Short Communications

Effect of Certain Immunotherapeutics on Microbial Status of Endometritic Cows

Basanti Jena[1]*, D.N. Mohanty[1], D. Das[2] and S. Dash[1]

[1]*Department of Animal Reproduction, Gynaecology and Obstetrics, CVSc & AH, OUAT, Bhubaneswar, Odisha-751003, India*
[2]*Department of Pathology, CVSc & AH, OUAT, Bhubaneswar, Odisha-751003, India*

*Corresponding author

A B S T R A C T

The present investigation was carried out to study the effect of certain uterine immunomodulators on microbial status of endometritic cows. A total number of fifty endometritic cows selected after screening were allotted randomly into five equal groups (n=10). The Group I, II, III, IV Cows were intrauterinely administered with 100μg of *E. coli* LPS (single dose), 500 mg of oyster glycogen (OG) (single dose), 1000 mg Enrofloxacin (once daily for 3days) and 250×10^6 lymphocytes (single dose) each reconstituted with 30 milliliter of PBS. Ten cows suffering from endometritis as confirmed by alkaline pH of cervical mucus discharge and positive white side test along with ten no. of normal cyclic cows were also selected for the study. The mean

Introduction

Endometritis is termed as the inflammation of endometrium and underlying glandular tissue without any systemic signs, occurring at least 21 days after calving (Sheldon *et al.,* 2006). Clinical and subclinical endometritis resulting from postpartum infection of the uterus are common causes of infertility and subfertility in high producing dairy cattle by delaying the onset of ovarian cyclic activity after parturition, extending luteal phases, prolonging the parturition-first insemination and parturition - conception intervals and

reducing conception rates (Sheldon, 2009a and 2009b). Uterine infections develop upon the establishment of pathogenic microorganisms onto the mucosa, their colonisation and penetration of the epithelial layer and the production of toxins by these microorganisms (Sheldon *et al.,* 2006). Once absorbed from the uterine lumen, endotoxins released from the wall of gram negative bacteria pass into the peripheral blood circulation, and prevent the secretion of the Gonadotropin Releasing Hormone (GnRH) from the hypothalamus and the Luteinizing Hormone (LH) from the hypophysis. These

toxins also reduce the susceptibility of the hypophysis to the secretion of endocrine and exocrine GnRH (Sheldon *et al.,* 2004 and Mateus *et al.,* 2002a). Lipopolysaccharides (LPS) secreted by gram negative bacteria, inhibit the transcription of steroidogenic enzymes, including 17β-hydroxylase/17, 20-lyase and P_{450} aromatase, and thereby, inhibit follicular activity (Magata *et al.,* 2014). Uterine infections may cause the formation of chronic endometrial scars, the narrowing of the oviduct and the adherence of the bursa to the ovaries (at an approximate rate of 2%). The present report is attempted to evaluate the therapeutic effect of certain uterine immunomodulators on microbial environment of endometritic cows.

Materials and Methods

The present experiment was conducted in crossbred cows suffering from endometritis presented in the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar and cases attended at mobile health coverage scheme, OUAT at owner's residence in and around Bhubaneswar city. Endometritis in these cows was confirmed by alkaline pH and White side test of cervical mucus as described by Pateria and Rawal (1990). A total number of fifty endometritic cows selected after screening as per the technique laid down by Zemjanis (1970) were allotted randomly into five equal groups (n=10). The Group I, II, III, IV Cows were intrauterinely administered with 100μg of *E. coli* LPS (single dose), 500 mg of oyster glycogen (OG) (single dose), 1000 mg Enrofloxacin (once daily for 3days) and lymphocyte infusion each reconstituted with 30 milliliter of PBS. In Group V (n=10), cows suffering from endometritis were administered with 30 ml of normal saline through intra uterine route and considered as positive control animals. Group VI (n=10) constituted of normal cyclic animals at estrus

presented for artificial insemination (AI) without institution of any kind of therapy taken for comparative study and were considered as negative control. After proper restraining of animal, cervical mucus samples were collected as described by Steffan *et al.,* (1984). Each sample was subjected to bacteriological isolation and identification process and compared with the respective values in normal cyclic cows (n = 10). The bacterial load or the number of surviving bacterial Colony Forming Unit (CFU) in uterine sample was counted adopting "total plate count technique" as described by Malik (1967).

Results and Discussion

The bacterial load ($\times 10^4$CFU/ml) in estrual cervical mucus indicated a non-significant difference among group I (296.61 ± 20.26), II (305.92 ± 18.05), III (328.50 ± 26.67), IV (333.62 ± 25.01), and V (319.60 ± 22.11) prior to treatment. It is in accordance with findings of Deori (2002) who reported similar range of bacterial load in endometritic cases.

A significant decline (p < 0.01) was observed in post-treatment bacterial count in estrual cervical mucus of all groups. The bacterial count ($\times 10^4$CFU/ml) at subsequent estrus varied non-significantly among group I (0.47 ± 0.07), II (0.53 ± 0.12), III (4.15 ± 0.87) and VI (0.34 ± 0.06). Reduction in bacterial load in all the above groups varied significantly (p < 0.01) from that of group IV (159.33 ± 18.81) and V (182.78 ± 15.03). The percentage of reduction was highest in LPS and OG treated groups followed by antibiotic group. Similar reduction in bacterial load was also reported by Deori (2002) in cervical mucus and Singh *et al.,* (2001) in uterine secretions after LPS infusion in endometritic cows. Intrauterine infusion of LPS efficiently increases the influx of PMNs into uterine lumen (Klucinski *et al.,* 1990; Hussain and

Daniel, 1992). With increased neutrophil count, rate of phagocytosis has increased resulting in significant reduction in bacterial count. Further, the concentration of bacterial load of micrococcus was presumed to be a contamination. Bacterial elimination was highest in OG group justifying its better immunomodulatory effect to eliminate the bacterial infection (Sarma *et al.,* 2010). Biswal *et al.,* (2014) reported that bacterial elimination was highest in OG group justifying its effectiveness in eliminating the bacterial infection by the chemotactic action that increased the PMN cell migration to uterus. The decline in bacterial load after antibiotic treatment might be due to the fact that enrofloxacin worked effectively against the bacteria present in uterus. Reduction in bacterial load in control group may be attributed to natural defence mechanisms in uterus.

Prior to treatment all the estrual cervical mucus samples of endometritic cows selected for the study were positive for bacterial presence. In group I, *Escherichia coli*, *Staphylococcus spp.*, *Streptococcus spp.* and *Micrococcus spp.* were the predominant bacteria found prior to treatment. After treatment only one sample was positive for *E. coli*. In group II, *E. coli*, *Staphylococcus spp.*, *Bacillus spp.*, *Klebsiella spp.* were the predominant bacteria found prior to treatment. After treatment only one sample was positive for *Escherichia coli* and *Staphylococcus spp.* In group III, the predominant bacteria were *Escherichia coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Klebsiella spp., Proteus spp., Bacillus spp.* found prior to treatment. After treatment three samples were positive containing *E. coli, Staphylococcus spp., Proteus spp.* In group IV, *E. coli, Staphylococcus spp., Streptococcus spp., Bacillus spp.* and *Klebsiella spp.* were found prior to treatment. After treatment 80 per cent samples were found to be positive containing *E. coli, Staphylococcus spp., Streptococcus spp., Proteus spp., Klebsiella spp.* of bacteria. In group V, the predominant bacterial isolates were *E. coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Proteus spp.*, *Bacillus spp.*, *Klebsiella spp., Micrococcus spp.* and *Pseudomonas spp.* After treatment 80 per cent of samples were found positive containing *E. coli, Staphylococcus spp., Streptococcus spp., Proteus spp., Bacillus spp., Klebsiella spp., Micrococcus spp.* and *Pseudomonas spp.* of bacteria.

Intrauterine treatment with LPS and OG could not eliminate 10 % bacterial isolates in the present study. Similarly Singh *et al.,* (2000) also reported where LPS could not eliminate 25 % bacterial isolates in their study. Failure of treatment in such animals might be attributed to the special property of certain bacteria, which despite being engulfed by the polymorphonuclear cells, were not destroyed inside them (Tizard, 2012). According to Singh *et al.,* (2000), another reason for the failure of treatment could be that in some animals, chronic infections might have destroyed the endometrium and thereby resulting in poor stimulatory response. The impaired biological function of granulocytes and monocytes/macrophages in cows from the experimental group probably resulted in the reduced ability to directly eliminate the bacterial infection which is the predominant cause of subclinical endometritis (Kim *et al.,* 2005; Brodzki *et al.,* 2014).

The bacterial contamination of the postpartum uterus is a frequent finding which by itself does not disturb the anatomical and histological restoration of tubular genital tract. The improper balance between uterine infection and the intrauterine antimicrobial self-defense mechanism, however, often results in complications, such as puerperal endometritis, clinical endometritis, pyometra and subclinical endometritis. These are most

common forms of genital diseases in dairy cows, which may delay the complete regeneration of endometrium, and disrupt the resumption of cyclic ovarian function resulting in postponement of the first insemination (AI), increasing the number of services per conception, and thus prolonging the calving interval and decreasing the calving rate (Foldi *et al.,* 2006; Sheldon *et al.,* 2006).

The mean bacterial load of cervical mucus discharge at estrous decreased significantly in the endometritic cows after treatment with LPS and OG. *Escherichia coli* were the most common bacterial isolate followed by *Streptococcus spp.* and *Staphylococcus spp.* This study revealed that the endometritis in cattle is mainly caused by bacterial etiology which should be considered during selection of appropriate therapeutic measures.

Acknowledgements

References

Biswal SS, Das S, Balasubramanian S, Mohanty DN, Sethy K and Dasgupta M. 2014. Serum amyloid A and haptoglobin levels in crossbred cows with endometritis following different therapy, *Veterinary World,* 7(12): 1066-1070.

Brodzki P, Kostro K, Brodzki A and Lisieck U. 2014. Determination of selected parameters for non-specific and specific immunity in cows with subclinical endometritis, *Animal Reproduction Science,* 148: 109–114.

Deori S. 2002. Use of bacterial modulins in treatment of endometritis cows. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar (U.P.), India.

Foldi J, Kulcsar M, Pecsi A, Huyghe B, de Sa C, Lohuis JACM, Cox P and Huszenicza Gy. 2006. Bacterial complications of postpartum uterine involution in cattle, *Animal Reproduction Science,* 96: 265–281.

Hussain AM and Daniel RCW. 1992. Effect of intrauterine infusion of *E. coli* endotoxin in normal cows by experimental infection with *Streptococcus agalactiae, Theriogenology,* 37: 791-810.

Kim IH, Na KJ and Yang MP. 2005. Immune responses during the peripartum period in dairy cows with postpartum endometritis, *Journal of Reproduction and Development*, 51:757– 64.

Klucinski W, Targowski SP, Winnickg A and Mimik E. 1990. Immunological induction of endometritis model investigation in cows, *Journal of Veterinary Medicine,* 37:148-153.

Magata F, Horiuchi M, Echizenya R, Miura R, Chiba S, Matsui M, Miyamoto A, Kobayashi Y and Shimizu T. 2014. Lipopolysaccharide in ovarian follicular fluid influences the steroid production in large follicles of dairy cows, *Anim Reprod Sci,* 144: 6-13.

Malik BS. 1967. *Laboratory Manual for Bacteriology and Immunology*, 1st edn, British Book Depot, Lucknow.

Mateus L, Lopes da Costa L, Bernardo F and Silva JR. 2002a. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows, *Reproduction in Domestic Animals*, 37: 31–35.

Pateria AK and Rawal CVS. 1990. White side test for sub-clinical metritis in

buffaloes, *Indian Journal of Animal reproduction*, 11: 142-144.

Sarma OK, Singh B, Singh MP, Tiwary BK and Sinha MP. 2010. Efficacy of Immunomodulators for Treatment of Endometritis in Cows, *Indian Journal of Animal reproduction,* 31(2): 59-61.

Sheldon IM, Cronin J, Goetze L, Donofrio G and Schuberth HJ. 2009a. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle, *Biology of Reproduction*, 81(6): 1025–1032.

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. 2006. Defining post-partum uterine disease in cattle, *Theriogenology*, 65: 1516–1530.

Sheldon IM, Price SB, Cronin J, Gilbert RO and Gadsby JE. 2009b. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle, *Reproduction in Domestic Animal*, 44: 1–9.

Sheldon IM, Noakes DE, Rycroft A and Dobson H. 2004. Effect of intrauterine administration of estradiol on postpartum uterine bacterial infection in cattle, *Animal Reproduction Science*, 8:13-23.

Singh J, Dhaliwal GS, Nanda AS, Arora AK and Pangaonkar GR. 2001. Dynamics of uterine microflora in cows suffering from endometritis following intrauterine administration of *Escherichia coli* lipopolysaccharide, *Indian Journal of Animal Sciences,* 71: 1156–58.

Singh J, Sidhu SS, Dhaliwal GS, Pangaonkar GR, Nanda AS and Grewal AS. 2000. Effectiveness of lipopolysaccharide as an intrauterine immunomodulator in curing bacterial endometritis in repeat breeding cross-bred cows, Animal Reproduction Science, 59: 159–166.

Steffan J, Agric M, Adriamanga S and Thibier M. 1984. Treatment of metritis with antibiotics or PGF2α and influence of ovarian activity in dairy cows, *American Journal of Veterinary Research*, 45(6): 1090-1094.

Tizard I. 2012. *Veterinary Immunology*, 9th Edn. W.B. Saunders Company, Philadelphia.

Zemjanis R. 1970. Diagnostic and Therapeutic Technique in animal production, 2nd edn. The Williams and Wilkine Co., Baltimore.