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Intrauterine Proteolytic Enzyme Therapy Ameliorates Cervical Mucus Characteristics and Fertility in Buffaloes with Clinical Endometritis

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ABSTRACT

The present study was carried out to evaluate the efficacy of proteolytic enzymes on recovery in terms of cervical mucus characteristics and first service conception rates in postpartum endometritic buffaloes. A total of 30 pluriparous buffaloes with signs of clinical endometritis were randomly divided into three groups of ten animals each. The buffaloes of Group I were administered intra uterine with a single dose of proteolytic enzymes, Group II treated with ceftiofur sodium intra muscularly and Group III animals received no treatment. The cervical mucus was collected from the buffaloes of all groups before and after treatment during estrus for assessment of cervical mucus characteristics. After treatment, the mucus score of discharge was found to be clear in 90, 70 and 0 per cent in Group I, II and III buffaloes, respectively. The mean spinnbarkeit values in Group I, II and III before the treatment were 7.24±0.79, 7.23±0.82 and 7.09±0.89 cm, and after the treatment were 15.79±0.64, 12.90±0.71 and 7.33±0.90 cm respectively. The mean pH values of Groups I, II and III before the treatment were 8.22±0.08, 8.12±0.10 and 8.13±0.07, and after treatment, 7.10±0.03, 7.43±0.14 and 8.01±0.09, respectively. The difference in the mean spinnbarkeit values and pH were significant ($p<0.05$) in Group I and II in pre and post-treatment and between groups. The first service conception rate was higherin Group I (44.44%) followed by Group II (42.85%). It can be concluded that intrauterine infusion of proteolytic enzymes may improve cervical mucus properties and fertility in buffaloes with clinical endometritis.

Key words: Clinical endometritis, Mucus score, Proteolytic enzymes, Spinnbarkeit

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INTRODUCTION

Endometritis one of the major causes of infertility in bovines, characterised by the presence of purulent or foul discharge, or cervical diameter >7.5 cm between 20 and 33 DIM, or mucopurulent discharge after 26 DIM, when cows are examined between 20 and 33 DIM, and vaginoscopy is performed (LeBlanc *et al.,* 2002). Shelden *et al.* (2006) defined clinical endometritis as purulent (>50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days postpartum. Optimization of insemination success rate depends not on the suppression of inflammation but on its fine regulation. As the use of antibiotics and hormones in food producing animals is under critical discussion, finding novel therapeutic approaches have generated some attention, such as proteolytic enzymes. The proteolytic enzymes *viz* trypsin, chymotrypsin and papain not only have fibrinolytic and proteolytic activity but also supports cellular defence mechanisms (Singh *et al.,* 2017). These enzymes also have hydrolytic properties and cause breakdown of necrotized tissue and debris (Drillich *et al.,* 2005). Enzymes inhibited growth, behaviour and survival of micro-organisms in vitro e.g., Staphylococcus spp, streptococcus spp, E. coli (Kruger et al, 1999). The present investigation was carried out to study the efficacy of proteolytic enzymes in terms of cervical mucus characteristics and fertility in buffaloes with clinical endometritis.

MATERIALS AND METHODS

The present investigation was conducted in buffaloes suffering from clinical endometritis presented to the Department of Veterinary Clinical Complex, College of Veterinary Science, Proddatur and selected nearby Veterinary Dispensaries.

A total of thirty buffaloes, calved atleast once and between 90-180 days in postpartum examined by inspection and palpation of genital tract per rectum during estrum and were selected based on the presence of purulent or mucopurulent discharges from vagina. The cervical mucus was collected from all the buffaloes and examined for suppuration and colour reaction to white side test before selection.

The cervical mucus of buffaloes was collected by using sterile blue sheath fitted to universal artificial insemination gun (IMV Technologies India Pvt. Ltd.). The gun was placed either in the mid cervix or uterus, and mucus was sucked into blue sheath. The aspirated cervical mucus was transferred into a sterile tube aseptically for further examination.

The experimental buffaloes were randomly allocated into three equal groups of ten animals each. The Group I buffaloes with endometritis were administered intrauterine infusion with a single dose of proteolytic enzymes in combination of trypsin 16mg (HIMEDIA® Laboratories Pvt. Ltd. Indra Nagar, Bareilly, Uttar Pradesh), chymotrypsin 16mg (Sisco Research Laboratories Pvt. Ltd. Andheri (E), Mumbai-400 099, Maharashtra, India) and papain 8mg (Loba Chemie Pvt. Ltd. Mumbai, Maharashtra, India) as used by Drillich *et al.* (2005). The measured doses of proteolytic enzymes were taken in a single sterile centrifuge tube and diluted with 20 ml of phosphate buffer saline (PBS) having a pH of 7.4. The solution was infused into the uterus during estrum with the help of artificial insemination gun barrel and sterile sheath. Whereas, Group II buffaloes were administered with broad-spectrum antibiotic ceftiofur sodium (Xyrofur®, INTAS Pharmaceuticals Ltd., Matoda-382 210, Ahmedabad, Gujarat, India) @2.2mg/kg body weight, SID, intramuscularly for three consecutive days from the day of estrus and Group III buffaloes were recommended for sexual rest for one cycle without any treatment. The physical characteristics of cervical mucus *viz* consistency (Verma *et al.,* 2014), mucus score (Fig. 1) (Sheldon and Dobson, 2004), spinnbarkeit value (Verma *et al.,* 2014), were evaluated in subsequent estrus and first service conception rates were evaluated sixty days post AI. The pH of cervical mucus sample at pre and post treatment was measured by using a digital pH meter (HANNA Digital pH Meter 209). The data generated was analysed statistically according to the method suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Before treatment, consistency of cervical mucus in Group I was moderate viscous in 40 and thick in 60 per cent buffaloes. In Group II and III, 30 and 70 per cent and 60 and 40 per cent buffaloes expelled moderate and thick consistency of cervical mucus, respectively. Higher recovery rates in terms of consistency were seen in Group I buffaloes during subsequent estrus with thin cervical mucus consistency (80%) followed by Group II (60%) and Group III (20%). This might be due to action of enzymes as biological scalpels that results in breakdown of products of inflammation (Singh *et al.,* 2017) and anti-bacterial activity of plant protease papain (Silva-López and Gonçalves, 2019).

Highest recovery percentage in terms of cervical mucus score observed in Group I compared with Group II and Group III (Table 1). This might be due proteolytic and

hydrolytic properties of enzymes resulting in breakdown of necrotized tissue and debris (Kruger *et al.,* 1999) eventually reducing inflammation of uterine epithelium. The recovery rate in Group II was higher compared to controls might be due to broad efficacy of ceftiofur sodium on both gram-positive and gram-negative bacteria (Chenault *et al.,* 2004). The systemic administration might result in minimum inhibitory concentration for pathogens associated with uterine disorders (Drillich *et al.,* 2006). Present study results were in accordance with the findings of Kaufman *et al.* (2010) who reported clear mucus in 74.2% endometritic cows after treatment with ceftiofur sodium (@1 mg/kg body weight, IM). Sharma and Srivastava (2018) reported 100% clear cervico-vaginal discharge in endometritic buffaloes after treating with ceftiofur sodium (@500mg, IU).

Group I animals treated with proteolytic enzymes exhibited highest significant spinnbarkeit value compared with Group II and III animals (Table 2). Zakiuddin *et al.* (2022) reported mean spinnbarkeit value as 6.75±0.81cm in repeat breeding buffaloes with uterine infection. The variation in spinnbarkeit value might be due to difference in properties of cervical mucus and time of collection of samples (Sharma *et al.,* 2008). The difference in the mean spinnbarkeit values were significant ($p<0.05$) in Group I and Group II in pre and post-treatment and no significant difference was observed in Group III. Whereas between groups, a significant difference ($p<0.05$) was observed after treatment among all three groups.

In Group I, a non-significant recovery in terms of pH when compared to Group II and significant difference with Group III was observed. There was a significant ($p<0.05$) difference of pH observed in Group I and II in pre and post-treatment (Table 2). A higher significant recovery in terms of pH in Group I might be due to hydrolytic and proteolytic actions of proteolytic enzymes. This would clear the debris and bacteria leading to decrease the pH from alkaline side. The increased contractility of the uterine smooth muscles due to action of chymotrypsin (Gulia *et al.,* 2022) might dislodge the bacteria from endometrial epithelium. The papain promotes healing by breaking down cell fragments and necrotic tissue. It lowers the pH, which stimulates the synthesis of cytokines that aid in cell repair and decreasing the growth of microorganisms and inflammatory reactions (Silva-López and Gonçalves., 2019). The reduction in the pH is a sign of homeostasis. pH drops towards neutral side once infection from the uterus is eliminated (Markusfeld *et al.,* 1984). The findings in present study were in accordance with Kumar *et al.* (2013) and Venkatesh *et al.* (2021) who also reported similar pH values of cervical mucus in buffaloes with endometritis before treatment. In contrast, Kumar *et al.* (2004) and Kadam *et al.* (2019) reported pH values higher than present study in buffaloes with uterine inflammation. However, findings of Sharma and Srivastava (2018) revealed less pH values (<8) than present study before treatment in buffaloes with endometritis. These variations in different studies might be due to difference in severity or degree of endometritis and the type of pathogens involved. In contrast, lower pH value (6.93±0.03) compared to this study after treatment with intrauterine ceftiofur sodium was reported by Sharma and Srivastava (2018). This might be due to route of administration of the drug, severity and type of infection. In normal healthy buffaloes, the pH value of cervical mucus at the time of oestrus is slightly alkaline and near to neutral. The pH of cervical mucus indicates the status of the uterine environment existing at the time of oestrus and fertilization. Acidic or excessive alkaline pH reduces the sperm motility, thereby causing failure of fertilization. The pH value more than 8.5 is reported to be associated with infertility and indicative of uterine infection (Kumar *et al.,* 2013).

Buffaloes that recovered from endometritis based on cervical mucus characters and whiteside test were inseminated at subsequent estrus. The first service conception rates in Group I, II and III were 44.44 (4/9), 42.85 (3/7) and 0.0 (0/3), respectively. The higher conception rate in Group I might be due to the degradation of proteins and lipids of gram-positive and gram-negative bacteria, yeast and toxins directly by these enzymes leading to stasis in growth and death of bacteria (Kruger *et al.,* 1999). Drillich

Table 1: Effect of Proteolytic enzymes (Group I), antibiotic (Group II) and controls (Group III) on cervical mucus score in buffaloes with clinical endometritis

Groups	Pre-treatment (%)			Post-treatment (%)			
	Clear with flakes	Muco-purulent	Purulent	Clear	Clear with flakes	Mucopurulent	Purulent
I (n=10)	30 (3)	50 (5)	20 (2)	90 (9)	10 (1)	0 (0)	0(0)
II (n=10)	40 (4)	30 (3)	30 (3)	70 (7)	20 (2)	10 (1)	0(0)
III (n=10)	20 (2)	60 (6)	20(2)	0 (0)	50 (5)	30 (3)	20 (2)

Table 2: Effect of Proteolytic enzymes (Group I), antibiotic (Group II) and controls (Group III) on spinnbarkeit value and pH of cervical mucus in buffaloes with clinical endometritis

Groups	Spinnbarkeit value (cm)		pH	
	Pre treatment	Post treatment	Pre treatment	Post treatment
I (n=10)	$7.24\pm0.79^{A,a}$	$15.79\pm0.64^{A,b}$	$8.22\pm0.08^{A,a}$	$7.10\pm0.03^{A,b}$
II (n=10)	$7.23\pm0.82^{A,a}$	$12.90\pm0.71^{B,b}$	$8.12\pm0.10^{A,a}$	$7.43\pm0.14^{A,b}$
III (n=10)	$7.09\pm0.89^{A,a}$	$7.33\pm0.90^{C,a}$	$8.13\pm0.07^{A,a}$	$8.01\pm0.09^{B,a}$

Note: Mean ± SE bearing different superscripts (A, B, C column wise and a, b row wise) differ significantly ($p<0.05$).

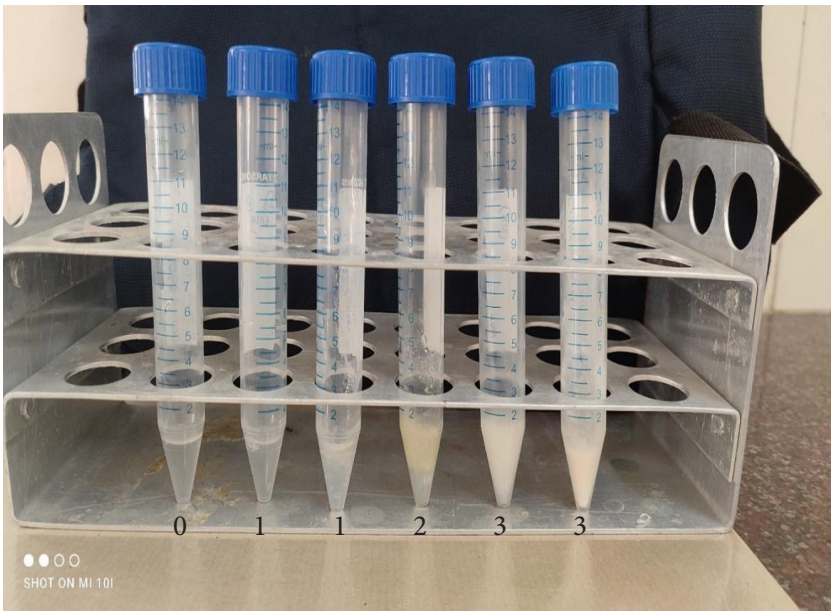

Fig 1: Scoring of cervical mucus from endometritic buffaloes (Score 0- clear mucus, Score 1- mucus with pus flakes, Score 2- mucopurulent discharge and Score 3- purulent discharge)

et al. (2005) and Singh *et al.* (2020) recorded lower first service conception rates using proteolytic enzymes (38.3% and 31.6%, respectively) in cattle with chronic endometritis and buffaloes with sub clinical endometritis, respectively compared to present study. Whereas, in Group II buffaloes, similar first service conception rates as that of Group I were obtained might be due to the broad efficacy of ceftiofur sodium on both gram-positive and gram-negative bacteria and its resistance to antibiotic resistance enzyme beta-Lactamase enzyme (Chenault *et al.,* 2004).

CONCLUSIONS

The use of hormones and antibiotics in the therapy of uterine infections is under critical public discussion. The alternative therapeutic approaches need to be evaluated. The present study used intrauterine infusion of proteolytic enzymes containing trypsin, chymotrypsin and papain to treat clinical endometritis in buffaloes and found improved cervical mucus properties and comparable first service conception rates with antibiotic ceftiofur sodium. and can be concluded that proteolytic enzymes can be used as an alternative to antibiotic treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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