

## EVALUATION AND MODULATION OF POSTPARTUM UTERINE HEALTH USING PROTEOLYTIC ENZYMES IN CROSSBRED DAIRY COWS

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Received: 14.06.2018; Accepted: 04.04.2019

### SUMMARY

The present study was conducted in 24 postpartum (pp) Holstein Friesian crossbred dairy cows. Clinical endometritis was detected using vaginal mucus scoring (VMS) done at 30±2 Days in milk (DIM). Cows with VMS=0 served as healthy control (HC, n=8) while with a VMS ≥ 1 were randomly allocated either untreated control (UTC; n=8 cows) group or proteolytic enzyme (PE; n=8 cows) group. PE cows received intrauterine infusion of proteolytic enzyme mixture viz. Trypsin-8 mg, Chymotrypsin-8 mg and Papain-4 mg in 20 ml Distilled Water as single dose while HC and UTC cows were kept untreated. Mean days to 1st pp estrus was <65 in PE cows while it was >65 in HC and UTC cows at 30±2 DIM. At 1st pp estrus, a clear and transparent Cervico-vaginal mucus (CVM) was observed in 100% PE, 66.67% HC and 37.5% UTC with recovery of 62.5, 33.33 and 0% in PE, HC and UTC cows, respectively. Mean pH of CVM in PE cows was significantly ( $p < 0.05$ ) lower than in HC and UTC cows ( $7.56 \pm 0.28$  vs.  $7.75 \pm 0.63$  and  $7.88 \pm 0.79$ , respectively). Higher overall conception rate with lesser no. of artificial inseminations (AIs) per conception and shortened calving to conception interval were observed in PE than HC and UTC cows. Results indicated that intrauterine infusion of proteolytic enzymes at 30±2 DIM enhance early commencement of postpartum estrus with estrual CVM characters indicative of improved uterine health and better fertility in crossbred dairy cows.

**Key words:** Cervico-vaginal mucus, Cow, Endometritis, Postpartum, Proteolytic enzymes

Postpartum endometritis affects the endometrial lining of the uterus leading to microbial growth, decreased fertility and increased calving to conception interval. The character score of vaginal contents at 21-28 DIM and thereafter correlates with the presence of the main pathogenic organisms associated with uterine disease (Williams *et al.*, 2005). Some previous studies recommended that cows with mild clinical endometritis should not be treated as there is a spontaneous recovery without any ill effect on reproductive performance later on (Paisley *et al.*, 1986; Knutti *et al.*, 2000; LeBlanc *et al.*, 2002). Indiscriminate use of antibiotics for the treatment of clinical endometritis in dairy cows leads to development of antibiotic resistance and requires compulsory milk disposal which is hardly practiced. Modulation of uterine immunity in crossbred cows with microbial endometritis has been proposed as an alternate to conventional treatment with antibiotics or antiseptics (Nanda and Singh, 2008). Proteolytic enzymes have been used intrauterine in the form of mastivexym ointment in endometritic repeat breeder cows (Honparkhe *et al.*, 2005) and buffaloes (Honparkhe *et al.*, 2014) with high cure and pregnancy rates. Studies related to the effect of these proteolytic enzymes during early post-partum period in cattle are scarce. Thus, the present study aimed to evaluate the effect of intrauterine infusion of proteolytic enzymes on resumption of postpartum estrus and estrual CVM characteristics indicative of uterine health and fertility in cross-bred dairy cows.

A total of 24 postpartum pluriparous (2-4 parity) Holstein Friesian crossbred dairy cows with the history of normal calving were selected. Genitalia of each cow were palpated per rectally at four weeks postpartum to rule out abnormality, if any. Clinical endometritis was detected on the basis of vaginal mucus scoring done at 30±2 DIM. For this, a clean, lubricated, gloved hand was inserted through the vulva of each cow and vaginal mucus was scooped out and scored for character as per Williams *et al.* (2005). Eight cows with a VMS=0 served as healthy control (HC) while cows with VMS ≥ 1 were randomly allocated either untreated control (UTC; n=8 cows) group or proteolytic enzyme (PE; n=8 cows) group. Control group cows were not given any treatment while PE group cows received intrauterine infusion of Proteolytic enzyme mixture viz. Trypsin-8 mg, Chymotrypsin-8 mg and Papain-4 mg in 20 ml DW as single dose at 30±2 DIM.

At the 1st post-partum estrus, cervico-vaginal mucus (CVM) was collected from each cow using sterile catheter as per method described by Dabas and Maurya (1988). A narrow range pH paper was used to note the pH of CVM. For assessment of recovery, CVM was subjected to white side test using 5% NaOH and colour reaction, if any was noted (Raja *et al.*, 2012). Reproductive parameters viz. days to 1st estrus, days to 1st service, first service conception rate (FSCR), overall conception rate (OCR), services per conception and calving to conception interval (CCI) were recorded. Statistical analysis was done using SPSS 16.0 software using independent t-test.

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There had been a consistent controversy regarding whether mild endometritis should be treated (Knutti *et al.*, 2000; LeBlanc *et al.*, 2002) and a previous study in postpartum cows (Drillich *et al.*, 2005) had failed to clarify it due to absence of untreated control group in the study. Thus, both healthy control (VMS=0) and untreated control (VMS $\geq$ 1) groups were included in the present study for a better comparison and understanding.

All cows (100%) of HC and an equal proportion of cows (87.5%) of both PE and UTC groups exhibited estrus within 90 DIM but mean days to 1st postpartum estrus were lesser ( $p>0.05$ ) in PE than HC and UTC cows i.e.  $57.62\pm11.18$  vs.  $66.38\pm4.70$  and  $65.50\pm6.39$ , respectively. At 1st post-partum estrus, a clear and transparent CVM was observed in 100%, 66.67% and 37.5% cows of PE, HC and UTC groups, respectively. A pH of  $\leq 8.0$  was recorded in CVM of 100% of PE, 50% of HC and 37.5% of UTC cows. The CVM of PE cows showed a significantly ( $P<0.05$ ) lower mean pH than HC and UTC cows i.e.  $7.56\pm0.28$  vs.  $7.78\pm0.94$  and  $7.88\pm0.79$ , respectively. A more alkaline pH of CVM in UTC and HC is indicative of uterine infection and is known to be associated with the metabolites of bacteria and inflammatory exudates (Salphale *et al.*, 1993). Based on a positive colour reaction of CVM of 1st postpartum estrus to white side test, 37.5% of PE, 66.67% of HC and 100% of UTC cows were endometritic which could be possibly explained on basis of number of leukocytes present in uterine discharge (Popov 1969) which is indicative of infection. The pH and WST indicated better uterine health in PE cows as compared to controls.

In the present study, days required for 1st service in PE cows ( $68.25\pm7.81$ ) were significantly ( $p<0.05$ ) lesser than UTC cows ( $87.88\pm5.26$ ) and numerically lesser than

HC cows ( $71.88\pm8.43$ ). Lower first service conception rates but higher overall conception rates were obtained in PE (12.5%; 40%) than HC (42.86%; 35.29%) and UTC (37.5%; 35%) cows. In a recent study on subclinical endometritis in 30 cows (Singh *et al.*, 2017), intrauterine infusion of proteolytic enzyme and vitamin mixture both in the form of Mastivexym ointment as well as in crude form resulted in successful treatment of subclinical endometritis in cows and yielded significantly ( $p<0.05$ ) higher first service pregnancy rates (50% in both groups) and overall pregnancy rates (60 and 80%, respectively) as compared to untreated control cows (both FSCR and OPR of 20%). A previous study (Honparkhe *et al.*, 2014) recorded similar pregnancy rates in proteolytic enzyme treated (60%) and control repeat breeder cows (20%). In our study, lesser number of services/AIs per conception with a shorter CCI was recorded in PE (2.5;  $120.86\pm11.78$ ) than HC (3.0;  $134.29\pm19.75$ ) and UTC (3.17;  $144.12\pm14.59$ ) cows which are indicative of higher fertility in PE cows after treatment with proteolytic enzyme mixture. On the other hand, Drillich *et al.* (2005) recorded better reproductive performance in healthy (VMS=0) cows than the endometritic (VMS  $\geq 2$ ) cows treated with proteolytic enzyme mixture but comparison with UTC was not reported in that study. CVM characteristics at 1st postpartum estrus and reproductive parameters recorded in cows administered PE mixture at  $30\pm2$  DIM as compared to HC and UTC are presented in table 1.

Improvement of uterine health after intrauterine infusion of proteolytic enzyme mixture in present study could be attributed to the fibrinolytic and proteolytic activity of these enzymes in inflamed tissue which is supposed to support cellular defense mechanism responsible for inhibition of microbial growth and thus have been suggested as an alternative to antibiotics in

**Table 1**  
**CVM characteristics at 1st postpartum estrus and reproductive parameters in cows administered PE mixture intrauterine at  $30\pm2$  DIM as compared to HC and UTC cows**

Parameter	HC (n=8)	UTC (n=8)	PE (n=8)
Days to 1st estrus	$69.25\pm3.41^a$	$65.50\pm4.37^a$	$57.63\pm7.64^a$
Cows in estrus within 90 DIM (%)	100	87.5	87.5
Cows with transparent CVM at 1stpost-partum estrus (%)	66.67	37.5	100
Cows with CVM pH of $\leq 8.0$ (%)	50	37.5	100
White Side Test negative cows at 1stpost-partum estrus (%)	33.33	0	62.5
Mean pH of CVM at 1stpost-partum estrus	$7.78\pm0.94^a$	$7.88\pm0.79^a$	$7.56\pm0.28^b$
Days to 1st service	$71.88\pm8.43^{ab}$	$87.88\pm5.26^a$	$68.25\pm7.81^b$
FSCR (%)	42.86	37.5	12.5
Overall CR (OCR, %)	35.29	35	40
Services per conception	3.0	3.17	2.50
CCI (days)	$134.29\pm19.75^a$	$144.12\pm14.59^a$	$120.86\pm11.78^a$

Values bearing different superscripts (a, b) within a row differ significantly ( $P<0.05$ )

subclinical endometritic cows (Singh *et al.*, 2017). Cross-breeding in India has resulted in increased milk production but has reduced immunity of crossbred generations than the natives leading to increased incidence of endometritis (Nanda and Singh, 2008). This might be the reason of failure of control cows to show spontaneous recovery and the cows having VMS=0 at 30±2 DIM becoming endometritic later on in the present study. As cows treated with proteolytic enzymes at 30±2 DIM achieved better uterine health and fertility traits than that of HC and UTC cows, proteolytic enzymes seem to have therapeutic as well as preventive property against endometritis.

To the best of our knowledge, this paper reports the use of proteolytic enzymes for uterine immunomodulation in postpartum crossbred cows in India for the first time.

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