



## Review Article

# An insight into the Recent Advances on the Physiology and Treatment of Retention of Fetal Membranes in Cattle

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### ABSTRACT

The fetal membranes which are commonly called placenta are the connections between dam and fetus for the normal circulation of nutrients to fetus. If the placenta is not expelled within normal time, it is called as retention of fetal membrane. Retention of fetal membrane is the common cause of reduction of fertility in cattle. For the management of this disease, it is necessary to understand the etiology and pathophysiology of this disease. The normal separation of fetal membranes consists of complex hormonal process that starts before parturition in cattle. The causes of retention of fetal membranes include immature placentomes due to infections, induction of parturition, difficult birth, hormonal imbalance and nutritional deficiencies. The treatments which are commonly used for the retention of fetal membranes do not show the significant effects. The use of antibiotics minimizes the growth of microorganism in retained fetal membrane but they have not shown improvement in the subsequent fertility of affected animals. Recently, new treatments like injection of collagenase enzyme into umbilical arteries and intrauterine use of ozone gas produces useful results but these therapies are costly. The objective of this review is to understand the physiological processes of fetal membranes expulsion, causes and treatments of retention of fetal membranes.

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### INTRODUCTION

The retention of fetal membrane can be defined as the failure of expulsion of fetal membranes (Drillich *et al.*, 2006). The normal time for expulsion of fetal membrane varies from 8–48 hours after parturition (Lee *et al.*, 1989). However, in one study 66% cattle expelled their fetal membranes 6 hours after parturition (Van *et al.*, 1992). The adverse effects of RFM on reproductive performance of cattle are: delay in first service (Stevens *et al.*, 1997), reduction of pregnancy rate (McDougall *et al.*, 2001) increase in services per conception (Holt *et al.*, 1989). The RFM also leads to endometritis, puperal metritis and mastitis (Bruun *et al.*, 2002) and these diseases ultimately cause the reduction in the fertility and milk production of cattle (Laven *et al.*, 1996). Number of factors like difficult birth, twinning of calves, abortion, and increase in the age of animal are responsible for delayed expulsion of fetal membranes (Han and Kim, 2005). Recently, some vitamins and minerals are also identified as cause of RFM (Akar and Yeldiz, 2005).

Inadequate supplementation of a ration with vitamins A and E,  $\beta$ -carotene, iodine, selenium, copper and zinc may also induce abortion in cows with increase incidence of RFM (Markiewicz *et al.*, 2001). The exact cause of RFM is still not known and this hampers the search for preventive and therapeutic measures (Holt *et al.*, 1989). Various prophylactic and therapeutic approaches have been

postulated by many workers ranging from no treatment to hormonal, chemotherapeutic and manual removal (Majeed *et al.*, 1991). Knowledge of the placental anatomy and physiology is helpful to understand causes of RFM and formulate treatment plans accordingly. The following review focuses on the normal placental detachment, causes and risk factors for RFM, and therapeutic options.

#### *Physiology of Placental Maturation and Separation*

The type of placenta in cattle is cotyledonary which is formed by the fetal cotyledons and maternal cruncles making new structure called placentomes. Many villi are present at this connection for the interaction between these two structures. The connection sites are enriched with collagens which play an important role for the separation of cruncles from cotyledons at the time of fetal expulsion (Eiler *et al.*, 1993). The normal events of parturition are initiated by placental enzymes for the conversion of the progesterone into estrogen (Flint *et al.*, 1979). The increased level of estrogen causes the sensitivity of receptors for oxytocin on myometrium as well as increased concentration of prostaglandin F<sub>2</sub> alpha (Fuchs *et al.*, 1999). This increased prostaglandin results in the contraction of myometrium and regression of corpus luteum (CL) (Janszen *et al.*, 1993). The luteolysis of CL results in the secretion of relaxin hormone along with a decline in the progesterone levels (Mush *et al.*, 1987). These two conditions are responsible for the initiation

of collagenase activity. Relaxin causes the lysis of collagen, softening of the cervix and expansion of pelvic ligaments. On the other hand, progesterone during the pregnancy inhibits the myometrial contraction and collagenase activity and reduction in the level of progesterone at parturition might allow the activity of enzymes which are necessary for the separation of placental membranes (Maj *et al.*, 1997). The normal process of placental separation is multifactorial and starts before the process of parturition (Figure 1). It is also suggested that serotonin is necessary for the normal attachment of placental membranes (Fecteau, 2001). High level of fetal and placental serotonin might help for the maintenance of placental attachment and cell proliferation during the process of pregnancy (Fecteau *et al.*, 2001) and inhibiting the activity of proteinase (Eiler *et al.*, 1993). The increase in the activity of enzyme at the time of parturition causes the increase in metabolization and reduction of serotonin which might help in placental separation and expulsion (Fecteau *et al.*, 2001). Besides the changes in the profile of hormones that cause breakdown of cotyledon–cruncle linkages through enzymes, the immune response of dam plays an important role for the breakdown of placenta. The chemotactic activity of leukocytes is increased in those cows which normally expel the placenta (Gunnink *et al.*, 1983); (Kimura *et al.*, 2002) and neutrophil are attracted by the cytokine interleukin-8 in cotyledons in the process of parturition (Kimura *et al.*, 2002). The trophoblast cells activate the immune response through major histocompatibility proteins that help in the separation of placenta (Gunnink *et al.*, 1983). The level of these proteins increase during the last trimester (Davies *et al.*, 2004).

The labor is characterized by the high level of prostaglandin along oxytocin and creation of high mechanical contraction within the uterus which is necessary for normal expulsion of fetal membranes (Janszen *et al.*, 1993) and (Lye *et al.*, 1996). The contraction of uterus ends into the third stage of parturition which causes the expulsion of placenta (Laven *et al.*, 1996). Still the role of contraction of uterus in the separation of fetal membranes is not clear. The release of fetus from the uterus results in the reduction of blood circulation within the placenta and subsequently shrinkage of villi takes place (Laven *et al.*, 1996). The forces of uterine contraction might cause disconnection between cruncles and cotyledons, even though the absence of any harm to villi of fetal in normal parturition suggests the evidence that this process is not mechanical (Bjorkman *et al.*, 1960). It is though currently that contraction of uterus is important for the expulsion of fetal membranes; the early lack of myometrial contraction is not the prerequisite of retained fetal membranes (Paisley *et al.*, 1986; Grunert *et al.*, 1986).

#### **Causes of RFM**

The number of factors which are responsible for the RFM are induction of parturition (Terblanche *et al.*, 1976), short gestation period (Muller *et al.*, 1974), abortion of fetus (Joosten *et al.*, 1987); (Roberts *et al.*, 1986) twin fetus (Muller *et al.*, 1974; Erb *et al.*, 1958) difficult birth (Joosten *et al.*, 1987; Rajala *et al.*, 1988) fetotomy (Joosten *et al.*, 1987; Wehrend *et al.*, 2002), mineral and vitamin deficiency like vitamin E, selenium and vitamin A (Julien *et al.*, 1976; Ronning *et al.*, 1953). The RFM is also caused by infectious

diseases like bovine viral diarrhea (Niskanen *et al.*, 1995) and immunosuppression (Laven *et al.*, 1996). Whereas the exact mechanism responsible for these factors is not completely known, the complex process of multiple hormones and biochemical events leads that disturbance in these events causes the RFM. The studies of physiological factors which are responsible for the detachment of fetal membranes help in the diagnosis of etiologies of RFM. The function of immunosuppression during the retention of fetal membrane is not entirely understood (Gunnink *et al.*, 1984; Kimura *et al.*, 2002; Peter *et al.*, 1987). To maintain pregnancy, it is necessary to suppress the immune response to save the fetus from rejection and RFM may be the due to the result of failure of switching off this protective activity of immune system.

The cows which suffer from RFM subsequent to normal delivery found with decrease amount and activity of leukocytes prior to parturition process (Gunnink *et al.*, 1984; Kimura *et al.*, 2002). Particularly, the cows which suffer from RFM were found with reduced chemotaxis activity of neutrophils from one week prior to one week following parturition and reduced myeloperoxidase function from two weeks prior to two weeks subsequent parturition (Kimura *et al.*, 2002). The interleukin-8 which has an important chemotactic activity for neutrophils was less in cows which suffer from RFM as compared to those cows which were normally expelled their placenta. These studies found that reduced activity of neutrophil might be the cause of retention of fetal membrane. Reduction of antioxidant enzyme activity is also found during the RFM (Wischrall *et al.*, 2001; Gupta *et al.*, 2005). Less level of estrogen and superoxide dismutase was found in RFM (Wischrall *et al.*, 2001). It is also proposed that there is an imbalance of antioxidant activity of placenta which results into decrease production of estrogen followed by the less level of PGF2a in the placenta (Wischrall *et al.*, 2001). The analysis of (44) studies by comparing the incidence of RFM in treated cows with vitamin E and cows without any treatment found that vitamin E reduced the incidence of RFM (Bourne *et al.*, 2007). Vitamin E improves the antioxidant activity and also increases the leukocytosis and chemotaxis at placentomes which help in the normal expulsion of fetal membranes (Bourne *et al.*, 2007). The differences in the activity of protease in retained and non-retained placenta (Gross *et al.*, 1985; Eiler *et al.*, 2007) indicate the changes in the activities of enzymes play the important roles in the cause of RFM. For instance, the collagenase is reduced but collagen of type III remains in normal level in cows with RFM. Disturbances in the normal profile of hormones within the uterus can stop the secretion of protease from the placentomes epithelium and inhibition of activity of leukocyte protease by immunosuppression. Either condition can cause the reduction of activity of protease. The initiation of third stage of parturition with the help of dexamethasone, alongwith or without prostaglandin is the major cause of retained placenta and the exact mechanism responsible for this cause is not known (Gross *et al.*, 1985). The glucocorticoids could inhibit the activity of collagenase (Guerin *et al.*, 2004). Dexamethasone also inhibits the secretion of PGF2a in the cells of cotyledons (Izhar *et al.*, 1992) and injecting the PGF2a and dexamethasone could reduce occurrence of RFM but not completely (Gross *et al.*, 1985; Gross *et al.*,

1986). The induction of parturition causes the RFM but can be reduced if relaxin is injected along with dexamethasone. Various relations between the hypocalcemic level and RFM are made (Curtis *et al.*, 1983; Melendez *et al.*, 2004). The cows which suffer from RFM have lower level of plasma calcium as compared to cows without RFM (Melendez *et al.*, 2004). Calcium is necessary for the collagenase action but reduced level of calcium found in retained placenta is not so low to exclude the activity of collagenase (Gross *et al.*, 1985). In addition, only total calcium is observed in retained and without retained animals (Melendez *et al.*, 2004). For instance, the level of calcium can be affected by the level of other minerals such as hypoalbuminemia. No reduction of RFM was found in cows which were fed calcium orally (Melendez *et al.*, 2003). But the deficiency of calcium might predispose the cattle to dystocia (Correa *et al.*, 1993) and loss

of uterine contractions subsequent to the failure of fetal expulsion but the direct role of calcium in separation of fetal membranes is not understood. The risk factors which are responsible for RFM are injury to uterus, difficult birth and caesarian section. Injury can cause edema of villi which could interfere in separation of cruncles of dam from the cotyledons of fetus (Laven *et al.* 1996); (Grunert *et al.*, 1986). The normal disconnection process of placenta involves the separation of villi of cotyledons from the crypts of carucles. So the edema of villi could hinder the separation villi from crypts. In addition, the injury to uterus might results in increase secretion of heparin from the mast cells. Heparin causes the inhibition of collagenases (Au *et al.*, 1992) and delay of involution of uterus and both these factors result into RFM (Eiler *et al.*, 2007)

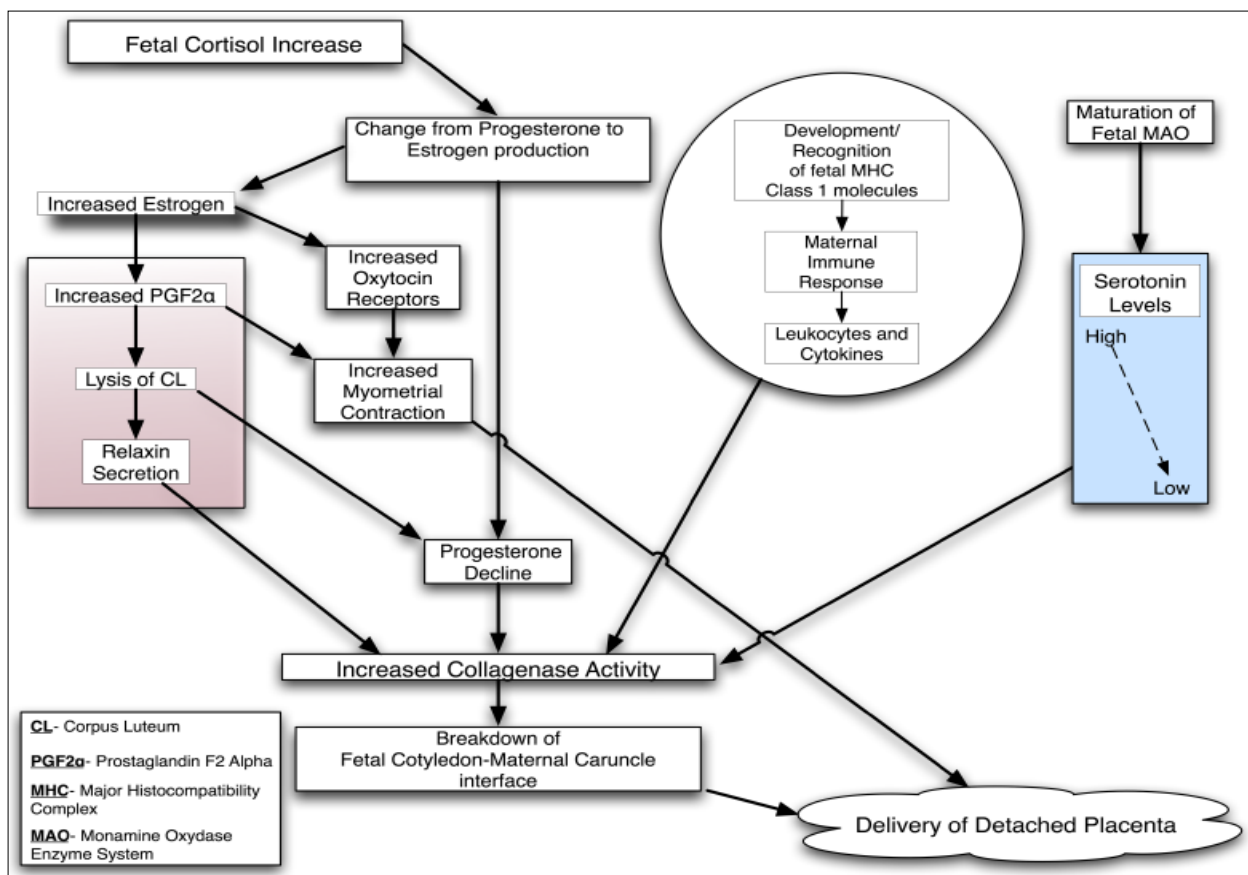


Figure 1: Physiologic processes of separation of the placenta in cattle.

### Treatments

Unfortunately, very fewer treatments are commonly used for the RFM and these treatments are based on traditional evidences. The manual handling of placenta is commonly used but no beneficial effects are reported on reproduction of animal or milk (Muller *et al.*, 1974). The benefits of recent studies like manual removal and systemic antibiotics have not been reported on the reproductive performance of cattle when compared without treatment (Drillich *et al.* 2006; Drillich *et al.*, 2007). The manual removal can cause more uterine infections when compared to traditional treatments

(Bolinder *et al.*, 1988). Whereas, the evidences collected of current studies do not support to manual handling of fetal membranes (Peters *et al.*, 1996). Certainly, the manual removal of placenta has more chances of injury to endometrium and reduces the phagocytic activity of leukocytes (Vandeplasseche *et al.*, 1982) and these two factors encourage the invasion of bacteria (Paisley *et al.*, 1986; Peters *et al.*, 1996). On the other hand, it is difficult to remove the whole placenta from the uterus, while some necrotic parts

could be removed leading to bacterial infections (Paisley *et al.*, 1986) or development of postpartum metritis.

Recently, new therapies have been introduced without the use of antibiotics. The use of ozone gas is also one of them. It has ability to inhibit the growth of microbes and fungi. The ozone gas has the ability of oxidative functions which is created by peroxides to destroy the microbes (Bocci *et al.*, 2009; Travagli *et al.*, 2009). Ozone gas does not develop the resistance as in antibiotics. Ozone gas is unstable and it dissociates into the powerful reactive molecules of oxygen under the usual pressure and temperature of atmosphere. It has properties of gas molecules and is combination of three oxygen atoms. It is a strong oxidant and can cause the activation of lymphocytes or monocytes to secrete many cytokines like interferon  $\alpha$ ,  $\beta$  and  $\gamma$ , tumor necrosis factor (TNF)  $\alpha$ , interleukins (IL) 1, 2, 4, 6, 8 and 10, granulopietins (GM-CSF), and transforming growth factor (TGF)  $\beta$  (Ducusin *et al.*, 2003 and (Ohtsuka *et al.*, 2006). Ozone gas optimizes the regeneration of tissue by creating the epithelialization and granulation. Djuicer *et al.*, (2012) studied the effect of two preparations of ozone gas on the reproductive performance of cattle suffering from RFM. The gas preparations were applied into body of uterus and the parameters of reproductive efficiency like calving to first insemination, interval from calving to pregnancy, relative pregnancy rate and number of services per conception was measured. The cows which were treated ozone spray have the same or improved reproductive performance parameters when compared to control group. The ethno veterinary practices have significant importance to veterinary treatment (Lin *et al.*, 2003) due to low cost and clear effectiveness (Mwale *et al.*, 2005). It is the common perception of the farmer that RFM is due to weakness and difficult birth of animal. Therefore, mixture of oil and milk is administered as source of energy. The milk of camel is preferred due to high mineral contents. The common salt is also rubbed on the back of animal for the expulsion of fetal membranes with this idea that it stimulates the uterus for contraction (Dilshad *et al.*, 2008)

## CONCLUSION

The RFM is an important reproductive disorder in dairy animals. It causes the great economic losses via reduction in milk production and reproductive efficiency of dairy cattle. The exact etiology of RFM is not completely understood due to multiple causes. The incidence of RFM can be reduced by improving the management and adopting the new techniques of treatments.

## CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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