INTRAUTERINE PROTEOLYTIC ENZYME THERAPY FOR SUBCLINICAL ENDOMETRITIS IN DAIRY CATTLE

J. SINGH[1], M. HONPARKHE[2*], S.P.S. GHUMAN[3], A. KUMAR[4], S. DHINDSA[5] AND M. CHANDRA[6]

Department of Veterinary Gynaecology and Obstetrics
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004

Received: 02.05.2016 Accepted: 16.06.2016

ABSTRACT

Intrauterine (I/U) administration of proteolytic enzymes for treating subclinical endometritis was investigated in dairy cattle exhibiting subclinical endometritis that was diagnosed on the basis of apparently clear cervico-vaginal mucus and uterine cytobrush cytology (≥4% PMN cells). At spontaneous estrus, subclinical endometritic cattle were administered either single I/U infusion of Mastivexym ointment (trypsin 8 mg, chymotrypsin 8 mg, papain 4 mg, α-tocopherol acetate 120 mg and retinyl palmitate 58.83 mg; Group I, n=10) or single I/U infusion of crude formulation of aforesaid enzymes and vitamins in similar quantities dissolved in 10 ml distilled water (Group II, n=10). Ten subclinical endometritic cattle were kept as control (Group III). On day 12 after spontaneous estrus, all these animals were administered (i.m.) a $PGF_{2\alpha}$ analogue (Cloprostenol 500 μg). This followed administration (i.m.) of GnRH analogue (Buserelin acetate, 20 μg) at the start of induced estrus. About 12-14 h after GnRH analogue, cattle were artificially inseminated twice at 12 h interval. At spontaneous and induced estrus, all these cattle were subjected to cytobrush technique for assessing PMN%, and microbial assay of cytobrush for bacterial load. Following enzyme therapy at spontaneous estrus, a reduction ($p<0.05$) in PMN% and bacterial load was observed at subsequent induced estrus in treated animals. Also, first service pregnancy rate were higher ($p<0.05$) in treated groups compared to control counterparts (50 *vs.* 20%). It was concluded that intrauterine proteolytic enzymes could be used to improve pregnancy rate in subclinical endometritic dairy cattle.

Keywords: Cytobrush, Dairy cattle, Endometritis, Proteolytic enzymes, Uterine health

INTRODUCTION

Dairy cattle with 75% exotic blood have lower uterine immunity leading to higher incidence (18-25%) of endometritis (subclinical 29.4%, clinical 21.7%), a major cause of repeat breeding syndrome (Nanda and Singh, 2008 and Singh *et al.,* 2016). Alternative therapies for the treatment of endometritis remain in focus because anti-biotherapy tried in endometritic dairy animals (Singh *et al*., 2009) often require frequent administration and compulsory milk disposal. The use of proteolytic enzymes (chymotrypsin, trypsin and papain) was described as non-antibiotic therapy (Kruger *et al*., 1999). These enzymes not only have fibrinolytic and proteolytic activity but also support the cellular defense mechanisms and inhibit the growth of microorganisms (Drillich *et al*., 2005). Therefore, we hypothesized that intrauterine proteolytic therapy would enhance pregnancy rate in subclinical endometritic cattle.

MATERIALS AND METHODS

Repeat breeding crossbred cattle (n=170) at spontaneous estrus were examined for the status of genitalia (through rectal palpation) and cervico-vaginal mucus (CVM) i.e. clear, cloudy, thick and turbid. Cytobrush technique was used to detect endometrial cytology for the confirmation of type of endometritis on the basis of apparently clear CVM and ≥4% polymorphnuclear cells, PMN, in uterine cytobrush cytology and microbial assay (Singh *et al*., 2016). Thirty subclinical endometritic cattle were randomly divided in to three groups (n=10 each). At spontaneous estrus, group I cattle received intrauterine treatment with

[1]M.V.Sc. Scholar, [2]Assistant Gynaecologist, [4]Assistant Professor; [3]Professor cum Head, Department of Teaching Veterinary Clinical Complex; [5]Assistant Animal Scientist, Department of Animal Genetics and Breeding; [6]Assistant Scientist, Department of Veterinary Microbiology; *honparkhem@rediffmail.com

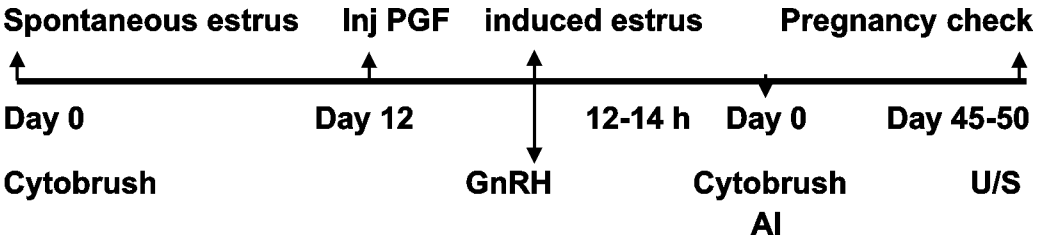

Figure 1: Experimental design for subclinical endometritic dairy cattle

Mastivexym ointment (Veyxpharma Gmbh, Germany; containing trypsin 8 mg, chymotrypsin 8 mg, papain 4 mg, α-tocopherol acetate 120 mg and retinyl palmitate 58.83 mg), whereas, group II was administered I/U with crude formulation of aforesaid enzymes and vitamins (Sigma-Aldrich, USA) in similar quantities dissolved in 10 ml distilled water. Group III cattle were not given any intrauterine therapy and were kept as control. On day 12 after spontaneous estrus, all the cattle were administered prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (cloprostenol 500 µg, i.m.). Estrus detection was done by visual signs viz. CVM discharge, cajoling and mounting behavior. At induced estrus, all the cattle were subjected to cytobrush technique and microbial assay. Also, at the start of induced estrus, in order to ensure ovulation all the cattle received GnRH analogue (Buserelin acetate 20 µg, i.m.). About 12-14 h after GnRH analogue administration, all the animals were artificially inseminated (AI) twice at 12 h interval (Figure 1). Pregnancy diagnosis in each cow was completed between day 45-50 post AI by ultrasonography. Statistical analysis was done with one-way ANOVA to find out differences between groups (SPSS 16.0). First service pregnancy rates were compared by chi square test.

RESULTS AND DISCUSSION

The bacterial contamination of uterus in postpartum dairy cattle is ubiquitous and the endometrium is the first line of defense against bacterial invaders (Galvao *et al.,* 2011). In case of endometritis, the pre- and post-treatment bacterial load is an important key to assess the level of infection and treatment efficacy (Dhaliwal *et al*., 2001). In the present study, the percentage of PMN cells and bacterial load at post-treatment induced estrus were reduced ($p<0.05$) in the proteolytic enzyme treated subclinical endometritic cattle compared to untreated controls (Table 1). The proteolytic enzymes viz. chymotrypsin, trypsin and papain are considered as biological scalpels and have fibrinolytic and proteolytic activity in the inflamed tissue resulting in breakdown of products of infection, damaged cells and tissues. The gram-positive and gram-negative bacteria, yeasts, and toxins contain proteins and lipids that are degraded directly by these enzymes leading to stasis in growth or death of

Table 1: Pre- (at spontaneous estrus) and post-proteolytic enzyme treatment (at induced estrus) %PMN cells and bacterial load in subclinical endometritic cattle (n=10 in each group)

Group	%PMN cells		Bacterial load (log_{10}CFU/ml)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I (T)	5.5±0.78^{a}	2.7±0.34^{b,A}	5.80±0.24^{a}	4.28±0.52^{b,A}
II (T)	4.6±0.22^{a}	2.3±0.26^{b,A}	5.76±0.17^{a}	4.55±0.54^{b,A}
III (C)	5.4±0.52	4.8±0.63^{B}	6.04±0.25^{a}	6.58±0.31^{B}

$^{a\ vs.\ b}p<0.05$ within a row for a parameter; $^{A\ vs.\ B}p<0.05$ within a column for a parameter; T - Treatment, C - Control

Table 2: Pregnancy rates in subclinical endometritic cattle subjected to proteolytic enzyme treatment

Group	First service pregnancy rate (%)	Cattle pregnant after 2nd/3rd AI	Total pregnant cattle (%)
I (T)	5/10 (50%)^{A}	1	6 (60%)^{A}
II (T)	5/10 (50%)^{A}	3	8 (80%)^{A}
III (C)	2/10 (20%)^{B}	0	2 (20%)^{B}

$^{A\ vs.\ B}p<0.05$; T - Treatment, C - Control

bacterium (Kruger *et al.,* 1999). The pregnancy rate in subclinical endometritic cattle treated with proteolytic enzymes was better compared to controls (70 *vs.* 20%, Table 2), as reported in an earlier study in buffalo using Mastivexym ointment (Honparkhe *et al*., 2014). Pregnancy rate following proteolytic enzyme therapy also improved in chronic endometritic cases (Drillich *et al*., 2005). The outcomes of this study supported our hypothesis that intrauterine proteolytic therapy would enhance pregnancy rate in subclinical endometritic cattle. Therefore, intrauterine proteolytic enzymes can be used as an alternative to the antibiotherapy in cattle suffering from subclinical endometritis.

ACKNOWLEDGEMENTS

The authors are thankful to University Grant Commission, New Delhi for providing funds under major research project. Authors are also highly thankful to Veyx-Pharma GmbH, Germany for providing Mastivexym.

REFERENCES

Dhaliwal, G.S., Murray, R.D. and Waldehiwet, Z. (2001). Some aspects of immunology of bovine uterus related to treatment for endometritis. *Anim. Reprod. Sci.,* **67**: 135-152.

Drillich, M., Raab, D., Miriam, W. and Heuwieser, W. (2005). Treatment of chronic endometritis in dairy cattle with an intrauterine application of enzymes; A field trial. *Theriogenology,* **63**: 1811-1823.

Galvao, K.N., Santos, N.R., Galvao, J.S. and Gilbert, R.O. (2011). Association between endometritis and endometrial cytokine expression in postpartum Holstein cattle. *Theriogenology*, **76**: 290-299.

Honparkhe, M., Ghuman, S.P.S., Singh, J., Dhindsa, S.S., Kumar, A., Chandra, M. and Brar, P.S. (2014). Diagnosing subclinical endometritis through uterine cytobrush cytology and its treatment with proteolytic enzymes in buffaloes. In: *XXIX Annual Convention and National Symposium of ISSAR at Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra*, Jan. 8-10, RFF 055, pp 147.

Kruger, M., ThiHien, T., Zaremba, W. and Penka, L. (1999). Untersuchungenzumeinfluss der proteolytischen enzyme trypsin, chymotrypsin und papain auf euterpathogenemikroorganismen. *Tiera¨rztlPrax,* **27**: 207–215.

Nanda, A.S. and Singh, J. (2008). Factor responsible for increased calving intervals in cross breed cow in India. In: *Proceeding of workshop on factors affecting reproductive performance in the cow at XXV jubilee World Buiatrics Congress (WBC*) at Budapest, Hungary, July 6-11, pp. 100-107.

Singh, J., Honparkhe, M., Chandra, M., Kumar, A., Ghuman, S.P.S. and Dhindsa, S.S. (2016). Diagnostic efficacy of uterine cytobrush technique for subclinical endometritis in crossbred dairy cattle. *Indian Vet. J.,* **93**(02): 11–13.

Singh, J., Ghuman, S.P.S., Dadarwal, D., Honparkhe, M. and Singh, N. (2009). Evaluation of systemic antibiotic treatment of chronic endometritis in buffaloes. *INTAS Polivet*, **10**(1): 23-26.