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EFFICACY OF BACTERIAL COLLAGENASE THERAPY IN RFM COWS ASSESED THROUGH BACTERIAL LOAD

Mohan P[1], Krishnakumar.K[2], Kulasekar.K[3], Karthickeyan.SMK[4], and Murugan. M [5]*

[1]*Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Shivamogga, KVAFSU.*
[2]*Professor and Section Head, Cattle and Buffalo Breeding Unit, Post Graduate Research Institute in Animal Sciences, Kattupakkam*
[3]*Professor, Department of Veterinary Gynaecology and Obstetrics, Madras Veterinary College, Vepery*
[4]*Professor, Department of Animal Breeding Genetics, Madras Veterinary College, Vepery*
[5]*Professor and Head, Department of Livestock Production and Management, Veterinary College and Research Institute, Tirunelveli*

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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. Hence, to determine the bacterial collagenase treatment effect on bacterial count in all the different treatment groups. Further, collected data were compared and analyzed; which showed that the bacterial load was significantly (P<0.01) lower in group I; higher in group IV on day 0 and group II on day 7 and 14. The overall mean bacterial load was significantly (P<0.01) lower in group I and III. These study of bacterial load results revealed that the administration of collagenase through intravenous route had achieved better healing and recovery.*

Keywords: *bacterial load, RFM, bacterial collagenase response, Cows.*

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 to16 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, $PGF_{2\alpha}$, 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 10^6 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, this study was formulated to determine the effect of bacterial collagenase through intravenous route instead of umbilical arteries. Further, the efficacy of different treatment regimens studied and the healing processes were assessed through bacterial count (Ahmadi *et al.,* 2006).

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, and 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).

Collection of lochia

Thirty ml of uterine lochia was collectedon days 0, 7 and 14 postpartum with the help of Ramson's children's enteral feeding tube and kept in a sterile screw capped vials, lochial sample was immediately utilized for bacteriological study **(**Cruikshank *et al.,* 1974) in all the groups.

Lochial examination

The collected lochia on days 0, 7 and 14 postpartum were utilized for the study of bacterial load

Preparation of lochia for bacterial load

One ml of uterine lochia was inoculated with 5 ml of nutrient broth and incubated at 37^{o}C for overnight and kept ready for bacterial load estimation of Cruickshank *et al.* (1974).

Bacterial load

Uterine lochia prepared for the estimation of bacterial load was estimated as per the method of Cruikshank *et al.* (1974).

Statistical Analyses

The bacterial load was analysed by one way ANOVA

Results and Discussion

The high growth rates of bacterial load encountered on day 0 were significantly ($P<0.01$) declined towards the days 7 and 14 postpartum in all the groups (Table and Figure). These findings were in agreement with the observations of Bekana *et al.* (1997) and Archbald *et al.* (1998) who reported that large and diverse number of bacteria observed in the uterus during the first 2 weeks of postpartum have been eliminated within the next 2 weeks of postpartum.

On day 0, the bacterial load was significantly ($P<0.01$) lower in group I (15.35 ± 0.57 10^4/ml) than the groups II (44.89 ± 0.39 (10^4/ml), III (44.61 ± 0.39 (10^4/ml) and IV (55.58 ± 0.39 (10^4/ml). Group IV had significantly ($P<0.01$) higher bacterial load than group II and III; however, group II and III did not differ significantly. These findings were in agreement with the observations of Balasubramanian (1994) that the mean bacterial count on day 1 postpartum was 16.86 ± 3.31 (10^4/ml) in normal puerperium and 57.38 ± 3.86 (10^4/ml) in RFM buffaloes. Similar observations were made by Ambrose (1984) that the average lochial bacterial load was 48.36 ± 23.6 million at 16 h after placental retention in bovine. Relaxation of the vulva and cervical dilation occurs during parturition, which allows the entrance of the bacteria into the uterus in buffalo cows (Azawi, 2008) might be the reason for the elevated bacterial load. However, Trauma, inter-concurrent infections and failure of local immune mechanisms lead to bacterial multiplication due to severe handling and tissue damage while attending dystocia. These might be the other reasons for the increased bacterial count in group IV than group II and III.

Days 7 and 14 postpartum in group II (34.28 ± 0.41 and 32.87 ± 0.92 (10^4/ml) had significantly ($P<0.01$) higher bacterial load than the remaining groups. Among the remaining groups, group IV (28.25 ± 0.41 and 16.02 ± 0.92 10^4/ml) had significantly ($P<0.01$) higher bacterial load than groups I (9.24 ± 0.60 and 3.14 ± 1.35 (10^4/ml) and III (19.17 ± 0.41 and 8.41 ± 0.92 10^4/ml); however,

group I had significantly (P<0.01) lower bacterial load than group III. These findings were in agreement with the observations of Balasubramanian (1994) that the mean bacterial count on days 7 and 14 postpartum was 9.29 ± 2.55 and 3.43 ± 4.67 (10^4/ml), respectively in normal puerperium; and 28.05 ± 8.98 and 13.81 ± 7.1 (10^4/ml), respectively in RFM buffaloes.

The higher concentration of bacterial load on days 7 and 14 postpartum in group II, might be due to increased cortisol concentration resulting in accumulation of immunosuppressive proteins in the uterine lumen, which makes the uterus susceptible to infections and persistence of bacteria (Azawi *et al.,* 2008). Specific factors that may delay the elimination of bacteria from the postpartum uterus includes nature of placental shedding, level of bacterial contaminations, degree of uterine involution and cows immune status (Sheldon *et al.,* 2006), these might be the other reasons for elevated bacterial load in group II.

Further, the presence of decomposing placental tissues with long expulsion time in group II provides favourable environment for bacterial colonization and higher concentration of endotoxins present in the lochia, these were potent inducers of prostaglandins and cytokines, favouring development of uterine infections (Dohmen *et al.,* 2010) and inflammation (Dobson and Hill, 2009). These accumulated endotoxins interact with RFM to secrete PGE_2, which predisposes the uterus to infection, resulting in elevated level of bacterial load in group II. In addition to that the irritant nature of proteolytic drugs damages the uterine endometrium, that leading to suppress the uterine leucocytic phagocytosis (Vandeplassche and Bouters, 1982) lead to higher bacterial invasion (Peters and Laven, 1996) in group II.

The overall mean bacterial load concentration was significantly (P<0.01) lower in groups I (9.24 ± 0.56 (10^4/ml) and group III (18.07 ± 0.38 (10^4/ml) than groups II (33.01 ± 0.38 (10^4/ml) and IV (25.62 ± 0.38 (10^4/ml). The uterine infections involved in the adherence of pathogenic organisms to the uterine mucosa, colonization and penetration of the epithelium and release of bacterial toxins leads to establishment of uterine infections (Sheldon *et al.,* 2006) and elevated bacterial load in groups II and IV. Group I and III had significantly (P<0.01) lower bacterial load in the present study, suggested that the quicker time taken for the expulsion of placenta and hasten the uterine involution, which improved the efficiency of uterine defense mechanism, resulting in elimination of bacterial contamination (Sheldon *et al.,* 2006). From the above study, it was concluded that the bacterial load was significantly (P<0.01) lower in group I; higher in group IV on day 0 and group II on day 7 and 14. The overall mean bacterial load was significantly (P<0.01) lower in group I and III.

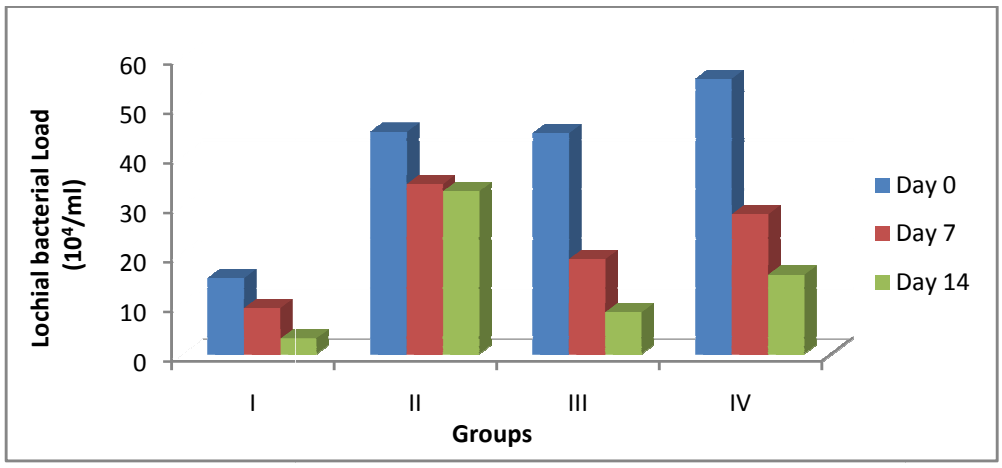
Table: Mean (± SE) lochial bacterial load during different phases of postpartum with different treatment regimes of RFM cows

Groups / Days	Bacterial load (10^4/ ml)			
	0	7	14	Overall mean
I (n=7)	15.35 ± 0.57^{aC}	9.24 ± 0.60^{aB}	3.14 ± 1.35^{aA}	9.24 ± 0.56^{a}
II (n=15)	44.89 ± 0.39^{bB}	34.28 ± 0.41^{dA}	32.87 ± 0.92^{dA}	33.01 ± 0.38^{d}
III n=15)	44.61 ± 0.39^{bC}	19.17 ± 0.41^{bB}	8.41 ± 0.92^{bA}	18.07 ± 0.38^{b}
IV (n=15)	55.58 ± 0.39^{cC}	28.25 ± 0.41^{cB}	16.02 ± 0.92^{cA}	25.62 ± 0.38^{c}

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)

Figure: Lochial bacterial load during different phases of postpartum with different treatment regimens of RFM cows



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