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Research Paper

IN-VITRO STUDY OF PLACENTAL TISSUE SEROTONIN IN RESPONSE TO TREATMENT WITH BACTERIAL COLLAGENASE INTRAVENOUS THERAPY 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 placental tissues of bovines that are presented within 12 to 24 hours after parturition to Obstetrics Unit of Madras Veterinary College, Chennai to determine the presence of serotonin in the placental tissue of all the different treatment groups and the experimental animals were divided in to four different treatment groups (Gp I:n = 7; Gp II:n = 15; Gp III:n = 15 and Gp IV:n = 15) and placental tissue samples were collected accordingly from all groups. The data were collected, compared and analyzed; further, the findings showed that the placental tissue serotonin concentration was significantly lower (P<0.01) in group I (1181.38 ± 16.45 ng/ml) than group II (1357.28 ± 76.7 ng/ml), III (1239.11 ± 77.34 ng/ml) and IV (1645.77 ± 21.44 ng/ml).

Keywords: Placental tissues, Serotonin concentration, Cows

INTRODUCTION

Retained Foetal Membrane (RFM) is one of the most important post parturient disease (Stephen, 2008), leading to reproductive problems and economic losses in dairy industry (Pathak *et al.,* 1991). The incidence of RFM ranges from 3 to 15% following normal parturition in dairy cows (Sheetal *et al*., 2015). 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, immune modulators, etc., (Amin *et al.,* 2013), although the efficacy of these treatments are questionable (Eiler, 1997). The concentrations of 5-HT in cotyledons in the expelled foetal membranes of cows within 3 h postpartum were clearly

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decreased from prepartum values and which might be due to increased degradation of 5 HT in the postpartum placenta (Okatani *et al.,* 1990). Eiler and Hopkins (1993) reported that high foetal and placental serotonin during pregnancy could help to maintain placental attachment by promoting placental cell proliferation and inhibiting matrix metalloproteinase activity in cattle. Fecteau and Eiler (2001) reported that the mean cotyledon concentration of serotonin was 1176 ± 199 ng/ml within 3 to 6 h postpartum in dairy cows and suggested that serotonin might play a role in regulating bovine placental attachment. Moore *et al.* (2015) stated that concentrations of 5HT decreased near parturition (on day 1) compared with prepartum (day 5) by 57.9 to 29.5% in dairy cows.

Hence, Based on these and to ascertain the concentration of serotonin in the placental tissues of different treatment groups this *in-vitro* study was formulated to determine the effect of collagenase on placental tissues.

MATERIALS AND METHODS

Placental tissue samples from 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 with RFM 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.

Materials Collected and Prepared

Placental Tissue Serotonin Estimation

Preparation of Serotonin Buffer

Serotonin buffer was prepared with 0.437 ml of concentrated HCl, 146.1 mg of EDTA and 380.2 mg of sodium metabisulfite dissolved in 500 ml of distilled water which comprises of 0.01 mM HCl, 1 mM EDTA and 4 mM sodium metabisulfite (Schraenen *et al.,* 2010).

Preparation of Placental Tissue Sample Forserotonin Estimation

Three grams of homogenised placental tissue was mixed with 10 ml of serotonin buffer for 3 minutes and centrifuged at 20000 rpm for 15 minutes at 4 ºC. After centrifugation, 0.1% ascorbic acid was added with the homogenate and stored at -20 °C until estimation of serotonin.

Placental Tissue Serotonin

Concentration of serotonin in the placental tissue homogenate was estimated using bovine 5-HT commercial ELISA kit supplied by Blue Gene, Shanghai, China.

Data on concentration of tissue serotonin *in-vitro* were collected, compared and analyzed as per the standard procedure outlined by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Placental Tissue Serotonin

The placental tissue serotonin concentration within 24 h after parturition was 1181.38 ± 16.45, 1357.28 ± 76.70, 1239.11 ± 77.34 and 1645.77 ± 21.44 ng/ml in groups I, II, III and IV, respectively (Table 1 and Figure 1). The mean placental tissue concentration was significantly (P<0.01) lower in group I than remaining groups. However, group IV had significantly (P<0.01) higher placental

tissue serotonin concentration than the groups II and III.

The concentration of placental tissue serotonin in group I concurred with the observations of Fecteau and Eiler (2001) that the mean cotyledon concentration of serotonin was 1176 ± 199 ng/ml within 3 to 6 h postpartum in dairy cows. It is suggested that high foetal and placental serotonin during pregnancy could help to maintain placental attachment by promoting placental cell proliferation, maturation of the foetal monoamineoxidase system close to parturition, resulting in the metabolization and subsequent decrease in serotonin, which in turn promote the placental separation and might be the reason for the early placental separation in group I. Similar reports were made by Moore *et al.* (2015) that concentrations of 5 HT decreased near parturition compared with prepartum by 57.9% on day 1 and 29.5% on day 5 in dairy cows.

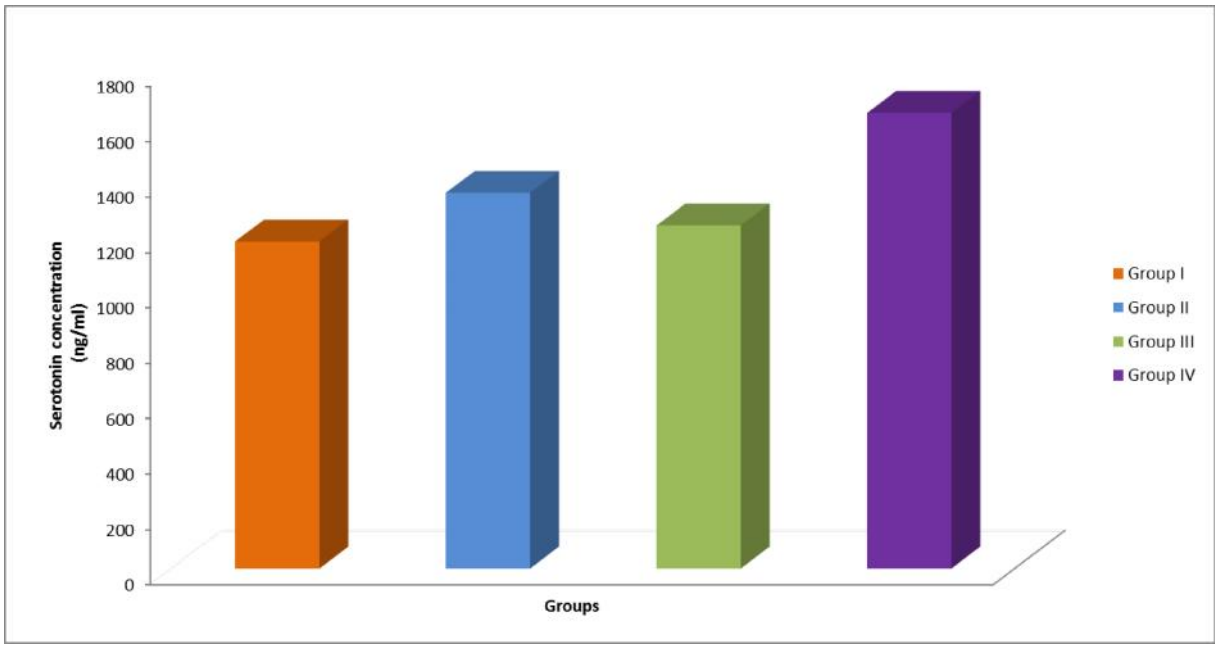
Table 1: Mean (±SE) in vitro Placental Tissue Concentration of Serotonin Concentration with Different Treatment Regimens of RFM Cows

Groups	Serotonin (ng/ml)
I	1181.38±16.45[a]
II	1357.28±76.7[b]
III	1239.11±77.34[b]
IV	1645.77±21.44[c]

Note: Means bearing different superscripts (A-B) in each row differ significantly (P<0.01).

The abundant level of serotonin in foetal blood, but not in neonatal calf blood, causes release of collagenase by uterine cells and serotonin has been proposed as a signal to begin the massive collagen degradation in the postpartum uterus. Further, it was suggested that the roles of serotonin during delivery were

Figure 1: In vitro Placental Tissue Serotonin Concentration with Different Treatment Regimens of RFM Cows



to stop blood circulation between the placenta and the foetus to trigger uterine proteolysis (Eiler and Fecteau, 2007) and the uterine smooth muscle contraction in response to serotonin via activation of 5 HT T2 receptors in bovine (Jim and Papinch, 2013), which might be the another reason for the foetal membrane expulsion in group I.

The significantly ($P<0.01$) higher concentration of placental tissue serotonin within 24 h of parturition in groups II, III and IV might be the reason for the retained placenta. In addition, the dystocia followed by RFM had higher tissue damage leading to increased concentration of serotonin which might be the reason for non-expulsion of foetal membrane immediately after parturition in group IV. Further, elevated level of placental tissue serotonin concentration, prevents the activity of collagenase in isolated placentomes and inhibited collagenase secretions by placentome cells, thus the higher level of serotonin is suspected to favour placental attachment in bovines (Dohmen *et al.,* 2000; and Fecteau and Eiler, 2001) causing for RFM in groups II, III and IV. Similar reports were made by Youngquist and Threlfall (2006) that serotonin has a proliferative effect on numerous cell types including cultured bovine placental cells which might favour attachment and inhibit secretions of proteolytic enzymes in bovine placentome cells, being the reason for the retained placenta in the present study.

CONCLUSION

From the above study it was concluded that Group I had significantly lower ($P<0.01$) placental tissue serotonin concentration and group IV had significantly higher ($P<0.01$) placental tissue serotonin concentration.

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