EFFECT OF UTERINE IMMUNOMODULATION ON SERUM AMYLOID - A CONCENTRATION AND CONCEPTION RATE IN CYCLIC NON BREEDING COWS

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Abstract: Twenty one repeat breeding cows were screened by white side test to eliminate endometritis and they were assigned to three treatment protocols with equal number of seven animals in each group. Cows of control group were administered with 50 ml of normal saline, cows in the group second received 20 ml of fresh colostrum and in group third 10 ml of non pathogenic E. coli. All treatments were given as intra uterine medication. Blood samples from each experimental animal were collected for evaluation of serum amyloid A concentration during pre- and post- treatment periods, consequent to various therapies as per standard procedures. In addition to this, the conception rate for each treatment regimen was evaluated for efficacy. The serum amyloid A values showed a receding trend in all the experimental groups with significant variation (p<0.01) was marked in the colostrum and non pathogenic E. coli treated group.

Key words: Serum amyloid, Cyclic non breeder

INTRODUCTION

Discovery of acute phase proteins in the blood is a major breakthrough in the history of clinical medicine. Rise of these protein molecules and their quantitative detection have significant application in different disease conditions including anomalies in reproduction [1]. These are considered as biomarkers which differentiate clinically between healthy and unhealthy irrespective of disease condition of any organ. The major acute phase proteins are C-reactive protein, haptoglobin (Hp) and serum amyloid A. Serum amyloid A (SAA) is a protein belongs to a family of lipo proteins and produced by liver and is particularly of paramount importance in bovines [2]. These proteins are secreted during acute phase reaction and mostly dedicated with immune factors of the body [3]. Besides this, it transports the cholesterol to liver for secretion of bile and recruitment of immune cells to inflammatory sites. There are many variants of SAA such as SAA1, SAA2 and SAA4. The SAA4 is normally present in serum but, other variants are produced by fibroblast and macrophages [4]. In cattle SAA and Hp are major acute phase proteins. So their variation in the blood has a major diagnostic value [1,5].

MATERIALS AND METHODS

Twenty one repeat breeding cows were selected after meticulous screening based on white side test according to Anilkumar and Devanathan [6] and Laing’s [7] criteria. They were divided into three equal groups and subjected to three treatment protocols. Cows in control group were administered
with 50 ml of normal saline, cows in the group second were received 20 ml of fresh colostrum and in group third 10 ml of non pathogenic *E. coli*. All were given as intra uterine medication. 10 ml of blood sample collected aseptically from the jugular furrow of each experimental animal on day 0, day 7, day 14 and day 21. Serum was harvested and the concentration of bovine serum amyloid A was estimated by using ELISA kit prepared by Uscn Life Science Inc. and expressed as microgram/ml. The microtiter plate of this kit was pre coated with an antibody specific to SAA. Samples were added to wells with a biotin conjugated antibody preparation specific for SAA. Seven wells for standard, one well for blank, and rest for samples were determined and 100µl each of dilutions were added into appropriate wells. The plate was sealed with plate sealer and incubated for 2 hours at 37°C. The liquid of each well were removed and 100µl of detection reagent A working solution was added to each well and incubated for 1 hour at 37°C. The plate was washed with wash buffer for 5 times. Then 100µl of detection reagent B working solution was added to each well and incubated at 37°C for 30 minutes. Repeated washing was done for total 5 times. 90µl of substrate solution was added to each well and again incubated for 20 minutes at 37°C. The liquid were turned blue by addition of substrate solution. 50µl of stop solution was added to each well which turned yellow colour in short time. Reading was taken by micro plate reader at 450 nm immediately.

**Conception Rate**

The conception rate was calculated following the forty-five days insemination.

\[
\text{Conception Rate} = \frac{\text{Number of Conception}}{\text{Number of inseminations}} \times 100
\]

**RESULTS**

Perusal of table 1 revealed the SAA values were 31.55 ± 1.99, 29.06 ± 1.71, 27.84 ± 1.47 and 26.07 ± 1.30 (µg/ml) respectively for 0, 7, 14 and 21 day of sampling with respect to intra uterine application of normal saline. Analysis of variance revealed no significant effect within group values. Concurrently, in colostrum treated cows, it recorded a value of 34.12 ± 1.57, 30.77 ± 1.30, 29.55 ± 1.24 and 27.65 ± 0.99 (µg/ml) respectively for same days of observation. Comparison of SAA values within the colostrum treated group revealed highly significant difference. Test of significance between days showed a highly significant difference (p<0.01) between 0 Vs 7, 0 Vs 14 and 0 Vs 21 day sampling. However, the 7th day value shows significantly (p<0.05) higher concentration of SAA than 21st day. SAA value recorded a highly significant difference within non pathogenic *E. coli* treated groups and it revealed a value of 32.49 ± 1.43, 29.82 ± 1.30, 27.74 ± 1.09 and 25.87 ± 1.01 (µg/ml) respectively for 0, 7, 14 and 21 day of collection. Comparison of sampling values on different days, revealed a highly significant difference (p<0.01) between 0 Vs 14, 0 Vs 21 and 7 Vs 21 day. Other interactions were not significant.

The cows subjected to normal saline treatment (control group) projected a first insemination conception rate of 42.86%, out of seven cows. Seven cows intrauterine treated with colostrum (second group) recorded a pregnancy rate of 57.14%. The nonpathogenic *E. coli* induced group (third group), three cows became pregnant (42.86%) out of seven inseminations (Table 2).

**DISCUSSION**

As a biomarker, serum Hp and SAA are considered to be superior to CRP in bovines [8] from the specialized group of acute phase proteins. Both Hp and SAA are very sensitive to inflammatory process of uterus, as they show significant variations in bovine metritis against normal cow. These proteins were significantly higher in cows even with subclinical endometritis [1]. It can also contribute to evaluate the degree of involution of the uterus and establishment of conception following post partum [9]. Levels of APPs are preferentially elevated during acute bacterial infections and less pronounced or even missed during viral infections [10]. This protein not only provides indication of inflammation, but also provides an insite to know the degree of recovery after medication [8]. However, this protein attains its peak value after 10-15 hours in the blood circulation [11]. The present value of SAA projected a normal range with corroborating the report of Ceciliani et al [1]. Concentration at different days of sampling showed a declining trend irrespective of drugs used. Further, it can be implied that the values did not escalate, as the experimental cows were suffering from subclinical endometritis.
Table 1: Mean and test of significance (F-test) of serum amyloid A concentration (µg/ml) in various experimental groups within days of sampling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Saline</th>
<th>Colostrum</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Amyloid A (µg/ml)</td>
<td>31.55 ± 1.99</td>
<td>29.06 ± 1.71</td>
<td>34.12 ± 1.57</td>
</tr>
<tr>
<td></td>
<td>27.84 ± 1.47</td>
<td>26.07 ± 1.30</td>
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<td>29.55 ± 1.24</td>
<td>27.65 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>27.65 ± 0.99</td>
<td>27.04 ± 1.09</td>
<td>25.87 ± 1.01</td>
</tr>
<tr>
<td>F-Value</td>
<td>1.95</td>
<td>4.43*</td>
<td>5.406**</td>
</tr>
</tbody>
</table>

Table 2: Conception rate in different protocols

<table>
<thead>
<tr>
<th>Test protocol</th>
<th>Group</th>
<th>Total number of insemination</th>
<th>Animals conceeded</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
<td>I</td>
<td>7</td>
<td>3</td>
<td>42.86 %</td>
</tr>
<tr>
<td>Colostrum</td>
<td>II</td>
<td>7</td>
<td>4</td>
<td>57.14 %</td>
</tr>
<tr>
<td>Non Pathogenic E. Coli</td>
<td>III</td>
<td>7</td>
<td>3</td>
<td>42.86 %</td>
</tr>
</tbody>
</table>
Perusal of table 2 revealed numerically higher conception rate in colostrum treated group followed by non pathogenic *E. coli* and normal saline. The higher conception in third group rate compared to other groups might be due to efficient microbial elimination by the presence of high immunoglobulin in the colostrum, which favored opsonisation of bacteria by PMN cells, mostly macrophages and leucocytes [12] recorded a 60 % conception rate following treatment with autologus plasma. The non pathogenic *E. coli* treated group showed a moderate conception rate. It can be presumed that, the conception rate with this therapy is lower than the application of *E. coli* LPS as it is a crude form, therefore the desired pregnancy rate is not achieved. Sharma et al. [12] reported a higher conception rate of 80% with *E. coli* LPS. The normal saline treated group recorded a similar conception rate with that of group third, suggesting its efficacy to dilute and clear bacterial contamination with restoration of normalcy in internal milieu of the uterus [13]. These immunomodulation offers timely uterine involution, resolution of the local uterine inflammatory process postpartum and resumption of ovulatory oestrous cycles and thus improves the overall conception rates [14].

The ultimate goal of our research is to develop biomarkers of early detection of uterine disease in particular subclinical endometritis which sometimes occurs due to different opportunistic bacteria [15]. Systemic detection of these biomarkers (e.g., in serum) would lead the way towards the development of diagnostics for uterine disease in cows. Identifying infection earlier prevents the reliance on the development of clinical symptoms, and is more likely to result in a favorable outcome in terms of fertility as well as cost. It could be concluded that the mode of the treatment with non conventional drugs, might act as an alternative choice in treating repeat breeders against routine use of antibiotics, which has got many limitations. The scope of widened by considering a larger population for validation. Use of biomarkers (serum amyloid A) could be beneficial for diagnosis and study of course of the disease. Analyses of SAA have gained more importance due to its application for diagnosis of various animal diseases like mastitis, metritis, pyometra and other systemic complications [16]. These indices could be very helpful for monitoring not only the course of infection but also stage of recovery.

REFERENCES