EFFICACY OF INTRAUTERINE INFUSION OF IMMUNOMODULATORS AND $PGF_{2\alpha}$ IN THE TREATMENT OF ENDOMETRITIS IN BUFFALOES

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ABSTRACT

A total of 72 buffaloes suffering from endometritis were utilized for the study. The affected buffaloes 12 each were treated with PBS, Lipopolysaccharides (LPS), Lysozyme (LZ), Oyster glycogen (OG), Vit. E and Selenium (SE); and $PGF_{2\alpha}$ (PG) i/m. Higher percentage of buffaloes suffering from endometritis was recovered with LPS (83.33), LZ (75.00), OG (66.67) and $PGF_{2\alpha}$ (66.67) than the SE (50.00) and control PBS (16.67). The conception rates were 53.33, 41.67, 33.33, 50.00, 25.00 and 16.67 in buffaloes treated with LPS, LZ, OG, SE, PG and untreated (PBS) groups, respectively. The bacterial colony counts were 79.58 ± 8.10, 77.92 ± 5.15 , 89.67 ± 5.05, 73.67 ± 7.33, 83.50 ± 6.19 and 70.86 ± 5.14 before treatment in PBS, LPS, LZ, OG, SE and PG groups of buffaloes, respectively while the same after treatment were 126.83 ± 8.27; 6.67 ±1.39, 12.87 ± 3.35 ; 21.00 ± 5.93; 27.75 ± 4.10 and 24.75 ± 5.38, respectively. The progesterone (P_4) profiles were 1.07±0.24; 0.81±0.10; 1.07±0.18; 1.03 ± 0.19; 0.98 ± 0.14 and 1.09 ± 0.14 ng /ml before treatment and 1.13±0.22; 0.32 ± 0.03; 0.37 ± 0.05; 0.48 ± 0.07; 0.48 ± 0.08 and 0.40 ± 0.05 ng /ml after treatment in PBS control, LPS, LZ, OG, SE and PG groups, respectively.

Key words: Endometritis, Buffaloes, Immunomodulators, PGF_2 alpha

Reproductive health of buffaloes reflects on the productive part of the animal i.e. milk production. But little attention is being given to improve their reproductive capabilities. Among various reproductive disorders, uterine infection is a major problem. The endometritis influence fertility in two ways, first in the short term, it reduces fertility by extending the calving to conception interval and increasing the number of services per pregnancy and secondly in the long term, it can result in sterility due to irreversible changes of genital tract. Most of the present forms of therapy either local or systemic with antibiotics, sulfonamides and antiseptics have a limited success rate in combating uterine infection and may disturb the uterine defense mechanisms. Besides this, intrauterine treatments result in some absorption of the drug, adulteration of meat and milk and bacterial resistance that lead to other complications. Hence, the role of immune system in the control of uterine infections in farm animals is attracting attention of researchers in recent times. Further, specific modulations of early migration of polymorphonuclear cells into the uterus as well as selective modification of functions of polymorphonuclear cells are clinically advantageous. Conversely, unphysiological and ill-defined exogenous substances such as oyster glycogen, bacterial endotoxin and lysozymes can induce migration of leucocytes into the uterus. Hence, the present paper reports the efficacy of immunomodulators in the treatment of endometritis in buffaloes.

The estrual cervical mucus of samples were collected aseptically from repeat breeding buffaloes and were screened by white side test as described by Pateria and Rawal (1992).

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The positive reactors were selected for the study. Seventy two buffaloes affected with endometritis were selected raddomly and divided into six groups i.e. each group consisting 12 buffaloes. These were treated with 50 ml of phosphate buffer solution (PBS) alone as control, 100 mcg of LPS (*E. coli* serotype O26:B6; Sigma Chemical Co., P.O.Box 14508, St Louis, MO 63178; USA 314-771-5750) in 50 ml PBS, 2 mg of lysozyme in 50 ml PBS (RM 074 from chicken egg white, Lyophilized, HiMedia Laboratories Limited, Mumbai - 400 086, India), 1 percent of OG solution (RM 379 from oysters, (C6H10O5 n); HiMedia Laboratories Limited, Mumbai - 400 086, India), 3 mg of selenium and 100 mg of tocopherol (E-Care-Se®, Health Line Pvt Ltd. 24, C&D, Kumbalgod Ind Area, Mysore Road, Bangalore – 560 074) in 50 ml of PBS and 0.75 mg of tioprost (Iliren®, Intervet International B.V, Boxmeer, Holland, Each ml contains 0.150 mg tioprost). All therapeutic substances were infused intrauterine except $PGF_{2\alpha}$ which was given i/m. The immunomodulators were infused at the time of estrus and the $PGF_{2\alpha}$ was given on day 10 of estrous cycle.

Bacterial colony count: The cervical mucus was collected aseptically and bacterial colony count was recorded as per standard plate count before and after treatment (M091, Plate Count Agar®, Standard methods of agar, HiMedia Laboratories Limited, Mumbai - 400 086, India).

Progesterone (P_4) profiles: The serum P_4 profiles were estimated by EIA method before treatment and at subsequent estrus (Pathozyme progesterone®, M/S Omega diagnostics limited, Omega house, Carse bridge court, Whins road, Alloa, Scotland, UK.).The efficacy of treatment was estimated in terms of number of buffaloes recovered and conceived.

The bacterial colony counts before vs. after treatment were 79.58 ± 8.10 vs. 126.83 ± 8.27 in PBS control; 77.92 ± 5.15 vs. 6.67 ±1.39 in LPS group, 89.67 ± 5.05 vs. 12.87 ± 3.35 in LZ group; 73.67 ± 7.33 vs. 21.00 ± 5.93 in OG group; 83.50 ± 6.19 vs. 27.75 ± 4.10 in SE group and 70.86 ± 5.14 vs. 24.75 ± 5.38 in PG group, respectively. The bacterial colony counts were significantly increased in untreated buffaloes and vice versa in treatment received buffaloes. Agarwal *et al.* (2002) recorded the bacterial colony counts in cervical mucus of buffaloes. The higher P_4 concentration in endometritis affected buffaloes was found to create alkaline pH in the uterus which leads the uterine epithelium more permeable to bacteria (Irwin, 1986).

The progesterone (P_4) profiles before treatment were 1.07±0.24; 0.81±0.10; 1.07±0.18; 1.03 ± 0.19; 0.98 ± 0.14 and 1.09 ± 0.14 ng /ml and after treatment were 1.13±0.22; 0.32 ± 0.03; 0.37 ± 0.05; 0.48 ± 0.07; 0.48 ± 0.08 and 0.40 ± 0.05 ng /ml in PBS (control), LPS, LZ, OG, SE and PG groups, respectively. The P_4 levels were significantly ($p<0.01$) decreased to lower than the basal levels after treatment in buffaloes while the P_4 levels maintained above basal level in untreated PBS buffaloes. This might be due to the insufficient luteolysins in buffaloes affected with endometritis. Similar studies were also reported by Shalaby (1997).

The recovery vs. conception per cent in buffaloes affected with endometritis were 25.00 vs. 16.67; 83.33 vs.58.33; 75.00 vs. 41.67; 66.67 vs. 33.33; 50.00 vs. 25.00 and 66.67 vs. 50.00 respectively, in PBS control, LPS, LZ, OG, SE and PG groups of buffaloes.

Once the physical defense mechanisms were breached, the next line of defense in endometrium was innate immune system which includes polymorphonuclear cells (PMN), lymphocytes, monocytes and eosinophils (Frank *et al.,* 1992) which might be responsible for recovery of buffaloes. Singh *et al.*(2000) and Methai and Rajasundaram (2003) used the LPS to treat the endometritis in bovines with various success rates. However the LPS could bring cellular influx in the uterus due to the chemotactic activity (Hussain and Daniel, 1992). The LPS was noticed to act on progestenoids of serum to facilitate to decrease P_4 levels (Peter *et al.,* 1990). Bizulevichius and Lukauskas (1998) treated the endometritis in cows with lysozyme enzyme. The LZ itself is having bactericidal activity (Hussain and Daniel, 1992 and Haribabu, 2003).

The OG was used to treat the animals basing on its chemotactic property to bring cellular influx as reported by Subandrio and Noakes, (1997) and Singh *et al.* (2000). In the present study, the SE was given

locally i.e. intrauterine. The efficacy might be due to increased killing capacity of PMN cells within the uterus (Grosso, 1987) due to glutathione peroxidase (antioxidant) dependent enzyme. The recovery might be due to properties of SE and Vit.E.

In $PGF_{2\alpha}$ treatment, the clearance of bacteria from uterus might be due to luteolytic and myometrial affect. The P_4 block on uterine defense mechanism was relieved thereafter improved uterine defense mechanism (Gilbert, 1992).

However failures of the treatment to certain extent recorded in all treatment groups might be due to special properties of certain bacteria (Tizard, 1996) and the conception also depend on the extent of damage to endometrium (Singh *et al.*, 2000) in endometritis affected buffloes.

The study concluded that LPS and LZ can be used effectively in treating subclinical endometritis in bovines as well as to avoid antibiotic residues in meat and milk of the treated bovines.

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REFERENCES

Agarwal, P., Devendra Kumar. and Shiv Prasad. (2002). Therapeutic evaluation of certain drugs for treatment of endometritis in cross bred cows. Indian Vet. J.,**79**: 502-503.

Biziulevichius, G.A. and Lukauskas, K. (1998). In vivo studies on lysosubtilin 2. Efficacy for treatment of post partum endometritis in cows. Vet. Res. **29**: 47-58.

Frank, T., Anderson, K.L., Smith, A.R., Whitmore, H.L. and Gustafsson, B.K. (1992). Phagocytosis in the uterus: A review. Theriogenology., **20**: 103-110.

Gilbert, R.D. (1992). Bovine endometritis: the burden of proof. Cornell Vet. **82**: 11-14.

Grosso, P.J., (1987). Phagocytosis bactericidal activity and oxidative metabolism of mammary neutrophils from selenium adequate and selenium deficient dairy cows. M.S Thesis. The Pennsylvania University, Univpark.

Haribabu, Y. (2003). Veterinary Immunology-Basic concepts and application, 1st edn. Jaypee brothers, Medical Publisher, New Delhi, pp: 33-116.

Hussain, A.M. and Daniel, R.C.W., (1992). Effects of intra uterine infusion of *Escherichia coli* endotoxin in normal cows and in cows with endometritis induced by experimental infection with *streptococcus agalactia*. Theriogenology., **37**: 791-810.

Irwin, K.M.Liu. (1986). Immunoglobulin and neutrophil defense against uterine infection in mares resistant and susceptible to chronic endometritis: A review. J. Ame. Vet. Med. Assoc.: 700-703.

Methai, A. and Rajasundaram, R.C. (2003). Histopathological studies on uterine endometrium in cross bred cows with endometritis pre and post treatment with intrauterine lipopolysaccharides. XIX Annual convention and National symposium on current reproductive technologies for improvement of livestock production in India. 22-24 August, Kolkotta. pp: 54.

Pateria, A.K. and Rawal, C.V.S. (1992). White side test for sub clinical metritis in buffaloes. Indian J. Anim. Reprod.**11**: 142-144.

Peter, A.T., Bosu, W.T.K. and Gilbert, R. O. (1990). Absorption of *E.Coli* endotoxin (lipopolysaccharides) from the uteri of post partum dairy cows. Theriogenology., **33**: 1011-1014.

Shalaby, S.I.A. (1997). Some clinic pathological changes in buffaloes suffering from chronic endometritis. Egyptian J. Comp. Path. Clin. Path.**10**: 1-16.

Singh, J., Sidhu, S.S., Dhaliwal, G.S., Pangoankar, G.R., Nanda, A.S., Grewal, A.S. and Singh, J. (2000). Effectiveness of lipopolysaccharides as an intra uterine immunomodulator in curing bacterial endometritis in repeat breeding crossbred cows. Anim.Reprod. Sci.**59**: 159-166.

Subandrio, A.L. and Noakes, D.E. (1997). Neutrophil migration into the uterine lumen of the cow: the influence of endogenous and exogenous sex steroid hormones using two intra uterine chemo attractants. Theriogenology., **47**: 825-835.

Tizard, I.R. (1996) Hypersensitivity in Veterinary Immunology – An introduction. WB Saunders co pp: 343-401.