RESEARCH ARTICLE

# Different Treatment Protocols of Retained Fetal Membranes and its Effect on Serum Profile, Uterine Involution and Postpartum Fertility in Cows

AI Shah[1], DM Patel[2], NP Sarvaiya[3], SP Madhira[4]

## Abstract

This study was undertaken on 36 freshly calved cows randomly divided into 6 equal groups under field conditions. Cows of group-VI that shed placenta within 8-12 hours postpartum naturally served as healthy control. The cows with retained fetal membranes (RFM, n = 18) for more than 12 hrs were managed either by manual removal of placenta without antibiotics (group-I), parenteral antibiotic (Ceftiofur 1 g i/m) for three consecutive days (group-II) or a combination of both (group-III). In group-IV and group-V, cows were administered with Inj. Oxytocin @ 50 IU i/m and Inj. Dinoprost tromethamine ($PGF_2\alpha$) @ 25 mg i/m, respectively, immediately after parturition and time of placental shedding was recorded. The overall prevalence of Brucellosis by RBPT was found to be 5.55 % amongst these 36 animals. The placental expulsion in groups following medicinal treatment was found to be 50 (3/6) % in Ceftiofur alone by 3 days (group-II), and 66.67 (4/6) % in Oxytocin (group-IV) and 100 (6/6) % in $PGF_2\alpha$ inj. (group-V) groups within 12 hrs. The time of uterine involution in groups I to VI was found to be 42.00 ± 1.94, 39.50 ± 0.99, 40.67 ± 1.39, 38.33 ± 1.55, 37.50 ± 1.02 and 37.33 ± 1.76 days, respectively, while the interval for the appearance of first postpartum estrus was 54.83 ± 2.06, 51.00 ± 1.05, 52.17 ± 1.96, 50.17 ± 2.03, 48.67 ± 1.90 and 49.17 ± 1.55 days, respectively, which did not vary statistically. The mean serum progesterone profile obtained on day 0 and day 21 postpartum was statistically non-significant between groups. However, it was significantly ($p$ <0.05) lower on day 0 as compared to day 21 in group-I, II and VI. The levels on day 0 coincided with the time of blood sampling after calving. The high level of serum P4 on day 0 in group-IV and V could be due to sampling immediately after calving. The serum calcium and phosphorus levels were significantly ($p$ <0.05) lower on day 0 than on day 21, but not the magnesium. The group effect was however non-significant for any of three minerals. It was observed that manual removal of RFM without parenteral antibiotics, resulted in puerperal metritis, cervicitis, pyometra which ultimately resulted into delayed uterine involution, delayed first postpartum estrus and thus, reduced the postpartum reproductive efficiency. It was inferred that the $PGF_2\alpha$ and Oxytocin injections could be used as a treatment of choice for prevention of RFMs in cattle.

**Keywords:** Cows, Retained fetal membranes, Therapeutic approaches, Serum progesterone and Minerals, Postpartum fertility.

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## Introduction

Retention of fetal membranes (RFM) is the most important postpartum complication and has great economic importance in the dairy industry. The state of placental maturity and the pathology of the fetal and maternal placenta are considered to be principal factors in the retention of the placenta, or it may arise out of insufficient uterine contraction, nutritional deficiency, hormonal imbalance, and reproductive diseases. Since RFM leads to uterine infection and a delay in the involution of the uterus, the future fertility of the animal could be adversely affected (Drillich *et al.,* 2006). RFMs have been associated with increased risk for endometritis, metritis, ketosis, and mastitis. These diseases can, in turn, lead to decreased fertility and potential losses in milk production (Laven and Peters, 1996). Reasonable and timely use of preventive and corrective measures, mainly with the object to remove the exciting causes could be considered as a rational way to deal with the condition. Oxytocin is one of the oldest drugs, most frequently used to stimulate uterine contractility in early postpartum cows (Bajcsy *et al.,* 2006). There are enough evidence that PGF2α has a direct effect on the bovine ovary apart from those of luteolysis, particularly with regard to the postpartum resumption of ovarian activity. The first postpartum dominant follicle has a slower growth rate and secretes less estradiol at the end of the growth phase (Sheldon and Dobson, 2004). Considering these facts, the

[1-4]Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Corresponding Author:** AI Shah, Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India, e-mail: adiishah@icloud.com

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present study was aimed to know the effect of RFM, use of oxytocin and PGF2α in the prevention of RFM, and their influence on serum progesterone and mineral profile, uterine involution and postpartum estrus in cows.

## Materials and methods

The study was undertaken on 36 freshly calved cows in villages of Anand and Dakor talukas of Gujarat. The cows were divided into six equal groups. Cows of group-VI (n = 6) that shed placenta within 8-12 hrs postpartum naturally served as healthy control. The cows with retained fetal membranes (RFM, n = 18) for more than 12 hrs were managed either by only manual removal of placenta without antibiotics (group-I) or with only parenteral antibiotic Ceftiofur 1 g (Wofur, Vetoquinol India Animal Health Pvt. Ltd.) i/m for three consecutive days (group-II), or a combination of manual removal plus Inj. Ceftiofur 1 g i/m for three days (group III). While cows in group-IV and group-V were administered with Inj. Oxytocin (Gynotocin, Svizera, Maneesh Pharmaceuticals) @50 IU i/m and Inj. Dinoprost tromethamine ($PGF_2α$, Lutalyse, Pfizer) @25 mg i/m, respectively, immediately after parturition, and time of placental shedding was recorded. The cows were followed for uterine involution by fortnightly rectal palpation and the occurrence of first postpartum estrus.

Blood samples were collected through jugular venipuncture, after the expulsion of a placenta in control group and before treatment in cases of RFMs, using 22 gauge needles in 9 ml serum clotting vacutainers on day 0 and again on day 21 postpartum. Serum samples were stored in deep freeze (–20°C) with a drop of sodium merthiolate (0.01%) until analyzed. The paired sera samples (both 0 and 21 days) were tested for the prevalence of Brucellosis by Rose Bengal Plate Test (RBPT). Serum progesterone concentrations were estimated by employing standard RIA technique of Kubasic *et al*. (1984). Labeled antigen (I125), antibody-coated tubes and standards were procured from Immunotech-SAS, Marseille Cedex, 9, France. The sensitivity of the assay was 30 pg/mL; the intra-assay coefficient of variation was 5.4 %, while the inter-assay variation was 9.1%. The levels of serum calcium, phosphorus and magnesium were estimated by Arsenazol-III method, Molybdate U.V. method, and Calmagite method, respectively, using standard procedures and assay kits procured from Coral Clinical System, Goa, with the help of Chemistry Analyser (Mindray, BS 120). The data were analyzed statistically using one way ANOVA, DMRT, and student's t-test to know the variation, if any, between and within groups.

## Results and discussion

The paired sera samples of the 36 cows under various groups including control tested by RBPT showed the prevalence of Brucellosis in 5.55 % (2/36) cows belong to oxytocin treatment group. The present incidence of brucellosis was found to be higher than the findings of Amin *et al.* (2004) and Degefa *et al.* (2011) from different parts of the globe.

### Placental Expulsion Time and Milk Production

The placental expulsion in Ceftiofur treated group-II was found to be in 50% (3/6) cows by 3 days, whereas in oxytocin and $PGF_2α$ Inj. groups IV and V, it occurred in 66.67% (4/6) and 100 % (6/6) cows within 12 hours of treatment. The effect of various treatment protocols on milk production was recorded for one month. There was a drastic reduction in milk production of the group treated with manual removal of RFM without antibiotic, whereas marginal lowering of milk production was noted in group-III with manual removal combined with a parenteral antibiotic for 3 days. There was an occurrence of puerperal metritis, cervicitis and pyometra in cows with manual removal without an antibiotic group, whereas no presence of such case was noted in other groups. The treatment with Inj. Ceftiofur sodium, Inj. Oxytocin and Inj. $PGF_2α$ did not affect the milk production, and the placenta was expelled within 12 hours of treatment.

Ceftiofur sodium inj. after calving reduces the chances of puerperal infections (Drillich *et al.,* 2006), manual removal of placenta was found to have an adverse effect on milk production and uterine involution (Majeed *et al.,* 2009). The $PGF_2α$ is given i/m shortly after parturition in buffalo significantly shortened the time taken to the expulsion of the placenta and also resulted in a highly significant reduction in the period for first postpartum estrus (Shalaby *et al.,* 1994).

### Uterine Involution and First Postpartum Estrus

The periods of uterine involution and occurrence of first estrus postpartum in cows with and without RFM under

**Table 1:** Uterine involution and occurrence of first estrus postpartum (days) in cows of various treatment groups for RFMs (Mean ± SE)

| *Sr. No.* | *Treatment group (n = 6 each)* | *Uterine involution (days)* | *First estrus postpartum (days)* |
|---|---|---|---|
| 1. | Manual removal without antibiotic | 42.00 ± 1.94 | 54.83 ± 2.06 |
| 2. | Inj. Ceftiofur sodium without removal | 39.50 ± 0.99 | 51.00 ± 1.05 |
| 3. | Manual removal with antibiotic | 40.67 ± 1.39 | 52.17 ± 1.96 |
| 4. | Inj. Oxytocin | 38.83 ± 1.55 | 50.17 ± 2.03 |
| 5. | Inj. PGF2α | 37.50 ± 1.02 | 48.67 ± 1.90 |
| 6. | Healthy control | 37.33 ± 1.76 | 49.17 ± 1.55 |

**Table 2:** Serum progesterone and minerals profile in cows treated with different protocols of retained fetal membranes (Mean ± SE)

| | | *RFM treatment groups* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Gr-I* | *Gr-II* | *Gr-III* | *Gr-IV* | *Gr-V* | *Gr-VI* |
| *Serum Parameter* | *Days post-partum* | *Manual removal of RFM without antibiotic* | *Inj. Ceftiofur without removal of RFM* | *Manual removal of RFM with Inj. Ceftiofur* | *Inj. Oxytocin soon after calving* | *Inj. $PGF_2\alpha$ soon after calving* | *Healthy control* |
| Serum P4 (ng/ml) | 0 | 0.36* ± 0.03 | 0.41* ± 0.05 | 0.40 ± 0.03 | 0.59 ± 0.03 | 0.63 ± 0.03 | 0.38**± 0.02 |
| | 21 | 0.51 ± 0.03 | 0.58 ± 0.06 | 0.47 ± 0.03 | 0.45 ± 0.04 | 0.54 ± 0.03 | 0.61 ± 0.04 |
| Serum Ca (mg/dl) | 0 | 8.45* ± 0.17 | 8.50* ± 0.11 | 8.32 ± 0.22 | 8.57* ± 0.19 | 8.53* ± 0.17 | 8.56* ± 0.09 |
| | 21 | 8.87 ± 0.18 | 8.87 ± 0.10 | 8.61 ± 0.20 | 8.93 ± 0.17 | 8.86 ± 0.13 | 8.89 ± 0.08 |
| Serum P (mg/dl) | 0 | 4.36* ± 0.10 | 4.36* ± 0.07 | 4.44* ± 0.10 | 4.48* ± 0.14 | 4.34* ± 0.11 | 4.43* ± 0.08 |
| | 21 | 4.79 ± 0.10 | 4.76 ± 0.08 | 4.74 ± 0.08 | 4.88 ± 0.11 | 4.76 ± 0.09 | 4.81 ± 0.08 |
| Serum Mg (mg/dl) | 0 | 2.24 ± 0.08 | 2.25 ± 0.07 | 2.29 ± 0.07 | 2.39 ± 0.11 | 2.36 ± 0.10 | 2.44 ± 0.09 |
| | 21 | 2.33 ± 0.07 | 2.33 ± 0.07 | 2.35 ± 0.07 | 2.44 ± 0.09 | 2.41 ± 0.10 | 2.46 ± 0.08 |

*Significant at $p$ <0.05; **Significant at $p$ <0.01 between days/periods
The variations between groups were statistically non-significant for all parameters

different treatment groups are presented in Table 1. The data did not vary statistically between groups for any of the traits. However, the mean values of both the traits in the control group were non-significantly shorter than other treatment groups, except those of $PGF_2\alpha$ and Oxytocin treated almost similar groups. These findings were in agreement with those of Nakhashi *et al.* (2006) and Dhami *et al.* (2012) in buffaloes.

**Serum Progesterone and Macro-Minerals Profile**

The mean levels of serum progesterone (ng/mL), calcium, phosphorus and magnesium (mg/dL) obtained on day 0 and on day 21 postpartum for cows under different treatment groups are shown in Table 2. The mean serum progesterone profile obtained on day 21 in group-I, II and VI was significantly ($p$ <0.05) higher than the values on day 0, whereas in group-IV and V, the trend was inversed without significant difference. The increased levels by day 21 could be due to luteinization of the first dominant follicle or silent ovulation resulting formation of CL in some animals by that day. The higher values of serum P4 on day 0 in group-IV and V was in fact due to much early blood sampling (soon after calving) before luteolysis of pregnancy CL, as treatment was initiated immediately after calving, compared to other groups where blood sampling was done some 8–12 hours after calving and natural expulsion of placenta or before start of treatment for RFM. However, at day 21 values of P4 were statistically at par in all the groups. Physiologically at parturition, the progesterone level drops which may explain the low value obtained on day 0 in most groups. Progesterone causes the uterine smooth muscles to be in a relaxed state, which may probably be the reason for the retention of fetal membranes. Present findings on serum P4 concurred well with those of Dhami *et al.* (2012) in Surti buffaloes with and without RFMs.

The mean serum calcium and inorganic phosphorus levels were significantly ($p$ <0.05) higher on day 21 in all groups as compared to those on day 0, except calcium in group III, but did not differ between treatment groups of RFM and healthy control at any of the periods. Similar non-significant variation in phosphorus levels has been reported by Sabry *et al.* (1997) and Semacan and Sevinc (2005) in cows with retained fetal membranes. Tiwari *et al.* (2001) however observed low levels of calcium in cows with retained fetal membranes. Mandali *et al.* (2002), El-Malky *et al.* (2010) and Thavani *et al.* (2011) reported low levels of calcium and phosphorus in buffaloes with RFM. The disturbance in the calcium metabolism and its utilization by the tissue results in the atony of the internal organs. During pregnancy especially at the last stage, there is the excessive mobilization of calcium, resulting in its less availability to the uterine tissue causing atony of uterus, leading to retention of fetal membranes. The significant rise in serum calcium level at day 21 over day 0, suggested activation of calcium homeostatic mechanism postpartum. The serum calcium and phosphorus profile, in general, show a gradual and significant rise with the advancement of the postpartum period in cows. Tillard *et al.* (2008) opined that low phosphorus values during the pre-calving and post-calving periods predispose the dam to retain fetal membranes. This may also be attributed to the decreased contraction of uterine muscles due to low phosphorus levels.

The differences in serum magnesium values obtained on day 0 and day 21 in all the treatment groups and those between treatment groups and the control group were

statistically non-significant ($p$ >0.05). Similar results were reported by Sabry *et al.* (1997) in cows. Mandali *et al.* (2002) also noted almost the same magnesium concentrations in the cases of retention of fetal membranes and control group of buffaloes. Thavani *et al.* (2011) however reported higher plasma magnesium levels on day 0, day 30 and day 45 than the present findings.

It can be concluded that the reproductive disorders like retention of fetal membranes which may occur after parturition leads to an increase in the number of days to first postpartum estrus. Hence, the postpartum breeding efficiency of the cows decreases significantly following RFM condition. It is also concluded that $PGF_2\alpha$ and Oxytocin can be used as a preventive measure of RFMs, while manual removal of the placenta with parenteral antibiotic therapy can be recommended in clinical cases of RFMs.

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