DETERMINATION OF SOME MINERALS CONCENTRATIONS IN COWS WITH RETAINED FETAL MEMBRANES IN KHARTOUM STATE- SUDAN

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ABSTRACT
This study was carried out to determine the concentration of some minerals in cows with retained fetal membranes (RFM) and to compare it with cows without RFM in Bahri locality, Khartoum, Sudan. This area contains a large number of dairy farms. A total of 60 cows (30 with retained fetal membranes and 30 without retained fetal membranes) were included. Data regarding the age, number of parities, and frequency of RFM were obtained during the visits to the farms. Blood samples were collected from jugular veins of these cows 12 to 24 hours after parturition. The estimation of serum Calcium, Iron and Phosphorus were done by using the spectrophotometric method, while Zinc was measured using Atomic absorption spectrophotometer. The mean values were compared using paired sample t test. Most visited farms reported having cases of RFM. The results showed that 63% of cows had retained placenta once, 28% had retained placenta twice and 8% had it three times. The mean values of Iron and Zinc were (83.70 ± 2.53 mg/dl), (0.25±0.03 mg/dl) consecutively and were significantly lower in cows with RFM than cows without RFM (86.1± 2.91 mg/dl), (0.28±0.03 mg/dl) at 12 to 24 hour after parturition. There was no significant difference between the mean value of Calcium and Phosphorus in RFM and Non RFM cows. In conclusion, minerals deficiency such as, iron, and zinc may be predisposing factor for the occurrence of retention of placenta in crossbred cattle. Maintaining an adequate dietary source of the minerals at the end of the pregnancy period is recommended.

KEYWORDS: Cows, minerals levels, retain of fetal membranes, Sudan.

I. INTRODUCTION
Retention of fetal membranes (RFM) is the failure to expel the fetal membranes within 12 to 24 hours after calving in bovines.[¹] It is one of the most common disorders affecting reproduction of dairy cattle.[²] It has a direct adverse effect on milk production and fertility of animals. It may result from several factors, such as abortion, forced labor, delayed gestation, early parturition, uterine atony, infections, seasonal and hormonal changes.[³]

It has been reported that the low serum concentrations of various minerals including Zn²⁺, Mg²⁺ and K⁺ in cows before parturition might cause or increase the risk of RFM.[⁴][⁵] Researchers reported that low serum Ca²⁺ concentration plays an important role in the development of RFM in cows,[⁶] whereas others[⁷][⁸] found that Ca²⁺ concentration was at the physiological level indicating that Ca²⁺, has virtually no role in the development of RFM.

Another important mineral is Zinc. It has a critical role in the repair and maintenance of the uterine lining following parturition and speeding return to normal reproductive function and estrus. Lower concentrations of zinc increase the chances of retention of fetal membranes in cows.[⁸] In Sudan, RFM is one of the conditions commonly seen in cattle with infertility problems. A previous study showed that the major reproductive problems were abortion, infertility stillbirth, metritis and retained placenta.[⁹] No other published data are available and therefore this study is one of the recent researches carried out to investigate the role of minerals deficiencies and its relation with reproductive disorders in Sudan.

The objective of this study was to determine the level of Ca²⁺, P⁴⁺, Fe⁵⁺ and Zn²⁺ in cows with RFM. Also to compare the concentration of these minerals with cows without RFM.

MATERIALS AND METHODS
i) Study area: This study has been conducted in the North district of Bahri locality where many dairy farms...
are established. The study was carried during the period from February 2018 to May 2018.

**ii) Experimental animals:** A total of sixty cows were included in this study. Thirty cows with RFM and 30 cows with no RFM.

**iii) Data collection**

a) **Questionnaires:** A pre structured questionnaire was carried out to collect data regarding: breed, the incidence of RFM in the farm, ages of cows and number of parities.

b) **Blood samples**

Thirty blood samples for sera preparation were collected from the jugular vein of animals using sterile disposable syringe. All samples were obtained within 12 to 24 hours after delivery. The samples were stored under cold conditions until delivered to the laboratory at University of Bahri, College of Veterinary Medicine. Serum was obtained after centrifugation and stored for further analysis.

c) **Mineral analysis**

Serum samples were analyzed at the Veterinary Research Laboratory - Soba using the spectrophotometric method for the determination of Phosphorus, Calcium and Iron while zinc was measured using Atomic absorption spectrophotometer (Uncan1 929 A.A UV spectrometer, England). These minerals were measured using Bio system estimation kit.

d) **Statistical analysis**

SPSS (statistical package for the social sciences) was used. Data was analyzed using Paired sample t test.

**RESULTS**

This study showed that 65% of cows were between three to five years old, 35% were above five years. Regarding the number of parities, 68% of cows were in one to three times of parity while 32% had more than three deliveries. Also it was found that, 64% of cows had retained fetal membrane once while 28% retained the membranes twice and 8% of cows retained their placenta's more than three times.

Mineral concentrations were measured in the serum of the two groups of cows. Calcium concentration in serum of cows with retained placenta was found to be 8.21±0.30 mg/dl while the calcium concentration in normal cows was found to be 8.3±0.29 mg/dl (Table 1 and Fig. 1). Using independent sample t test with confidence interval 95% and p value =0.248 there was no statistical significant difference between the means of Calcium in cows with RFM and normal ones.

The Phosphorus concentration of serum of cows with RFM was found to be 3.7±0.50 mg/dl while phosphorus concentration in normal cows was 3.73±0.17 mg/dl (Table 1 and Fig. 1). Using independent sample t test with confidence interval 95% and p value =0.060 there was no statistical significant difference between the means of phosphorus in cows with RFM and normal one.

The Zinc concentration in serum of cows with RFM was found to be 0.25±0.03 mg/dl while zinc concentration in normal cows was 0.28 ±0.03 mg/dl (Table 1 and Fig. 1). Using independent sample t test with confidence interval 95% and p value =0.000 there was a statistically significant difference between the means of Zinc in cows with RFM and normal one.

Finally Iron concentration in serum of cows with RFM was found to be 83.70±2.53mg/dl while Iron concentration in normal cows was found to be 86.10±2.91mg/dl (Table 1). Using independent sample t test with confidence interval 95% and p value =0.001 there was a statistically significant difference between the means of Iron concentration in cows with RFM and normal ones being lower in cows with RFM.

| Table 1: Concentration Ca$^{2+}$, P$^{3+}$, Zn$^{2+}$ and Fe$^{2+}$ in sera of RFM cows and non RFM cows. |
|-------------------------------------------------|----------|
| **Cow case** | **Mean ± SD(mg/dl)** |
| Ca$^{2+}$   | Non RFM: 8.30 ± 0.29 |
|            | Cows with RFM: 8.21 ±0.29 |
| P$^{3+}$    | Non RFM: 3.73 ± 0.17 |
|            | Cows with RFM: 3.70 ± 0.5 |
| Zn$^{2+}$   | Non RFM: 0.28 ± 0.03 |
|            | Cows with RFM: 0.25 ± 0.03 |
| Fe$^{2+}$   | Non RFM: 86.1±2.91 |
|            | Cows with RFM: 83.70 ± 2.53 |
DISCUSSION
Retention of fetal membrane (RFM) is a reproductive abnormality unique to cows' and buffalo among other domestic ruminants. RFM can result from various factors including deficiency in some vitamins and minerals. RFM can be a substantial risk factor for toxic puerperal metritis in buffaloes, can lead to economic loss due to infertility.

The cows involved in this study, were all cross breed and the age of the cows ranged between three to five years. A previous study reported the relation between the age of the animal and RFM, where the incidence increases with age. Also a previous study on 1401 cows, showed that the RFM rate in 2-year-old cows was lower than that in older cows. In addition retention of plaetza may also increase according to age and number of parities. In the present study, 68% of cows were in one to three time of parity while 32% had more than three deliveries which may increase the risk for RFM.

The four minerals understudy were Ca$^{2+}$, P$^{2+}$, Zn$^{2+}$ and Fe$^{2+}$. A number of studies reported the importance of minerals in the prevention of RFM, while other studies have found that additional supplementation of trace minerals can have positive effect on reproductive performance.

The mean concentration of Ca$^{2+}$ found in this study was higher than the study carried previously where it has been reported a concentration of 6.48 mg/dl ± 0.32 for cows with RFM compared to 9.41 mg/dl ± 0.39 in control group but still no significant difference between the two groups. The findings in this study are also similar previous studies which reported the presence of Ca$^{2+}$ at the normal levels, thus indicating that Ca$^{2+}$ has virtually no role in the development of RFM. On the other hand, some researchers reported that low serum Ca$^{2+}$ concentration plays an important role in the development of RFM in cows. The mean P$^{2+}$ concentration in cows with RFM was 3.66±0.15 mg/dl compared to cows without RFM (3.73±0.17mg/dl) with no significant difference between the two groups. The concentration of phosphorus found in this study is higher than that reported in an earlier study which reported low phosphorus concentration in cows with RFM. Phosphorus concentration during the pre-calving and post-calving periods predispose the dam to retain fetal membranes.

The mean Zn$^{2+}$ concentration in the current study was lower in cows with RFM being 0.25±0.03 mg/dl compared to 0.28±0.03 mg/dl in normal cows with a statistically significant difference between the two groups. This study came online with who reported that cows with RFM had low serum Zn$^{2+}$ concentration and this low levels can increase the chances of RFM.

Zinc is required to maintain epithelial tissue integrity and for keratin formation that provide a physiological barrier against infection. Zn$^{2+}$ can also affect the immune status via its role in cell proliferation. Cells deficient in Zn$^{2+}$ have decreased ability to proliferate.

The mean Fe$^{2+}$ concentration in cows with RFM was lower than the cows without RFM (83.7±2.5 mg/dl compared to 86.1±2.9 mg/dl, respectively) with a statistically significant difference. The present findings are in line with those reported in buffaloes and cows. The study reported a decreased concentration of Iron in cows and buffaloes retaining their fetal membranes. Feedstuffs usually may contain a significant amount of Fe$^{2+}$ and no supplementation may be needed, but Fe$^{2+}$ status may decline in late gestation.

CONCLUSION
RFM occur frequently in dairy cows in Bahri locality. Low concentration of Fe$^{2+}$ and Zn$^{2+}$ were found to be significantly different in cows with RFM compared to
cows with normal delivery. No difference was found with regard to Calcium and Phosphorus concentration.

The data presented in this study can be used as a baseline to study the serum macro and micro-minerals profile to reduce the incidence of RFM in crossbred cattle.

The concentration of minerals should be taken into consideration in the diet of cows. During the last gestation period supplement of macro and micro mineral should be considered.

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REFERENCES