EFFICACY OF CERTAIN INTRAUTERINE IMMUNOMODULATORS IN MANAGEMENT OF ENDOMETRITIS IN POSTPARTUM GRADED MURRAH BUFFALOES

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Received: 15 November 2019
Accepted: 25 March 2022

ABATRACT

The present study is conducted to evaluate the efficacy of certain immunomodulators on recovery and conception rate in post partum endometritic buffaloes. Total 50 buffaloes were selected for the study and randomly divided into five groups. The buffaloes of Group 1 were given with a single dose of 100 µg *E. coli* lipopolysaccharide as intrauterine, Group 2 buffaloes were treated with a single dose of 2 mg Lysozyme, Group 3 was treated with 4 g of Cephalexin for three consecutive days. While, Group 4 buffaloes were treated with a single dose of 2 mg Lysozyme + 4 g of Cephalexin and Group 5 was treated with 30 ml of normal saline and was kept as control. All therapeutics were infused into the uterus after dissolving in sterile water. The efficacy of treatment was assessed by declines in bacterial load and PMNL cell count after treatment. The overall conception rate was 88.88, 71.42, 66.66, 75.00 and 33.33% in Group 1, 2, 3, 4 and Group 5, respectively.

Keywords: *Bubalus bubalis*, buffaloes, endometritis, immunomodulators, bacterial load, conception rate

INTRODUCTION

Endometritis is one of the main reproductive problem in dairy animals and it causes more economical losses in India. Predisposing factors that are associated with the development of endometritis includes retained placenta, dystocia, multiple births, abortion, etc. (Noakes *et al.*, 2001). During parturition, the physical barrier of the cervix, vagina and vulva is compromised in providing opportunity to bacteria to ascend the genital tract. Normal uterine defense mechanism is essential to prevent colonization of invading bacteria (Hussain, 1989). Recently, an alternative approach has emerged for the effective treatment of endometritis involving stimulation of uterine defense mechanism, through uterine immunomodulation by chemotaxis of PMNL cells to uterine lumen has been reported to play important role in the resolution of endometritis.

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MATERIALS AND METHODS

The cervical mucus is collected from all experimental animals at the time of estrus with sterile A.I sheath by recto-vaginal method. White side test was conducted as per the procedure described by Pateria and Rawal (1990). The percentage of bacterial load and PMNL cell was estimated before and after treatment, PMNL cell count also estimated after 24 h of immunomodulators infusion. Total 50 post partum buffaloes were selected for this study and randomly divided into five groups. Group 1, 2, 4 and Group 5 were treated with a single dose of 100 µg *E. coli* lipopolysaccharide, 2 mg Lysozyme, 2 mg Lysozyme + 4 g of Cephalexin (Lixen-I.U.) and normal saline, whereas Group 3 was treated with 4 g of Cephalexin as intrauterine for three consecutive days. Bacterial count in cervico vaginal mucus of all buffaloes in the experiment was performed by the pour plate technique (Diliello, 1979). The collection technique of uterine flushing was done by aspiration of fluid aseptically by a sterile two-way, 20 gauge Foley's catheter at 24 h of treatment as per the procedure described by Anderson *et. al.* (1985). The uterine flushing fluid was centrifuged at 1,000 rpm for 15 minutes, after discarding supernatant, cytological smears were prepared from sediment on clean glass slide. The smears were air dried, fixed with methanol for 2 minutes and stained with Giemsa stain for 25 to 30 minutes. Then the slide was washed with running water and air dried. The stained smears were observed under oil immersion at 100x magnification, 200 cells were counted for per cent of PMN cells. A.I was done in animals which were recovered and diagnosed the pregnancy by ultrasonography at 28th day of insemination.

RESULTS AND DISCUSSIONS

After treatment, 10, 30, 40, 20 and 70% of the cervical mucus samples were shown positive for white side test in Group 1, 2, 3, 4 and Group 5, respectively. The positive result of white side test could be explained on the basis of number of leucocytes and cellular debris present in the cervical mucus. The normal cervical mucus has less number of leucocytes, which doesn't cause any change of colour in white side test. Whereas, in clinical or sub clinical endometritis, the cervical mucus contains increased number of leucocytes which causes colour reaction to white side test (Deori, 2002).

Before treatment the mean bacterial load was almost similar in all groups of buffaloes. The overall mean bacterial colony counts of all the groups before treatment was observed as 77.12±0.50x10^{6}/ml. The mean bacterial load at subsequent estrus was found to be 4.20±0.67, 6.20±0.82, 18.20±2.85, 5.30±0.91 and 48.90±5.86 in Group 1, 2, 3, 4 and Group 5, respectively. A significant ($P<0.05$) reduction in bacterial loads of the entire treatment group of buffaloes were observed at subsequent estrus. In the control group also the bacterial load declined at subsequent estrus after infusion of normal saline, it might be due to natural uterine defense mechanism. The reduction in bacterial load after treatment might be due to an infiltration of neutrophils into uterine lumen after infusion of immunomodulators, which are responsible for the clearance of bacteria from the uterus. During the period of investigation, the polymorphonuclear cells were observed in uterine flushing at day 0, 24 h after treatment and subsequent estrus were 23.76±0.40, 55.58±2.40 and 8.26±0.46. The percent of PMNL cells observed at 0 day of treatment was 23.76±0.40. The mean

Table 1. Bacterial load and percent of PMNL cells in cervical mucus of therapeutic buffaloes.

Groups	Bacterial load (x10^6/ml)		Per cent of PMNL cells			Conception rate (%)
	Day 0	At subsequent estrus	Day 0	After 24 h of treatment	At subsequent estrus	
1	75.30±1.29	75.30±1.29	23.50±1.50^{a,y}	74.20±1.10^{a,x}	6.20±0.62^{b,z}	88.88
2	76.60±1.11	76.60±1.11	23.10±0.67^{a,y}	57.10±1.97^{c,x}	7.10±0.62^{b,z}	71.42
3	78.20±1.32	78.20±1.32	24.1++0±0.78^{a,y}	48.70±0.80^{d,x}	8.20±0.32^{b,z}	66.66
4	77.10±0.91	77.10±0.91	23.70±0.73^{a,y}	69.80±0.66^{b,x}	6.70±0.44^{b,z}	75.00
5	78.40±0.89	78.40±0.89	24.40±0.61^{a,y}	28.10±1.08^{e,x}	13.10±1.12^{a,z}	33.33

^{a,b,c} Means sharing different superscripts in the same row differ significantly (P<0.05).

^{x,y,z} Means sharing different superscripts in the same column differ significantly (P<0.05).

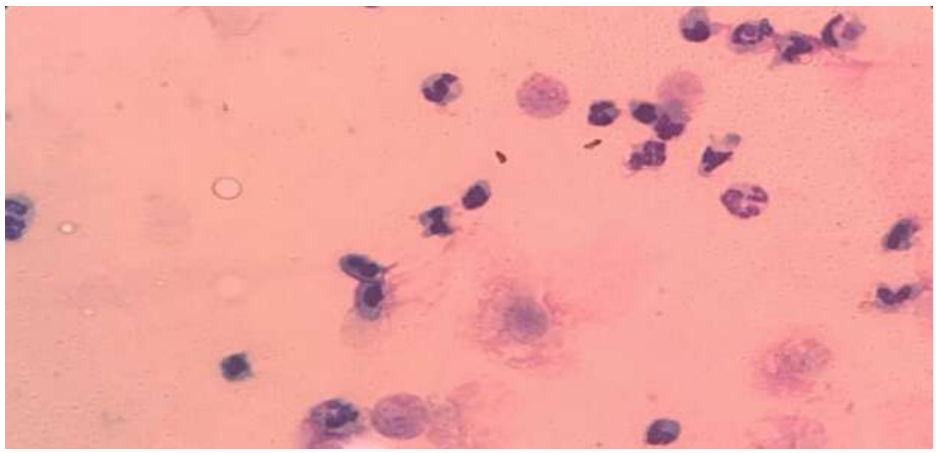

Figure 1. Neutrophils in uterine flushing after treatment with *E. coli* LPS (100X).

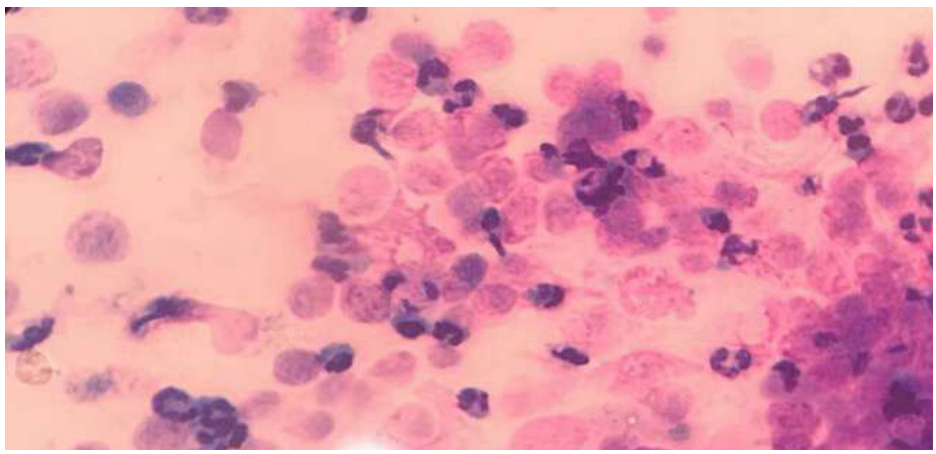

Figure 2. Neutrophils in uterine flushing after treatment with Lysozyme + Lixen-I.U. (100X).

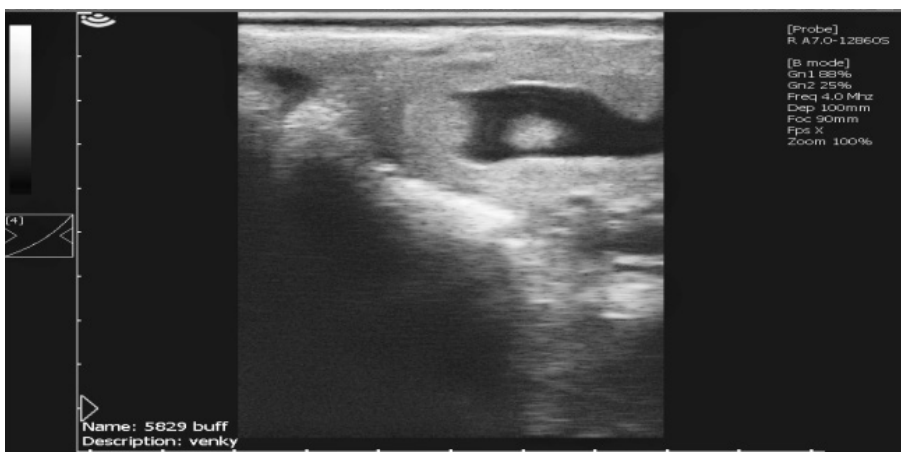

Figure 3. Ultrasonograpic images of gravid uterus at 28th day of pregnancy.

percentage of PMNL cells in uterine flushing of Group 1 (*E. coli* LPS) buffaloes were 23.50±1.50, 74.20±1.10 and 6.20±0.62 on day 0, 24 h after treatment and subsequent estrus, respectively. In Group 2 (lysozyme), the values were 23.10±0.67 and 57.10±1.97 and 7.10±0.62. Where as in Group 3 (Lixen-I.U.), the values were 24.10±0.78, 48.70±0.80 and 8.20±0.32, buffaloes of Group IV (Lysozyme + Lixen-I.U.), the values were 23.70±0.73 and 69.80±0.66 and 6.70±1.41 on day 0, at 24 h after treatment and subsequent estrus, respectively. There was a significant ($P<0.05$) increase in the percent of PMNL cells in the uterine flushing at 24 h after treatment and also at subsequent estrus the per cent of PMNL cells was declined significantly ($P<0.05$) in all therapeutic groups. The conception rate was assessed on the basis of pregnancy diagnosis by ultrasonography at day 29th of post insemination. The overall conception rate was 88.88, 71.42, 66.66, 75.00 and 33.33% in Group 1, 2, 3, 4 and Group 5, respectively.

CONCLUSION

The study was concluded that the immunomodulators can be used as alternative to antibiotics and antiseptics to treat endometritis in post partum buffaloes effectively.

REFERENCES

Anderson, K.L., N.A. Hemeida, A. Frank, H.L. Whitmore and B.K. Gustafsson. 1985. Collection and phagocytic evaluation of uterine neutrophilic leukocytes. *Theriogenology*, **24**(3): 305-317. DOI: 10.1016/0093-691x(85)90221-3

Denis-Robichaud, J. and J. Dubuc. 2015. Determination of optimal diagnostic criteria for purulent vaginal discharge and cytological endometritis in dairy cows. *J. Dairy Sci.*, **98**(10): 6848-6855. DOI: 10.3168/jds.2014-9120

Deori, S. 2002. *Use of bacterial modulins in treatment of endometritis cows*, M.V.Sc. Thesis, Indian Veterinary Research Institute, Uttar Pradesh, India.

Diliello, L.R. 1979. Antimicrobial chemical agents. *Clinical Microbiology*, Connecticut: AVI Publishing Company, Westport, USA. p. 120-125.

Galvao, K.N. and J.E.P. Santos. 2014. Recent advances in the immunology and uterine microbiology of healthy cows and cows that develop uterine disease. *Turk. J. Vet. Anim. Sci.*, **38**(6): 577-588. DOI: 10.3906/vet-1407-29

Hussain, A.M. 1989. Bovine uterine defense mechanisms: A review. *Zentralblatt fur Veterinarmedizin Reihe B.*, **36**(9): 641-651. DOI: 10.1111/j.1439-0450.1989.tb00657.x

Methai, A. and Rajasundram. 2002. Efficacy of Lipopolysaccharide and plasma in the treatment of endometritis in cows. *In Proceeding 18th Annual Convention and National Symposium of Indian Society for Study of Animal Reproduction*, Indian Veterinary Research Institute, Izatnagar, India.

Noakes, D.E., T.J. Parkinson, G.C.W. England and G.H. Arthur. 2001. *Arthurs Veterinary Reproduction and Obstetrics*, 8th ed. W.B. Suanders, Philadelphia, Pennsylvania, USA. 333p.

Pateria, A.K. and C.V.S. Rawal. 1990. White side test for subclinical metritis in buffaloes. *Indian J. Anim. Reprod.*, **11**: 142.