

## **Effect of Injection of Calcium Gluconate Solution on Fertility Traits of Postpartum Cross-Bred Dairy Cows in Khartoum, Sudan**

**Manal Sulum Sulieman**

*Department of Clinical Medicine, Faculty of Veterinary Medicine, University of Khartoum,  
P.O. Box 32, Khartoum North, Khartoum, Sudan.*

*Corresponding author: Tel.: +249912170901 E-mail: [vetuk1995@yahoo.com](mailto:vetuk1995@yahoo.com)*

### **Abstract**

The study aimed to evaluate the effect of subcutaneous injection of a solution contains calcium gluconate, magnesium hypophosphite and glucose into cross-bred dairy cows during the early postpartum period (PPP) on the rates of the first service non-return (FSN) and repeat breeding (RB). A total of 150 cows were selected randomly and divided into treated cows (no. = 90); these were the cows that were subcutaneously injected with 100 ml of the solution, and untreated cows (no = 60); these were the control cows that were not injected with the solution. Based on the number of parities, treated and untreated cows were further subdivided into primiparous and multiparous cows to compose four experimental sub-groups: treated primiparous (no. = 15 cows), treated multiparous (no. = 75), untreated primiparous (no. = 10 cows) and untreated multiparous (no. = 50 cows). The duration of postpartum period (PPP), the days open (DO), the number of services per conception (NSP), the rates of the FSN and RB were determined for each sub-group. The results revealed significantly ( $P < 0.0001$ ) shorter PPP, DO and NSC, in treated multiparous and untreated primiparous cows compared to cows in the two other sub-groups. The rate of the FSN was higher (70.0%) in untreated primiparous cows and treated multiparous cows (64.0%) than the rates (16.0%) of this trait in untreated multiparous cows and treated primiparous cows (6.6%). The rates of RB were lower (0.0%) in untreated primiparous cows and treated multiparous cows (13.3%) than the rates in treated primiparous cows (40.0%) and untreated multiparous cows (42.0%). The study concluded that subcutaneous injection of 100 ml of a solution contains calcium gluconate, magnesium hypophosphite and glucose during the early postpartum period could elevate the rate of the FSN and alleviate the rate of RB in multiparous cross-bred dairy cows, however; this treatment protocol had worsened the reproductive performance of primiparous cows. Further studies to measure the blood levels of calcium, phosphorous, magnesium, glucose and other blood constituents related to fertility after postpartum subcutaneous injection of a solution contains calcium gluconate, magnesium hypophosphite and glucose are recommended

**Keywords:** Calcium gluconate; Postpartum; dairy cows, first service non-return; repeat breeding.

### المستخلص

هدفت الدراسة إلى تقييم تأثير الحُثن تحت الجلد لمحلول يحوي الكالسيوم قلوكونيت والماغنيزيوم هايبوفوسفيت والجلوكوز لأبقار اللبن الهجين، خلال الفترة المبكرة بعد الولادة، على معدلات الحمل من التلقيحة الأولى وتكرار الشبق. أختبرت 150 بقرة عشوائياً وقُسمت إلى أبقار مُعالَجة (العدد = 90) وهذه مثلت الأبقار التي حُقنت 100 مليليتراً من المحلول تحت الجلد ، ومجموعة تحكم (العدد = 60)، وسُميت الأبقار غير المُعالَجة. بناءً على عدد الولادات، قُسمت الأبقار المُعالَجة إلى مجموعتين: مجموعة أبقار الولادة الواحدة المُعالَجة (العدد = 15)، ومجموعة الأبقار عديدة الولادات المُعالَجة (العدد = 75)، وأيضاً قُسمت الأبقار غير المُعالَجة إلى مجموعتين بناءً على عدد الولادات، مجموعة أبقار الولادة الواحدة غير المُعالَجة (العدد = 10)، ومجموعة الأبقار عديدة الولادات غير المُعالَجة (العدد = 50). تمَّ تحديد طول فترة اللاشبق بعد الولادة، وعدد التلقيحات اللازمة للحمل، و طول الفترة المفتوحة، ومعدلات الحمل من التلقيحة الأولى وتكرار الشبق لكل مجموعة. أوضحت النتائج وجود تدني معنوي ( $P < 0.0001$ ) في طول فترة اللاشبق بعد الولادة، وطول الفترة المفتوحة وعدد التلقيحات اللازمة للحمل في الأبقار عديدة الولادات المُعالَجة وأحادية الولادات غير المُعالَجة مقارنةً بالأبقار في المجموعتين الأخريتين. كان معدل الحمل من التلقيحة الأولى أعلى (70.0%) في أبقار الولادة الواحدة غير المُعالَجة وفي الأبقار متعددة الولادات المُعالَجة (64.0%) مقارنةً بمعدل 16% لهذه الصفة في الأبقار متعددة الولادات غير المُعالَجة و 6.6% في أبقار الولادة الواحدة المُعالَجة. كان معدل تكرار الشبق أدنى (0.0%) في أبقار الولادة الواحدة غير المُعالَجة وفي الأبقار متعددة الولادات المُعالَجة (13.3%) مقارنةً بمعدل 40.0% في أبقار الولادة الواحدة المُعالَجة و 42.0% في الأبقار عديدة الولادات غير المُعالَجة. خلصت الدراسة إلى أن حقن 100 مليليتراً تحت الجلد لمحلول يحوي الكالسيوم قلوكونيت والماغنيزيوم هايبوفوسفيت والجلوكوز خلال الفترة المبكرة بعد الولادة إستطاع أن يرفع معدل الحمل من التلقيحة الأولى و يقلل معدل تكرار الشبق في أبقار اللبن عديدة الولادات، في حين أن هذا البروتوكول العلاجي أدى إلى تدني الأداء التناسلي في أبقار الولادة الواحدة. التوصية بدراساتٍ أخرى لقياس مستويات الكالسيوم، والفوسفور، والماغنيزيوم والجلوكوز ومكونات الدم الأخرى ذات العلاقة بالخصوبة بعد حقن هذا المحلول خلال الفترة المبكرة بعد الولادة.

**الكلمات المفتاحية:** كالسيوم قلوكونيت، الفترة المبكرة بعد الولادة، ابقار اللبن، التلقيحة الأولى ، تكرار الشبق.

### Introduction

The optimization and maintenance of a high profit of a dairy herd are the goals of the dairy farmers and represent a continuous challenge especially when dairy cattle are reared under stressful conditions. To achieve these goals, farmers have to imply good dairy management practice for their herd by improving the overall health indices and optimizing the reproductive performance. The fertility problems of dairy cows are encountered among the most important factors that contribute to economic losses in dairy farming (Gross *et al.*, 2016; Karrar *et al.*, 2017). The reproductive efficiency of a herd is almost evaluated by the measurement of the fertility traits such as the duration of postpartum period (PPP), the days open (DO), the number of services per conception (NSC), and thereafter, the rates of the first service non-return (FSN) and repeat breeding (Walsh *et al.*, 2011; Sulieman *et al.*, 2017). The efficient reproductive performance of a dairy cow requires a short PPP of 60 -70

days, DO of 85-90 days, NSC of 1.5 and calving interval (CI) of about one year (DeJarnette *et al.*, 2007). The repeat breeding syndrome has been considered one of the major causes of economic losses in dairy farming due to extended length of the days open and, as a sequel, prolonged inter-calving (Wodaje and Mekuria, 2016). The incidence of repeat breeding syndrome in dairy cows is varying from less than 1% (Saraswat and Purohit, 2016) to more than 28% (Ahmed, 2009, Sulieman *et al.*, 2017). Any attempt for the optimization of the reproductive indices values is expected to result in a high rate of non-return rate to a low rate of repeat breeding syndrome. Calcium, magnesium and phosphorus are interrelated macrominerals that serve as important constituents of body fluids and they play important roles in the maintenance of acid-base balance and other vital functions in the body. Blood calcium level decreases in postpartum cows and reaches the lowest concentration within the first 24 hours postpartum and returns to normal, in

healthy cows, within 2-3 days postpartum (Martinez *et al.*, 2012). Dairy cows with decreased blood calcium levels during the early postpartum period may experience subclinical hypocalcemia (Reinhardt *et al.*, 2011) or milk fever (Braun *et al.*, 2004; Sulieman *et al.*, 2014). Subclinical hypocalcaemia has a negative effect on the health and postpartum reproductive performance of dairy cows (Caixeta *et al.*, 2017) and has been reported to affect more than 50% of dairy cows (Reinhardt *et al.*, 2011). In the Sudan, the effect of subclinical hypocalcaemia in dairy cows has not yet been studied. To maximize the profile of health and fertility indices, dairy cows have to be properly managed during the early PPP (transition period) because dairy cows, in this period, undergo severe negative energy balance. The relationships between blood metabolites measured during the transition period and fertility of dairy cows have been reported worldwide (Kurykin *et al.*, 2011; Obese *et al.*, 2015) and in Sudan (Sulieman *et al.*, 2017) but with a high degree of incompatibility. However, the beneficial therapeutic effects of combining magnesium hypophosphite and glucose in calcium gluconate or calcium borogluconate solutions on the concentration of blood in-organic phosphorus and magnesium represents a conflicting area among researchers (Braun and Jehle, 2007; Daunoras *et al.*, 2008). Therefore, this study is designed to evaluate the effect of Hipracal- FM<sup>®</sup>, a calcium gluconate solution, administered subcutaneously on the reproductive traits of cross-bred dairy cows in the Sudan during the first 24 hours postpartum.

#### **Materials and Methods:**

##### **Animals and Management:**

The animals used in this study were 150 clinically healthy cross-bred (Holstein Friesian X Zebu cattle) dairy cows of different ages (3-10 years) and parities (1-4) that calved between January and December 2008. The cows were raised in a commercial dairy farm in Khartoum North, Khartoum State, Sudan. The barns

were constructed from iron poles and corrugated iron sheets which provided inadequate shade. Two types of feed had been provided: green fodder, (*Medicago sativa*), which had been provided *ad libitum* two times a day, and a concentrate, which had been provided twice a day at 10kg/cow/day. Drinking water was freely accessed. One bull with known fertility record was moving freely within the herd and natural breeding was practised.

##### **The Treatment Protocol and Experimental Design:**

The drug used in this study was Hipracal-FM<sup>®</sup> (Laboratorios Hipra, S.A. 17170 Amep (Girona) Spain). The active ingredients contained in one ml of Hipracal-FM<sup>®</sup> were calcium gluconate: 175.0 mg, glucose: 100.0 mg and magnesium hypophosphite: 35.0 mg. A total of 150 cows were selected randomly and divided into treated cows (no. = 90); these were the cows that were subcutaneously injected with 100 ml of the solution, and untreated cows (no = 60); these were kept as control cows and were not injected with the solution. Based on the number of parities, the treated and control cows were further subdivided into primiparous (number of parity = 1) and multiparous (no. of parities  $\geq 2$ ) cows to compose four experimental sub-groups: treated primiparous (no. = 15 cows), treated multiparous (no. = 75), untreated primiparous (no. = 10 cows) and untreated multiparous (no. = 50 cows). The sorting of the cows to be injected or not injected was based only on the possibility of administration of the drug to cows during the first 24 hours postpartum which was controlled by the availability of the drug, the willingness of the owner and the ability to inject the cows during this certain period. The recommended dose of Hipracal-FM<sup>®</sup> is 100-500 ml per cow. In this study, the minimum dose range was selected (100 ml/cow as a single dose). The age and the number of parities for each cow were reported by reviewing the farm record. During the study, all selected

cows were monitored for their reproductive behaviour from the day of parturition until the next pregnancy was confirmed by rectal palpation. Certain fertility traits were monitored, including the duration of postpartum period (PPP), which was determined by counting the days from the day of parturition to the day of observable oestrous signs for each cow; the days open (DO), which was determined by counting the number of days from calving to the day of conception for each cow, and the average was calculated, the number of services per conception (NSC), which was the number of services required for pregnancy for each cow. The date of non-return oestrus in cows that were later diagnosed pregnant was regarded as the date of conception. The first service non-return rate (FSNR) for each group was calculated by dividing the number of cows within the certain group that conceived from the first insemination by the total number of cows inseminated within the same group. Diagnosis of pregnancy was carried out by rectal palpation between 60 and 90 days after mating. Based on the NSC, treated

and untreated cows were sorted into two categories: category-1; represented first service non-return cows (FSNC) and these were the cows that required one insemination to conceive and category-2; represented the repeat breeders (RP) which were the cows that required three or more than three inseminations to conceive.

#### Statistical analysis

Data were analysed with IBM SPSS software package for statistics 22 for Windows. Data were presented as means and standard deviation (SD). One- way ANOVA was used to compare means. Significant differences were considered when  $P$ -value  $\leq 0.05$ .

#### Results

The duration of postpartum period (PPP), the days open (DO), and the number of services per conception (NSC) for treated and untreated cows reported in this study are shown in table-1. The longest (82.1 days) and the shortest (53.5 days) durations of PPP were reported for untreated multiparous and untreated primiparous respectively. The treated primiparous exhibited the highest (2.5) NSC.

Table-1: Mean ( $\pm$ SD) Postpartum Period (PPP), Days Open (DO) and Nnumber of Services per Conception (NSC) in Cross-bred Dairy Cows Treated with a Calcium gluconate Solution during the First 24 Hours Postpartum

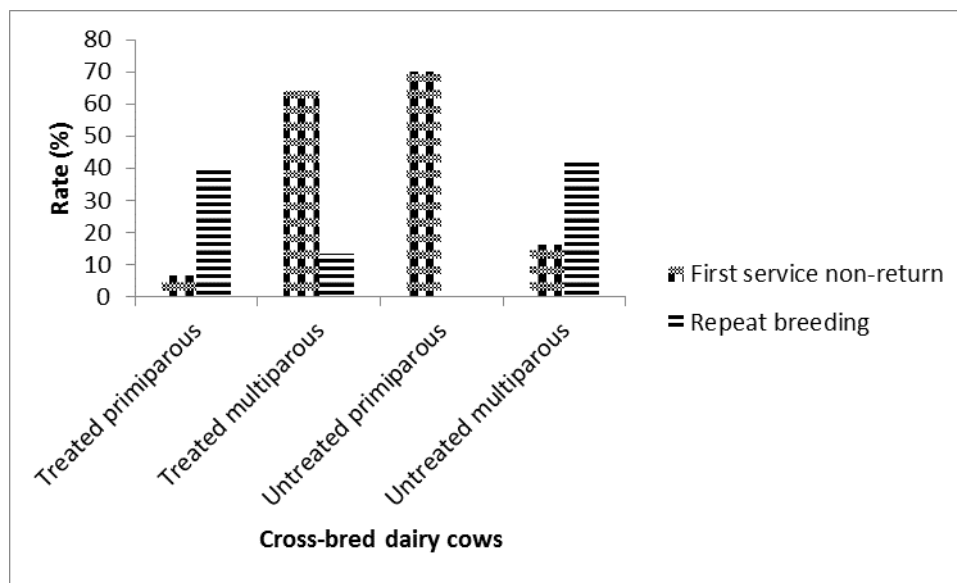
Fertility Trait	Treated cows (n = 90)		Control cows (n = 60)	
	Primiparous (n = 15)	Multiparous (no = 75)	Primiparous (no= 10)	Multiparous (no = 50)
PPP (days)	72.8 $\pm$ 12.6*	56.7 $\pm$ 7.1*	53.5 $\pm$ 4.3*	82.1 $\pm$ 15.2*
DO (days)	106.3 $\pm$ 19.9*	68.3 $\pm$ 17.8*	60.2 $\pm$ 7.8*	111.4 $\pm$ 28.0*
NSC	2.5 $\pm$ 0.8*	1.5 $\pm$ 0.8*	1.3 $\pm$ 0.4*	2.3 $\pm$ 0.8*

\* Means within the same row for each fertility trait differ significantly at  $P < 0.0001$ .

The findings of the rates of the first service non-return and the repeat breeding are shown in figure-1. The highest rate (70.0%) for the first service non-return was reported for untreated primiparous cows followed by the treated multiparous

cows which showed a rate of 64.0% for this reproductive trait. The treated primiparous cows exhibited the lowest rate (6.6%) for the first service non-return and the second highest rate (40.0%) for repeat breeding. The highest repeat breeding rate

(42.0%) was reported for untreated multiparous cows.



**Figure1:** The Rates of the First Service non-return and Repeat Breeding in Cross-bred Dairy Cows Treated with a Calcium Gsluconate Solution during the First 24 Hours Postpartum

### Discussion

The fertility traits (PPP, DO, NSC, the rates of FSN and RB) that were considered in this study are those which are usually determined by dairy practitioners to evaluate the reproductive performance of dairy cows (Walsh et al., 2011; Sulieman et al., 2017). The result of the current study revealed poor reproductive performance of multiparous dairy cows that did not receive Hipracal- FM<sup>®</sup> during the First 24 hours postpartum (untreated multiparous cows) indicated by the prolonged PPP (82.1 days), prolonged DO (111.4 days), increased NSC (2.3) and thereafter decreased FSNR (16.0%) and increased RBR (42.0%) when compared with standard values for reproductive performance reported by many authors (McDowell, 1985; Opsomer et al., 1996; DeJarnette et al., 2007). However; these values of fertility traits were within the standard values in multiparous cows that received the mentioned treatment protocol during the first 24 hours postpartum (treated multiparous cows). The poor reproductive performance of untreated multiparous dairy cows reported in this study is compatible with the previously reported findings for dairy cows reared

under tropical conditions of Sudan (Abdel Gadir et al., 2007; Sulieman et al., 2017). The poor reproductive performance of multiparous dairy cows is attributed to many factors such as uterine infections and other apparent postpartum reproductive problems. In this study, the cows that were selected were apparently healthy, this situation suggests the possibility of metabolic disorders. Untreated primiparous cows exhibited optimal reproductive performance values (53.5 days, 60.2 days, 1.5, 66.6% and 0.0%) for PPP, DO, NSC, FSNR and RBR consecutively. The reproductive performance of primiparous cows is still representing a matter of argument among researchers. Some researchers have reported the superseding of primiparous cows on biparous and multiparous ones regarding the reproductive performance (Balendran *et al.*, 2008, Marques *et al.*, 2015) and other studies have claimed the opposite (Asseged and Birhanu, 2004). Balendran *et al.* (2008) reported first insemination pregnancy rate of 42.9% to primiparous lactating cows compared to 20.0% in multiparous lactating cows of 2-4 parities. The differences in reproductive performance between primiparous and

multiparous dairy cows have been proposed to be due to differences in blood levels of certain metabolites between these groups of cows (Folnozic *et al.*, 2016), although some authors reported that the influence of parity on the resumption of ovarian cycle is modulated by factors different from the nutrition-related changes during the postpartum period in dairy cows (Tanaka *et al.*, 2008). The difference in concentration of blood metabolites between primiparous and multiparous cows is still representing a conflicting area. Most of the research findings confirmed that the multiparous cows are more vulnerable and susceptible to metabolic stress during the transition period (Wathes *et al.*, 2007). However; Folnozic *et al.* (2016) reported that the primiparous cows were more susceptible to metabolic stress during the transition period and their metabolic and endocrine profiles were more unbalanced compared to the multiparous cows. The subcutaneous administration of 100 ml Hipracal-FM<sup>®</sup> as a single dose during the first 24 hours postpartum, in this study, could elevate the rates of the first service non-return and alleviate the rate of repeat breeding in cross-bred multiparous dairy cows. However; this treatment had worsened the rates of the first service non-return and repeat breeding in primiparous cross-bred dairy cows. The positive effect of the treatment protocol used in this study on the reproductive performance of multiparous cows was in agreement with the results of many authors (Stevenson *et al.*, 1999, Feri *et al.*, 2007; Martienez *et al.*, 2016), However; the negative response of primiparous to the calcium treatment during the first hours of calving was in line with previously reported findings (Martinez *et al.*, 2016; Saraswart *et al.*, 2016) and controverted some other reports (Feri *et al.*, 2007). The somewhat controverting reports on the response of dairy cows to administration of calcium gluconate solution containing magnesium hypophosphite and glucose during the

early postpartum period suggest the effects of management and genetic differences in the responses of these individuals to lactation. Amanlou *et al.* (2016) suggested that prophylactic subcutaneous infusion of calcium at calving can improve postpartum calcium status in Holstein cows and intake at one day in milk. Alteration in Calcium and phosphorus ration may affect ovarian function through the blocking action on pituitary gland (Yasothi, 2014).

### Conclusion

The findings of this study concluded that a subcutaneous administration of a single dose of 100 ml Hipracal-FM<sup>®</sup>, a calcium gluconate solution containing magnesium hypophosphite and glucose during the first 24 hours postpartum could optimize the reproductive performance of cross-bred multiparous dairy cows and worsen the reproductive performance of primiparous cross-bred dairy cows in the Sudan. Further studies to evaluate the effect of administration of Hipracal-FM<sup>®</sup> during the first 24 hours postpartum on the concentration of certain blood constituents related to fertility are recommended.

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