

## SERUM CALCIUM AND MAGNESIUM CONCENTRATIONS AND THERAPY FOR RETAINED PLACENTA OF COWS.

M. TAWFIK and A.S.EL-BAKHMAY

Animal Reproduction Research Institute, Agriculture Research Centre Pyramids, Giza, Egypt.

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### SUMMARY

The purpose of the present study was to compare serum calcium and magnesium concentrations in different ages in cows with or without retained placenta (RP) and to evaluate treatment of cows with RP with oxytocin versus oxytocin combined with Cal- Bor- Mag solution. Blood samples were obtained within 12 hours of parturition from different breeds of cows with and without RP (n=38 and 30 respectively). Serum Ca and Mg concentrations were analyzed by atomic absorption spectrophotometry. 38 cases of RP in different breeds of cows were treated by infusion of either oxytocin dissolved in normal saline solution or oxytocin dissolved in Cal-Bor-Mag solutions. The intervals between parturition and initiation of treatment between 12-24 hours P.P. RP is the failure to expel all or part of the fetal membranes up to 12 hours after parturition. The positive response to the treatment was considered as the ex-

pulsion of the entire placenta within 2 hours after infusion.

Ca levels in cows with retained placenta were significantly lower than those in the control group (without RP). Cows in the control group and those with retained placenta were divided into subgroups based on age (3-4 , 5-7 years old). The serum Ca concentration of 3-4 years old cows with retained placenta was significantly higher ( $p<0.05$ ) than those of 5-7 years old. Serum magnesium levels showed no difference. Sixty percent of the cows treated with oxytocin in Cal-Bor-Mag solution responded positively to the treatment, compared to approximately thirty-nine percent of the cows treated with oxytocin in saline solution ( $p<0.05$ ).

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### INTRODUCTION

Retained placenta (RP) may result from a number

of factors, such as early parturition, uterine atony, abortion, forced labor, infections, and seasonal and hormonal disorders (Laven and Peters, 1996). In addition, it is well known that deficiencies of some vitamins and minerals induce or predispose animals to RP (Alacam, 2002, Hurley and Doane, 1989).

The physiological drop of the placenta after parturition requires adequate and regular uterine contractions (Morrow, 1980). The deficiency in secretion of PGF<sub>2</sub>, oxytocin and serum Ca concentration, which maintain adequate contraction of the uterus may increase the risk of dystocia, cause RP, and delay the involution of the uterus (Miller and Lodge, 1984 and Mutiga et al., 1993,).

Squire, (1980), Zhang, et al., (1992) reported that a low serum Ca concentration plays an important role in the development of RP in cows, while others (Lotthammer, 1983) found that the Ca concentration was at the physiological level indicating that Ca has virtually no role in the development of RP.

Mean while, Carson et al., (1978) found that when these animals were fed a ration enriched with supplemental bone meal for last 3 months of gestation the incidence of dystocia was reduced from 75% to 10%, the RP rate from 35% to 8% and the puerperal metritis rate from 70% to 10%. The serum Ca concentration in these cows was

reported to increase from 8.98 mg / dl to 10.26 / dl with this type of diet.

It had been reported that the low serum concentrations of various minerals including Zn, Mg. and K in cows before parturition might, cause or increase the risk of RP (Vandeplassche, et al., 1971, Stancioiu and Constantinescu, 1983 and McDowell, 1992,).

Holda et al;(1996) stated that oxytocin stimulates the final myometrial activity and the expulsion of the foetus after the Ferguson's reflex is induced during parturition, while Russe, (1982) add that oxytocin released must be sustained there after to expel the after birth placenta, Therefore, exogenous oxytocin had been suggested for prevention of retained placenta immediately postpartum (Curtis et al., 1973).

Actually, the systemic use of oxytocin was considered to be the most appropriate therapy in cases of RP (Roberts, 1971). Firmly attached placentas, which are difficult or impossible to remove prior to oxytocin infusion, become easily detachable directly after treatment (Mutiga et al., 1993). The objectives of the present study were to compare calcium and magnesium concentrations in serum, in cows with or without RP and with regard to age and to determine the relative effectiveness of intravenous administration of oxytocin dissolved in normal saline as compared to oxytocin dissolved in Cal-Bor-Mag solution, as a treat-

ment of RP in cows.

## MATERIALS & METHODS

Sixty eight cows 3-7 years old were used. Thirty eight of the cows had RP and thirty cows as control (without RP). Out of 68 (28 were indigenous breed cows, 12 were Brown -Swiss, 13 were Holstein-Friesian and 15 mixed breeds) belonged to 3 different private dairy farms free from Brucella as indicated by periodic investigation in Giza Governorate, during the years between 2006-2007.

Animals were fed a ration of wheat straw, concentrated feed and barseem or darawa, according to the system of feeding in each farm. The cows with the undropped placenta within the first 12h of parturition were assigned to the RP group; while the drop of the placenta within 12h were assigned as control group. Cows in the control group and those with RP were divided into subgroups based on age (3-4 , 5-7 years old).

38 cows with the RP group were divided into 2 subgroups (group A, n = 18) (group B, n = 20 ). 10 ml blood sample from each animal were taken from jugular vein within 12h of delivery, using an evacuated sterile blood collecting tubes. The samples were centrifuged at 3000 rpm for 20 min and serum samples were stored at - 20°C until analysed.

Serum Ca and Mg concentrations were analysed with atomic absorption spectrophotometer (Perkin Elmer 370 Model) (Henry, et al.,1974) before an eventual treatments of RP.

Cows with RP were treated intravenously with either 50 IU, oxytocin (5ml of oxytocin® of (ADWIA Co.) dissolved in 500 ml saline solution (group1) i.V. infused 0.9% sodium chloride (EL Nasr Pharmaceutical & chemicals Co.), while group B infused by 50 IU oxytocin dissolved in 500 ml Cal-Bor-Mag solution (CAL-BOR-MAG injection, ADWIA Co.), containing 24.17 gm calcium gluconate and 2.09 gm magnesium chloride (anhyd).

All infusions lasted about 15 min. The positive response to treatment was the expulsion of the entire placenta within 2 h of the infusion without additional treatment. The interval between parturition and initiation of treatment must be in the range between 12-24 h of parturition.

### Statistical analysis

The relationships between serum calcium, magnesium levels and age of the cows with and without RP using student's t-test (Sokal and Rohlf, 1987) The differences between the results of the two treatments for RP were tested using Pearson's chi-square analysis on contingency tables (Fleiss, 1981). The results were expressed as mean  $\pm$  SEM.

## RESULTS

The determination of the mean serum concentration of Ca in RP cows was significantly lower than those of control group as shown in table 1 , whereas serum magnesium was not significant ( $p < 0.05$ ).

Serum Ca concentration in 3-4 year-old cows with RP was higher than those of 5-7 years old (table 2) but no differences were seen according to age in the control group (table 3).

There were more positive responses to treatment among cows treated with oxytocin in Cal-Bor-Mag solution than among cows treated with oxytocin in saline (table 2).

There were more positive responses to treatment among cows treated with oxytocin in Cal-Bor-Mag solution than among cows treated with oxytocin in saline (table 2).

Table 1 : Serum Ca and Mg concentration in the cows with and without RP.

Group	RP	Control
Parameters	Mean $\pm$ SE	Mean $\pm$ SE
Ca (mg / dl)	6.65 $\pm$ 0.69a	9.42 $\pm$ 1.60b
Mg (mg / dl)	2.09 $\pm$ 0.42	2.12 $\pm$ 0.34

(a,b) :  $p < 0.05$  the difference between the values marked with various letters in the same line is significant.

Table 2: mean serum Ca and Mg concentrations in cows of different ages with RP.

Group	3-4 years old (n = 15)	5-7 years old (n = 23)
Parameters	Mean $\pm$ Se	Mean $\pm$ SE
Ca (mg / dl)	7.29 $\pm$ 0.78a	6.21 $\pm$ 0.38b
Mg (mg / dl)	1.98 $\pm$ 0.21	2.12 $\pm$ 0.14

(a,b): ( $p < 0.05$ )

The difference between the values marked with various letters in the same line is significant.

Table 3: Mean serum Ca and Mg concentration in cows of different ages in the control group.

Group Parameters	3-4 years old (n = 17)	5-7 years old (n = 13)
	Mean ± SE	Mean ± SE
Ca (mg / dl)	9.95 ± 0.75	10.37 ± 1.79
Mg (mg / dl)	2.08 ± 0.27	2.23 ± 0.38

Table 4 : Results of treatments with oxytocin in saline or oxytocin in Cal-Bor-Mag solution of cows with retained placenta.

treatment	Response to treatment			
	Positive* (n,%)		Negative (n,%)	
Oxytocin in saline n = 18	7	39c	11	61
Oxytocin in Ca-bor-Mag n = 20	12	60d	8	40

(a, b, c, d) Numbers within a column with different letters are significant (P<0.05)

\* Expulsion of the placenta within 2h of treatment

## DISCUSSION

The results from this study show that cows with RP have lower serum calcium concentrations within 12h of delivery than control cows without RP. This results are in agreement with that obtained by ( Shukla et al., 1983 and Zhang et al., 1992). They suggested that the serum Ca. concentration of RP animals ( $6.27 \pm 0.18$  mg/dl) was lower than postpartum cows without RP ( $7.40 \pm 0.18$  mg/dl). In other species, decreased calcium levels can lead to reduced myometrial activity. In sows, induced hypocalcaemia led to significantly reduced uterine activity during and after parturition (Ayliffe, et al., 1984). In cows, induced hy-

pocalcemia led to rapid reduction in the frequency and amplitude of uterine contractions, which closely paralleled blood calcium concentration (Al-Eknaah and Noakes, 1989). When blood calcium concentrations increased, uterine activity recovered. Another report revealed that even mild hypocalcemia reduced or abolished the response of the uterus to oxytocin in parturient cows (Odegaard, 1977). Therefore, it would be interesting to compare recordings of myometrial activity and serum calcium concentration in cows with and without RP, the normal plasma concentration of Ca, Mg in cows were regarded as 8-12 mg / dl (Bari, et al., 1996), 1.8-2.3 mg / dl respectively (Braak et al., 1987). In this study the average serum Ca was  $6.65 \pm 0.69$  and  $9.42 \pm 1.60$  mg / dl in

RP and control cows respectively and the average serum Mg was  $2.09 \pm 0.42$  and  $2.12 \pm 0.34$  in RP and control cows respectively.

So the serum Ca concentrations were significantly lower in RP than in control cow while of serum Mg were within the normal physiologic values in RP and control cows. Skula et al. (1983) and Bari et al. 1996 suggested that there was a relation between the age of the animal and RP; thus RP incidence increases with age. In research conducted by Erb and martin, 1980 on 1401 cows, it was stated that RP rate in 2-years-old cows was lower than in 4-years old cows. Ocal et al. (1999) reported that cows 2-3 years old with RP had higher serum Ca (9.41 and 8.72 Mg / dl) levels than cows 3-6 years old. In this research, the serum Ca concentration of the cows with RP in the 3-4 years old group was higher than that in the cows in the 5-7 years old group ( $p < 0.05$ ). No difference was noted between age group in respect of Mg levels. Such a decrease in vitamin D receptors, which increase the Ca absorption from the intestine that occurred due to aging, may be considered as the result of low Ca levels in older cows.

In the present study, the interval between delivery of the calf and the initiation of injection of oxytocin infusion occurred after 12-24 hours P.P. these results of treatment are in agreement with squire, (1980) who reported that oxytocin increased the uterine tone and expulsion of fetal membranes

and improved reproductive efficiency. He added that, oxytocin was of little benefit after 24-28 hours P.P..

In vitro studies of rat and human myometrial tissue (Kawarabayashi, et al., 1997, Monga, et al., 1999) showed that oxytocin plays an important role in the regulation of uterine contractility by increasing intracellular free calcium through mobilization of calcium from extracellular and intracellular sources. In cows, hypocalcemia, which was often accompanied by hypomagnesemia (Braak, et al., 1987, Sansom, et al., 1983) was a risk factor for RP (Chassagne and Chacornac, 1994).

The Cal-Bor-Mag solution is used as therapy for periparturient paresis in cow. The possible effect of magnesium on uterine contractility in cows is unknown.

In conclusion, cows with RP had significant lower serum calcium levels, within 12hr of parturition, than cows without RP. Magnesium concentrations in serum, measured within 12hr of parturition, were not different between cows with and without RP.

Further more, significantly more cows with RP were responded positively to treatment with oxytocin in Ca-Bor-Mag solution than that treated with oxytocin in saline solution.

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# قياس تركيز محل الكالسيوم والمغنسيوم و علاج احتباس

## المشيمة في الأبقار

محمد توفيق سعيد / احمد شعبان البخمي

معهد بحوث التناسليات الحيوانية - الهرم - الجيزة مصر - مركز البحوث الزراعية

الهدف من إجراء هذا البحث هو مقارنة تركيز الكالسيوم والمغنسيوم في الأعمار المختلفة للأبقار التي تعاني من احتباس المشيمة وبدون احتباس مشيمة بعد الولادة وتقييم علاج الأبقار التي تعاني من احتباس المشيمة بواسطة هرمون الأوكسيتوسين المضاف إلى محلول الملح الفسيولوجي أو محلول الكال - بور - ماج .

تم أخذ عينات دم بعد ١٢ ساعة من الولادة من سلالات مختلفة من الأبقار التي تعاني من احتباس المشيمة (٣٨ بقرة) ومن المجموعة الضابطة (٣٠ بقرة لا تعاني من احتباس المشيمة). وتم قياس تركيز الكالسيوم والمغنسيوم باستخدام

### Atomic Absorption spectrophotometry.

قسمت الأبقار التي تعاني من احتباس المشيمة إلى مجموعتين مجموعة منها عولجت بواسطة ٥٠ وحدة دولية أوكسيتوسين المضاف إلى محلول الملح الفسيولوجي والأخرى بنفس الهرمون ولكن بإضافته إلى محلول الكال - بور - ماج وبدأ العلاج في الفترة من ١٢ حتى ٢٤ ساعة من الولادة. وقد تحدد الأبقار التي تعاني من احتباس لمشيمة بأنها الأبقار التي تفشل في قذف الأغشية الجنينية بالكامل أو أجزاء منها حتى مرور ١٢ ساعة من الولادة. مع ملاحظة مدى استجابة أو نجاح العلاج بأنه قدرة البقرة على قذف المشيمة بكاملها في مدى ساعتين من بعد المحاليل الوريديّة. أثبتت النتائج أن مستوى الكالسيوم في الأبقار التي تعاني من احتباس المشيمة أقل منه في أبقار المجموعة الضابطة.

وقسمت جميع الأبقار محل الدراسة في هذا البحث سواء في المجموعة التي تعاني من احتباس المشيمة أو في المجموعة الضابطة إلى مجموعات أخرى طبقاً لأعمارها من (٣-٤ سنة) وأخرى من (٥-٧ سنة).

أثبتت النتائج أن مستوى الكالسيوم في الأبقار التي أعمارها من ٣-٤ سنة والموجودة في مجموعة في مجموعة الأبقار التي تعاني من احتباس المشيمة أكبر منه في الأبقار التي أعمارها من ٥-٧ سنة بدرجة معنوية (على مستوى ٠,٠٥). في حين أن مستوى المغنسيوم لم يظهر أي اختلاف معنوي في أي مجموعة. وقد وجد أن ٦٠% من الأبقار التي عولجت بالأوكسيتوسين المضاف إلى محلول الكال - بور - ماج أظهرت استجابة إيجابية لهذا المحلول الوريدي بينما كانت نسبة الأبقار التي استجابت إيجابياً للأوكسيتوسين المضاف إلى محلول الملح هي ٣٩% تقريباً.