

Original Research Article

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Efficacy of Bacterial Collagenase Therapy in RFM Cows Assessed through Endometrial Biopsy

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ABSTRACT

The study was conducted in cases of RFM presented within 12 to 24 hours after parturition to Obstetrics Unit of Madras Veterinary College, Chennai. Group I received placebo treatment with one litre of normal saline intravenously. Group II cows, treated with intrauterine proteolytic bolus containing nitrofurazone, metronidazole and urea and antibiotic therapy (Inj. Streptopenicillin @ 20,000 units/kg body weight) without manual removal for 7 days. Groups III cows, received single dose of 2, 00,000 CDU of collagenase plus 40 mg of calcium chloride and 40 mg of sodium bicarbonate dissolved in one litre of normal saline at a pH of 7.5 intravenously through jugular vein (Eiler and Hopkins,1993). Group IV received single dose of 2, 00,000 U of collagenase intravenously. The endometrial histopathology findings on day 14 and 35 in group I revealed mild to moderate glandular activity with haemorrhage and vascular activity, hyperplastic changes in the mucosa and infiltration of mononuclear cells. The higher degree of focal necrosis of epithelial mucosa and infiltration of neutrophils were recorded in group II and IV than group I and III; group II had severe fibrotic necrosis than group IV. Group II had severe diffused cellular infiltration and increased inflammatory response. Group III had mild to moderate glandular activity, infiltration of bacterial colonies, mild necrotic tissues, neutrophilic and lymphocytic infiltration; group IV had higher leucocytic infiltration, bacterial load and focal necrosis than group III. These histopathological results were revealed that the administration of collagenase through intravenous route had achieved better healing and recovery.

Keywords

Uterine tissue biopsy, RFM, Bacterial collagenase response, Cows

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Introduction

If the placenta is retained longer than 8 to 12 h, it is considered pathological and referred as retention of foetal membrane (Hanafi *et al.*, 2011). The incidence of retained foetal membrane (RFM) varies from 4 to 16 per cent in cattle (Hossein and Ardalam, 2011), which leads to reduced milk yield, endometritis and poor fertility.

A variety of methods have been used in the treatment of RFM, which includes manual removal and / or administration of oxytocin, PGF_{2α}, antibiotics, immunomodulators *etc.*, although the efficacy of these treatments are questionable (Eiler, 1997). The alternate route for the collagenase administration instead of umbilical arteries was reported by Eiler and Hopkins (1993) that the dose of 2.2 x 10⁶ U in 1000 ml physiological saline solution over a period of 30 mts through jugular vein. The collagenase administration through umbilical artery is the effective treatment for RFM in dairy cows; however, such collagenase treatment is costly and administration through the umbilical cord is more difficult after 48hrs. Hence, the study was formulated to determine the effect of collagenase through intravenous route instead of umbilical arteries. Further, the efficacy of different treatment regimens was studied and the healing processes were assessed through the uterine biopsy with histopathological study of endometrial tissue (Chapwanya *et al.*, 2010).

Materials and Methods

Fifty two healthy and parous cows less than 10 years of age, presented to the Large Animal Obstetrics Unit, Teaching Veterinary Clinical Complex, Madras Veterinary College, Chennai-7 were utilized for the study. Seven healthy cows with normal calving and shedding of placenta were served as group I (control) and treated with one litre of normal

saline intravenously. Thirty cows with unassisted calving followed by retained foetal membranes between 12 and 24 h interval were selected and randomly allotted into groups II and III of fifteen each. Fifteen cows with difficulty in parturition followed by RFM were considered as group IV.

Group II cows, treated with intrauterine proteolytic bolus containing nitrofurazone, metronidazole and urea and antibiotic therapy (Inj. Streptopenicillin @ 20,000 units/kg body weight) for 7 days without manual removal. Groups III and IV cows, received single dose of 2, 00,000 CDU of collagenase plus 40 mg of calcium chloride and 40 mg of sodium bicarbonate dissolved in one litre of normal saline at a pH of 7.5 intravenously through jugular vein (Eiler and Hopkins, 1993).

Uterine endometrial biopsy samples were collected *in vivo* using Albuchin's uterine biopsy catheter on day 14 and 35 postpartum in all the groups. The collected tissue samples were trimmed, dehydrated, cleared and embedded in paraffin section at a thickness of 5 to 6 μ and stained with haematoxylin and eosin for histological examination as per the procedure described by Balasubramanian (1994) and Karunakaran *et al.*, (2012).

Results and Discussion

On day 14 and 35 postpartum, group I cows had mild to moderate glandular activity with haemorrhage and vascular activity, hyperplastic changes in the mucosa, and infiltration of mononuclear cells than the remaining groups (Plate 1). These findings were in concurrence with the observations of Dolatkah *et al.*, (2013) who reported that the number of endometrial blood vessels, necrotic regions, and endometrial neutrophilic infiltrations were slightly increased in the endometritic cows than healthy cows on days 27 to 79 postpartum. Similar reports were

made by Bonnett *et al.*, (1991) that the single columnar epithelium lining the endometrium of the uterus is denuded, allowing invading bacteria access to the underlying stromal compartment immediately after parturition. These mild inflammatory changes in the endometrium, such as focal areas of cellular infiltration have little effect on fertility (DeBois and Manspeaker, 1989) in group I cows.

The higher degree of focal necrosis of epithelial mucosa and infiltration of neutrophils were recorded in groups II and IV than groups I and III on days 14 and 35; however, group II had severe fibrotic necrosis than group IV (Plate 2, 3 and 4). These findings were in agreement with the observations of Singh *et al.*, (1987) and McEntee (1990) that the different degrees of periglandular fibrosis were produced by intrauterine infusions of iodine solutions due to inflammatory changes in dairy cows. Chronic signs of inflammation such as dilatation of endometrial glands and periglandular fibrosis leading to delayed involution (DeBois and Manspeaker, 1989) might be the reason in group II for the prolonged lochial discharge and delayed involution.

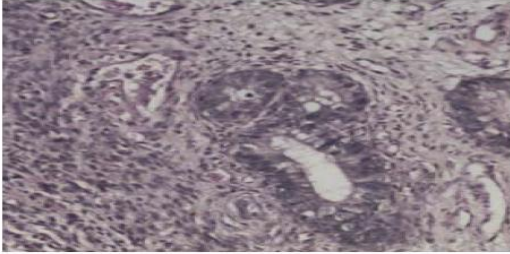
Group II had severe diffused cellular infiltration and increased inflammatory response due to severe irritation caused by the administration of intrauterine proteolytic drugs than the remaining groups. These results concurred with the observations of Raja *et al.*, (2012) who reported that cows with severe infection had characteristic changes in endometrium like glandular hyperplasia with periglandular fibrosis and necrotic changes with cystic dilatation which indicated poor fertility. These results revealed that the cows with RFM treated by an irritant intrauterine drugs causes uterine damage, which concurred with the observations of Schnyder *et al.*,

(1990) that irritant drugs like Lugol's iodine solution locally showed severe changes of degeneration and cellular infiltration in endometrial tissues, which led to reduced fertility. Further, the delayed involution in group II might be related to disruption of endometrial epithelium, infiltration and accumulations of inflammatory cells, vascular congestion and stromal edema (Polat *et al.*, 2015).

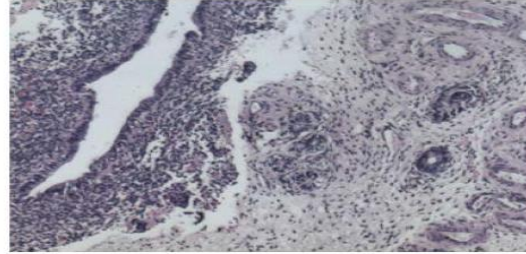
Group III had mild to moderate glandular activity, infiltration of bacterial colonies, mild necrotic tissues, neutrophilic and lymphocytic infiltration on days 14 and 35 postpartum, which was comparable with the results of group I. Though the group IV received collagenase administration, had higher leucocytic infiltration, bacterial load and focal necrosis than group III on days 14 and 35 postpartum. These findings were in agreement with the observations of Sheldon *et al.*, (2009) that the bacterial infection caused by the trauma, which disrupted the endometrial tissue structure and function. The tissue damage occurred while handling dystocia leading to inflammation, which favours elevated bacterial load (Herath *et al.*, 2009; Cronin *et al.*, 2012) might delay the collagenolysis efficiency, even with the exogenous administration of collagenase in group IV, this result in delayed uterine involution.

The higher degree of focal necrosis of epithelial mucosa and infiltration of neutrophils were recorded in group II and IV on day 14 and 35; however, group II had severe fibrotic necrosis with diffused cellular infiltration and increased inflammatory response than group IV. In group III had mild to moderate glandular activity, infiltration of bacterial colonies, mild necrotic tissues, neutrophilic and lymphocytic infiltration on day 14 and 35 postpartum. Group IV had higher leucocytic infiltration, bacterial load and focal necrosis.

Plate 1. Histopathology of uterine endometrium in Group I cows

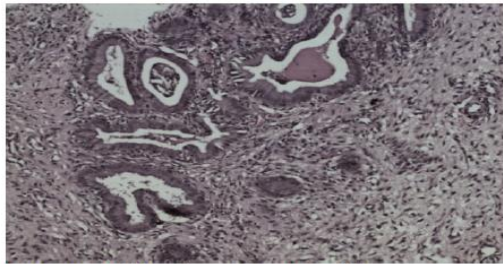


Day 14: Moderate glandular activity

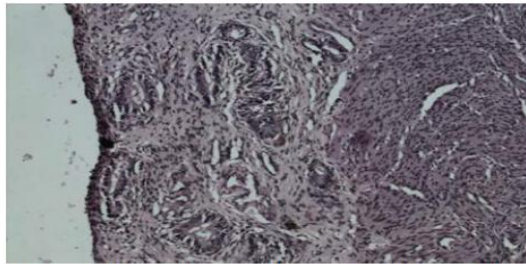


Day 35: Mild glandular activity, fibroblast, an infiltration mononuclear cells, heavy neutrophilic infiltration, bacterial colonies and necrotic tissue

Plate 2. Histopathology of uterine endometrium in Group II cows

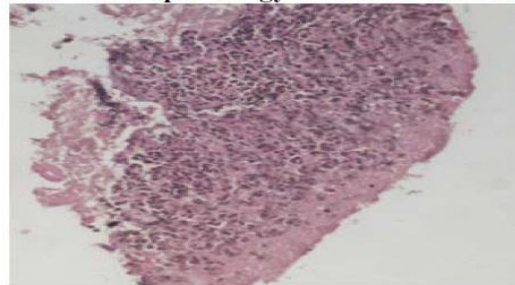


Day 14: Infiltration of neutrophils and lymphoplasmacytes

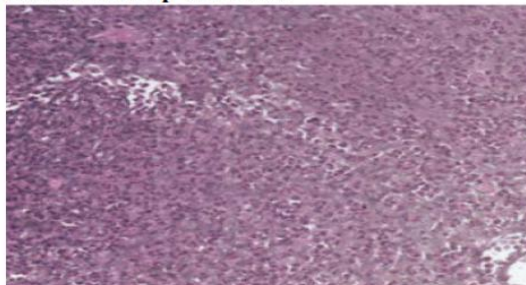


Day 35: Increased vascular activity, mild fibrosis and mild lymphocytes and focal necrosis

Plate 3. Histopathology of uterine endometrium in Group III cows

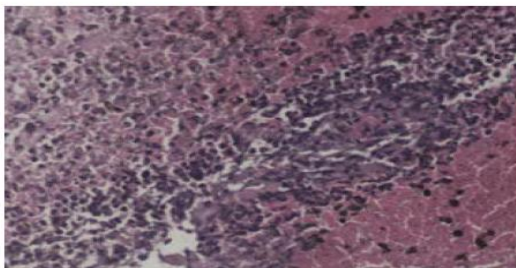


Day 14: Heavy neutrophil infiltration, bacterial colonies and necrotic tissues

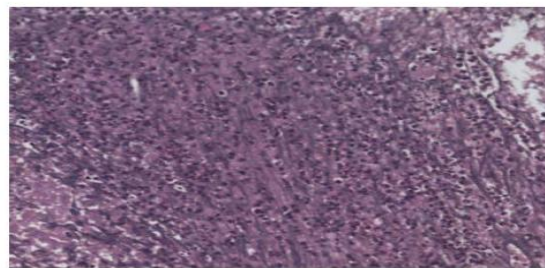


Day 35: Heavy neutrophil infiltration, sub mucosal mononuclear cells infiltration and bacterial colonies

Plate 4. Histopathology of uterine endometrium in Group IV cows



Day 14: Heavy neutrophil infiltration and necrotic tissue



Day 35: Heavy neutrophilic infiltration

These results showed that the administration of single dose of 2, 00,000 CDU of collagenase plus 40 mg of calcium chloride and 40 mg of sodium bicarbonate dissolved in one litre of normal saline at a pH of 7.5 intravenously through jugular vein is recommended for the treatment of RFM than the other intrauterine therapy.

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