A thin smear of uterine fluid was prepared on a grease free glass slide. It was air dried and subjected to Giemsa staining as per routine staining technique and PMN cells are counted under microscope. Hundred cells are counted and expressed in percentage.

Blood samples were collected from the jugular vein prior to institution of therapy and in succeeding estrus after therapy. The collected blood was left for two hours and then centrifuged at 2000 rpm for 10 minutes at room temperature for harvesting serum which was was stored in a cryovial at –40°C until further analysis. The concentration of haptoglobin and serum amyloid A were measured by solid phase sandwich ELISA kit (LIFE DIAGNOSTIC, Inc.).Total serum protein (TSP) and albumin was measured by biuret method using standard kit crest bio systems (a division of Coral Clinical Systems, Goa, India) .Total globulin concentration was determined by subtracting albumin from total protein. The concentration of SGPT (ALT), SGOT (AST) was estimated by using standard kit prepared by crest bio systems. Data generated from the present experiment was analyzed statistically as per the method suggested by Snedecor and Chochran (1994).

RESULTS AND DISCUSSIONS

The predominant bacterial species isolated from the pretreatment cervical samples were E.Coli, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus and Micrococcus with a count (10^6 CFU/ml) of 6.20, 0.86, 0.52, 0.93, 3.20, 4.50 and 8.60respectively, whereas only Micrococcus and E. coli were found in post treatment samples with a count of 0.24 and 0.63.In control cows the predominant bacteria isolated were E. coli, Bacillus and Micrococcus with a count of 2.10, 1.30 and 1.15 respectively. This type of bacteria isolates observed in the present study were in alliance with the results obtained by Rao *et al.*, (2001).This significant decrease ($P<0.01$) in microbial load was due to the leucocyte migration into uterine lumen which have a phagocytic action on microorganisms.

The PMN cell count before and 24hrs after treatment and in succeeding estrus was 13.14 ± 1.35, 73.71 ± 3.59 and 71.71 ± 2.15 respectively where as in control cows this count was 9.57 ± 2.09. PMN cell count 24hrs. post treatment revealed a highly significant difference ($p< 0.01$) between pretreatment and control groups. But there is no significant difference at the succeeding estrus between pretreatment and control groups. Singh *et al.* (2003) also reported the same finding with regard to PMN cell count in endometritic cows after intrauterine infusion with oyster glycogen. This increase in neutrophil count in uterine sample may be due to the chemotactic action of oyster glycogen which causes migration of neutrophils in to the uterine lumen showing immunomodulatory effect.

No significant alteration was observed in total protein concentration between pre and post treated cows (7.24 ± 0.18 vs. 8.04 ± 0.35).In case of control cows the value was 8.30 ± 0.34.The present finding corroborates with the finding of Khan *et al.*, (2010) while Ahmed *et al.*, (2003) reported higher protein value in normal cyclic cows. The serum albumin value also didn't vary significantly among control, pre and post treatment (4.61±0.15, 4.23± 0.10 and 3.86±0.18).The serum globulin concentration between pre and post treatment group showed significant difference ($p<0.01$) while it didn't vary significantly between control and pretreatment. The serum globulin concentration was 3.00 ± 0.07, 4.19 ± 0.19 and 3.53 ± 0.19 for pretreatment, post treatment and control cows respectively. The present finding is in consistent with Pandey *et al.*, (2009).The significant increase($p<0.01$) in globulin concentration during the post treatment period might be due to local immune stimulation of drug which is reflected in systemic vascular circulation. The comparison of pre and post treatment albumin: globulin ratio values by test of significance showed a highly significant difference ($p<0.01$).The respective values for pretreatment, post treatment and control cows were 1.41±0.02, 0.92±0.03 and 1.37±0.03.

The serum concentration (U/L) of aspartate amino transferase for pretreated endometritis cows was much higher than post treatment (78.28 ± 2.66 vs. 47.98 ± 2.95)while for cyclic cows its concentration was (39.87±1.66).There was no significant alteration in ALT values in pre and post treatment (32.58±2.26 vs.28.34± 2.37).The present serum AST and ALT values were comparable to the finding of Sattler *et al.*, (2004).The elevation in serum AST concentration during endometritis might be due to damage to uterine tissue and increased plasma membrane permeability. The significant reduction of this enzyme in the serum after treatment might be attributed to their recovery following immunomodulation therapy.

The mean concentration of haptoglobin (µg/ml) in serum was 81.30±3.98, 24.57± 3.65 and22.39± 1.93 for pretreatment, post treatment and control groups respectively. There was a significant reduction ($p<0.01$) in the haptoglobin concentration after treatment which is nearly equal to the concentration of control animals. It is an acute phase protein which is primarily synthesized by hepatocytes of liver and helps in optimization and trapping of microorganisms and their products .The mean concentration of SAA pretreatment value was 35.45±1.56 whereas for post treatment the concentration was 13.97±1.62.Simillarly the concentration for control animal was 16.80±1.62.There was a significant difference ($p<0.01$) between the pre and post treatment whereas no significant difference between post treatment and control. SAA has the potential to induce cholesterol metabolism at inflammatory site by chemo toxic phagocytic cells. Both in SAA and HP, there was significant reduction in post treatment values indicating substantial remission of the infective process. Estimation of these values served not only a diagnostic aid during pre-infective stage but can be monitored during the course of recovery (Smith *et al.*, 1997).

The conception rate following inseminations in post treatment cows is sixty percent where as in control cows it is seventy percent. The present finding corroborate with the observation of Sarma *et al.*, (2010) whereas high conception have been reported by Singh *et al.*, (2003).

Hence, it could be concluded that oyster glycogen could be used as an effective immunomodulator andalternative therapy against antibiotics.

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