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Research Paper

CHARACTERISTICS OF EXPELLED FOETAL MEMBRANE FOLLOWING TREATMENT WITH INTRAVENOUS BACTERIAL COLLAGENASE ON RFM IN COWS

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The collagenase administration through umbilical artery is the effective treatment for Retained Fetal Membrane (RFM) in dairy cows. RFM was treated with collagenase enzyme through jugular vein as it is easy route than previous study of experimenting on umbilical arteries which is very difficult in a delayed case in field conditions in bovines. The study was conducted in bovines with RFM and presented within 12 to 24 hours after parturition to Obstetrics Unit of Madras Veterinary College, Chennai). Data on time of expulsion of foetal membrane, duration of lochial discharge and gross appearances was collected, compared and analyzed. The experimental animals were divided in to four different treatment groups (Gp:n=7; Gp:n=15; Gp:n=15; Gp:n=15). The findings showed that the expelled foetal membrane were bulky and shiny appearance in group I; shreds of foetal membranes with voluminous discharge in group II, remnants of digested foetal membrane with resisted umbilical vessels in group III and dark coloured remnants of digested foetal membrane with resisted umbilical vessels in group IV. The mean time taken for the foetal membrane expulsion was significantly shorter ($P<0.01$) in group I (6.00 ± 0.69 h) and longer in group II (126.40 ± 6.8 h) than group III (38.93 ± 2.54 h) and IV (50.60 ± 0.76 h). The mean duration taken for lochial discharge in group I (5.00 ± 2.16 h) and III (6 ± 2.25 h) had significantly ($P<0.01$) shorter than group II (15.00 ± 0.78 h) and IV (10 ± 3.36 h).

Keywords: Foetal membrane expulsion time, Duration of lochial discharge, Gross appearance, Intravenous bacterial collagenase therapy, Cows

INTRODUCTION

Retained Foetal Membrane (RFM) is one of the most important postparturient disease (Stephen, 2008), leading to reproductive problems and

economic losses in dairy industry (Pathak *et al.*, 1991). A variety of methods have been used in the treatment of RFM, which includes manual removal and/or administration of oxytocin, PGF_{2 α} ,

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antibiotics, immune modulators etc. (Amin *et al.*, 2013), as the efficacy of these treatments are questionable (Eiler, 1997), bacterial collagenase from *Clostridium histolyticum* was used for the treatment of RFM as it could degrade several types of collagen (Azawi, 2013). However, collagenase administration after 48 h postpartum is ineffective due to residual blood clots in the placental vessels which causes anastomosis of umbilical arteries and tend to close (Eiler, 1997).

The alternate route for the collagenase administration instead of umbilical arteries was reported by Eiler and Hopkins (1993) that the injection of collagenase (2.2×10^6 U in 1000 ml of physiological saline solution over a period of 30 mts) through jugular vein caused release of foetal membrane within 36 h in experimentally induced retained foetal membrane. Hence, this study was formulated to determine the effect of collagenase through intravenous route instead of umbilical arteries on the foetal membrane expulsion time, duration of lochial discharge and gross appearance post treatment.

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). 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 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. Data on time of expulsion of foetal membrane, duration of lochial discharge and gross appearances were collected, compared and analyzed as per the standard procedure outlined by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

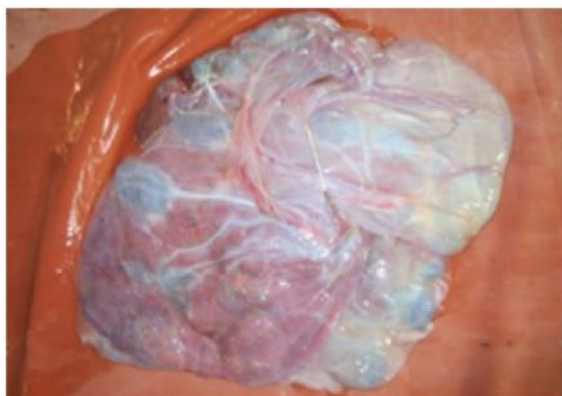
The mean (\pm SE) foetal membrane expulsion time with gross appearance of the expelled foetal membrane were significantly differed in all the groups (Table and Plate). In group I, the placental expulsion time was significantly ($P < 0.01$) shorter (6.00 ± 0.69 h); where, the gross appearance of the placenta was more bulky and shiny than the remaining groups. These findings were in agreement with the observations of Purohit and Borghese *et al.* (2013) that the expulsion time of placenta in dairy cows varied between 30 min to 8 h in normal calving. Similar observations were made by several researchers that the shedding of placenta occurs within 3 to 8 h after calving (Ahmed *et al.*, 1984; Eiler, 1992; Kunbhar and Memon, 2011; and Waheebet *et al.*, 2014). The hormonal changes associated with increased collagenase activity and declined progesterone level leads to reduced Bi-Nucleated Cells (BNC), might increase the activity of matrix

Table: Mean (\pm SE) Fetal Membrane Expulsion Time, Duration and Appearance of the Expelled Fetal Membrane with Different Treatment Regimens of RFM Cows

Group/Days	Fetal Membrane Expulsion Time (h)	Duration of Lochial Discharge (Days)	Gross Appearance of Fetal Membrane
I (n=7)	6.00 \pm 0.69 ^a	5.00 \pm 2.16 ^a	Bulky and Shiny
II (n=15)	126.40 \pm 6.8 ^d	15.00 \pm 3.78 ^c	Shreds of fetal membrane with voluminous lochial discharge
III (n=15)	38.93 \pm 2.54 ^b	6.00 \pm 2.25 ^a	Remnants of digested fetal membrane with resisted umbilical vessels
IV (n=15)	50.60 \pm 0.76 ^c	10.00 \pm 3.36 ^b	Remnants of dark coloured digested fetal membrane with resisted umbilical vessels

Note: Means bearing different superscripts (a-b) in each row differ significantly ($P < 0.01$); Means bearing different superscripts (a-b) in each column differ significantly ($P < 0.01$).

Plate: Gross Appearance of the Expelled Fetal Membrane with Different Treatment Regimens of RFM Cows



(a) Bulky, shiny fetal membrane expelled in normal calving



(b) Shreds of placenta in proteolytic treatment group



(c) Digested placenta with resisted umbilical vessels in normal calving with collagenase treatment group



(d) Digested placenta with dark coloured resisted umbilical vessels in dystocia with collagenase treatment group

metalloproteinase (MMP); which, influence the hydrolysis of collagen (Maj and Kankofer, 1997), resulted in easy separation and earlier shedding of placenta in group I.

In group II, the mean time taken for the expulsion of foetal membrane was significantly ($P < 0.01$) longer (126.40 ± 6.8 h); where, shreds of placenta with voluminous discharge was expelled than the remaining groups. These results were in agreement with the observations of Roberts (1986) who speculated that while controlling local bacterial growth, intrauterine antibiotics actually interfere with the necrotizing process at the caruncle-cotyledon interface which delays the release of placenta, which leads to putrefactive process in uterus (Al-Kennany *et al.*, 2010). Similar results were obtained by Chandraprasad and Mouli Krishna (2009) who observed that the placenta was expelled within 6.7 days (2 to 12 days) in cows followed by RFM treated with iodine preparations. However, these results were contradicted with the appearance of placenta as shreds of placenta with copious discharge observed in group II. Recent studies suggest that RFM might have a relationship with releasing of oxygen free radical that cause oxidative stress, which might be dangerous for cells and tissues (Kimura *et al.*, 2002), leading to delayed placental separation and shreds of placenta observed in the lochial discharge in group II.

The results obtained in group II were in contrast to the observations made by Dutta and Dugwekar (1988), Gurubulak *et al.* (2010) and Waheeb *et al.* (2014) that the expulsion time of foetal membrane was significantly ($P < 0.01$) shorter in cows treated with Furea bolus (38.25 ± 3.43 h), Chlortetracycline bolus (43.4 ± 1.9 h) and Oxytetracycline bolus (44.4 ± 1.9 h), respectively.

The increased duration of placental expulsion time in group II might be due to severe tissue damage caused by the intrauterine proteolytic drugs, favouring the release of higher concentration of placental tissue serotonin leading to the failure of detachment of placenta (Eiler and Fecteau, 2007). Further, the intrauterine proteolytic bolus causes irritation and increased cortisol concentration (Ahmed *et al.*, 1999) which has immunosuppressing inhibitory effect on leucocytic migratory activity (Engler *et al.*, 2004), leading to reduced collagenase release and increased expulsion time in group II.

In groups III and IV, the mean time taken for the expulsion of foetal membrane was significantly ($P < 0.01$) shorter as 38.93 ± 2.54 and 50.60 ± 0.76 h, respectively; where, the remnants of digested placenta with resisted umbilical vessels were expelled. The expelled digested placental membranes with resisted umbilical vessels were dark in colour in group IV than group III. These findings were in agreement with the observations of Eiler *et al.* (1997) that 80% of the cows with normal calving followed by RFM treated with 2,00,000 CDU of collagenase through umbilical artery expelled the placenta within 36 h. In group III and IV, the administration of collagenase leads to a marked reduction in retention time of RFM, may increase MMP enzyme activity and capable of breaking down the extracellular matrix (Dilly *et al.*, 2011), which results in hydrolysis of collagen (Maj and Kankofer, 1997) and subsequent release of equally digested placenta and remnants of foetal membrane with resisted umbilical vessels (Fecteau *et al.*, 1998).

These findings were in contrast to the observations of Eiler and Hopkins (1993) and Fecteau *et al.* (1998) that the mean placental detachment time was 27.9 ± 1.7 and 24 h,

respectively in RFM cows treated with 2,00,000 CDU of collagenase through umbilical cord. These variations in the present findings might be due to the time and route in which the drug was administered, environmental, climatic and managerial influence (Sheldon *et al.*, 2006; and Shivhare *et al.*, 2013).

However, group IV had significantly ($P < 0.01$) longer time of placental expulsion time than group III. These extended expulsion time in group IV with dystocia followed by RFM treated by collagenase might be due to the trauma occurs in the uterus during dystocia followed by RFM, resulting in oedema of chorionic villi, which impairs the separation of cotyledon and caruncle interface (Laven and Peters, 1996). Further, the bigger oedematous villi might not be able to disarticulate from the crypts as easily and trauma to the uterus might increase heparin release from the mast cells at the site of injury, which reduces the efficiency of the collagenase activity (Gross *et al.*, 1985; and Au *et al.*, 1992).

The expelled foetal membrane had bulky and shiny appearance in group I; shreds of foetal membranes with voluminous discharge in Group II, remnants of digested foetal membrane with resisted umbilical vessels in group III and dark coloured remnants of digested foetal membrane with resisted umbilical vessels in group IV. The mean (\pm SE) time taken for the foetal membrane expulsion was significantly shorter ($P < 0.01$) in group I and longer in group II.

The mean (\pm SE) duration of lochial discharge was 5.00 ± 2.16 , 15.00 ± 3.78 , 6.00 ± 2.25 and 10.00 ± 3.36 days in groups I, II, III and IV, respectively (Table). The mean (\pm SE) duration of lochial discharge in group I was 5.00 ± 2.16 days. These present findings concurred with the

observations of Noakes *et al.* (2009) that the greatest flow of lochia occurs during the first 2 to 3 days of postpartum, which reduces on day 8 in cows with normal puerperium. However, the present findings were in contrast to the observation of several researchers that the lochial discharge was usually completed within 12 to 43 days of postpartum in dairy cows (Okano and Tomizuka, 1987; McEntee, 1990; Mortimer *et al.*, 1997; and Noakes *et al.*, 2001;

Sheldon *et al.*, 2004; and Sheldon *et al.*, 2014). The shortest duration of lochial discharge in group I might be due to the variations in the species, breed, parity, feeding, managerial practices, climatic conditions and season (Shivhare *et al.*, 2013).

Group II (15.00 ± 3.78 days) had significantly ($P < 0.01$) longer duration of lochial discharge than the remaining groups. These findings were in accordance with the observations of Markandeya *et al.* (2014) who reported that the time taken for the cessation of lochial discharge was 15 days in bovines with normal calving followed by RFM treated with 4 number of intrauterine Ropitas bolus. However, the duration of lochial discharge in group II was longer than the remaining groups; which might be due to severe irritation, tissue damage and failure of detachment of placenta (Eiler and Facticeau, 2007), resulted in decomposed placental tissues with long expulsion time. This hostile environment in group II was favouring development of uterine infections (Dohmen *et al.*, 2010) and inflammation (Dobson and Hill, 2009) leading to prolonged lochial discharge. Further, the prolonged discharge in group II were associated with inflammatory cell infiltration and more fibrotic changes in the endometrium, involved in prolonged mucus production from the fibrotic

nests and inflamed endometrium (LeBlanc, 1994; and Causey *et al.*, 2000).

Groups III (6.00 ± 2.25 days) and IV 10.00 ± 3.36 days) had significantly (P<0.01) shorter duration of lochial discharge than the group II (15.00 ± 3.78 days). The administration of collagenase has acted primarily on the cotyledon matrix (Eiler, 1991) and cotyledon-caruncle interface leading to detachment of RFM (Eiler and Hopkins, 1992) by the hydrolytic release of the cotyledon (Takagi *et al.*, 2007) that secure the endometrium without causing any injury and acute inflammation (Eiler and Hopkins, 1993), results in early cessation of lochial discharge in group III and IV, from the results of study it was concluded that expelled foetal membrane had bulky and shiny appearance in group I; shreds of foetal membranes with voluminous discharge in group II, remnants of digested foetal membrane with resisted umbilical vessels in group III and dark coloured remnants of digested foetal membrane with resisted umbilical vessels in group IV. The mean (± SE) time taken for the foetal membrane expulsion was significantly shorter (P<0.01) in group I and longer in group II. The duration of lochial discharge in groups I and III had significantly (P<0.01) shorter. 🌐

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