

# The Influence of Subclinical Hypocalcemia on Production and Reproduction Parameters in Israeli Dairy Herds

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## ABSTRACT

A large percentage of mature dairy cows experience some degree of hypocalcemia during the first days post-calving. In some cases calcium concentrations decline to levels that disrupt neuromuscular function, resulting in the clinical syndrome known as parturient paresis or milk fever. Post-parturient hypocalcemia is divided into clinical and subclinical forms. It has been established that cows suffering from clinical milk fever are susceptible to a variety of secondary conditions, however to the best of the authors' knowledge there has been no evaluation of the impact of the subclinical form on production and reproductive parameters. The objective of this study was to investigate the association between subclinical hypocalcemia and post-parturient disorders, production and reproductive parameters in Israeli dairy herds. Blood results for corrected calcium concentrations were analyzed from 634 mature cows from 5 farms. The subclinical hypocalcemic cows produced 3.2, 2.7 and 1.9 kg more milk in the first three milk recordings than the normocalcemic cows. Subclinical hypocalcemic cows did not show an increased risk for post-parturient diseases nor compromised reproduction parameters in comparison to normocalcemic cows. It was concluded that there was no negative impact of subclinical hypocalcemia on production and reproductive parameters in Israeli dairy cows after parturition.

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**Keywords:** Bovine; Milk Fever; Calcium; Hypocalcemia; Milk Production.

## INTRODUCTION

Parturient paresis is a metabolic disorder occurring close to parturition especially in high producing dairy cows. The disease is characterized by a rapid decline in blood calcium (Ca) concentrations. Nearly all mature cows experience some degree of hypocalcemia during the first day after calving as the intestine and bone adapt to the Ca demands of lactation (1). In some cows, the mammary drain of Ca causes extracellular and blood Ca concentrations to decline to levels that disrupt neuromuscular function, resulting in the clinical syndrome of "Milk Fever". This Ca decline lasts in some cases for several days postpartum (2).

Post-parturient hypocalcemia is divided into clinical

and subclinical forms (3, 4). The literature indicates that the clinical form is associated with an increase in post-parturient diseases (5, 6, 7, 8). In large parts of the United States and some European countries it is assumed that the subclinical form is also related to post parturient diseases (5, 9, 10, 11) and has a negative impact on profitability. Therefore, many dairies use expensive feed additives to reduce the incidence of this form (12, 13). Fatty acid metabolism might differ between cows with subclinical hypocalcemia and their normocalcemic counterparts (14) however, there is conflicting evidence regarding the impact of subclinical blood calcium levels on milk production, reproduction parameters and post-parturient disorders (10, 14, 15). Most of the research

published targets methods to improve calcium homeostasis through manipulations of dietary cationic anionic difference or through calcium binders (16, 17, 18, 19) however to the best knowledge of the authors' the direct impact of the subclinical form on production and reproductive parameters has not been adequately investigated. The objective of this study to investigate whether the subclinical hypocalcemia state has an influence on post-parturient diseases, reproductive parameters and milk production parameters in Israeli dairy herds.

## MATERIALS AND METHODS

### Animals and study design

The study was comprised of two separate entities: The first study, the preliminary study was conducted on a 60 cow dairy farm in the northern part of Israel. Cows were housed in large covered loose housing systems and fed dry cow total mixed ration (TMR) pre-calving and a standard milking TMR post-calving both manufactured by Givaat Yoav Feeding Center located in Moshav Givaat Yoav, Israel. Blood was drawn from the coccygeal vein of 11 mature Holstein cows at a 4 hour interval from the beginning of first stage of labor and up to 12 hours postpartum. Blood was drawn once more at 24 hours postpartum. Samples were immediately centrifuged and serum was harvested and frozen at  $-20^{\circ}\text{C}$  for laboratory analysis at the Kimron Veterinary Institute, Beit Dagan. According to these results a post-calving calcium curve was prepared to determine the time of nadir levels of serum calcium.

The second study, the main study was conducted on 4 commercial Israeli dairy herds of 200-450 milking cows each between June 2006 and July 2007. Blood was drawn from 633 mature Holstein cows between 8-20 hours postpartum when serum Ca levels were expected to be the lowest based on the results of the first preliminary study. Samples were immediately centrifuged and serum was harvested and frozen at  $-20^{\circ}\text{C}$  for further laboratory analysis.

All cows were either housed in free stalls or large covered loose housing systems. All herds were fed dry cow TMR pre-calving and a standard milking TMR post-calving. All farms received their feed from different feed manufactures (Table 1).

In all herds cows were milked 3 times daily identified by ear tags and freeze marking in computer controlled milking parlors. The annual milk production was 10,000-

**Table 1:** Farm name and feed manufacture

Farm	Feed manufacturer
Kibbutz Geshur	Givaat Yoav Feeding Center, Moshav Givaat Yoav, Israel
Kibbutz Beit Zera	Amabar Feeding Center, Moshav Kefar Yehezkel, Israel
Kibbutz Givaat Haim Meuhad	Amatz Feeding Center, Moshav Amatz, Israel
Kibbutz Afikim	Kibbutz Afikim, Israel

12,500 kg/cow. The herds were within the practice area of the "HaChaklait", a mutual society for veterinary medicine which provides a complete herd-health service. Visits are conducted by a veterinarian to the farms during the study period at least twice weekly.

All cows were examined after calving by trained veterinarians who diagnosed, treated and recorded all the periparturient disease conditions. Cases of retained fetal membranes (RFM) were defined as the presence of placental tissues 24 hours or more after calving as observed by trained farm employees or the attending veterinarian. Animals with observed or suspected RFM were submitted for veterinary examination on the next routine veterinarian visit (1-4 days postpartum). Animals without a history or diagnosis of RFM were submitted for examination between 6 and 9 days postpartum. At this examination, body condition scoring (BCS) of all animals was recorded and cows were comprehensively examined by intravaginal palpation after thoroughly cleaning the perineal area. The diagnosis of clinical endometritis (CEM) was based on the combined characteristics of vaginal discharge obtained by manual examination of the vagina. Affected cows with CEM had a watery or purulent, fetid vaginal discharge as previously described (20).

All cows were examined for ketosis by placing a drop of urine obtained with a sterile disposable plastic catheter on a reagent strip (Ketostix, Bayer, Germany). The color reaction was compared to the standardized color chart after 15 seconds. Cows with urine aceto-acetate concentrations above 15 mg/dl were recorded as ketotic (3). Cows with lower than expected milk production and poor appetite were examined for displacement of the abomasum (LDA) by auscultation and percussion. BCS was further recorded approximately 40-60 days after calving and before the dry-off period. All animals not observed in estrus by the end of the voluntary waiting period at approximately 60 days postpartum were recorded and submitted for examination.

Clinical, reproductive, production and management data were computer recorded by the herd manager and the attending veterinarians. Cows not observed in estrus were recorded for further reproduction calculation as cows not showing heat. Once a month, each cow's milk was sampled and analyzed for fat, protein, lactose and somatic cell count by the Central Laboratory for Milk Recording at the national service for udder health and milk quality located in Caesarea industrial park, Israel.

Reproductive management was solely based on artificial insemination performed by trained technicians employed by "Sion" Israeli Company for artificial insemination and breeding, Migdal Ha'emek, Israel. In all herds, cows were mainly inseminated on observed estrus or computerized pedometry system. Conception rates and cumulative pregnancy were based on pregnancy diagnosis performed by rectal palpation of the uterus and its contents 40-50 days post-insemination.

### Blood analysis

Total serum calcium levels were determined using Arsenazo III method and calcium levels were corrected for serum albumin which was determined using the Bromocresol Green method. Correction was done using the following equation (3). Corrected Calcium (mg/dl) = Measured Calcium (mg/dl) – Albumin (g/dl) + 3.5

### Statistical analysis

All data editing and analysis were performed using SAS version 9.0 (21). Results were considered to be of statistical significance if the relevant *P*-value was < 0.05.

In general, data analysis followed a 3 step approach: (1). Descriptive statistics which included calculation of the mean, standard deviation and histogram for continuous variables, and frequency tables for other variables (2). Bivariate analysis in which associations between a dependent variable and an independent variable were assessed using the chi-square test for categorical variables and t-tests when one of the variables was on a continuous scale. For time to event data, i.e. days from calving to conception, survivor functions were compared using the Kaplan-Meier method and log-rank test (3). Multivariable analysis in which associations between the dependent variable and two or more independent variables were assessed. Multivariable analysis was only performed if, for the same dependent variable, two or more significant associations were found in the bivariate analysis. In our case,

this only occurred in the analysis of milk production data. For the latter, average test-day milk (kg) was estimated from monthly test-day data using a linear model with a marginal effect to account for repeated measurements from the same cow. A maximum of 10 test-days was allowed per cow. Lactation number was grouped into four categories, i.e. second, third, fourth and fifth, or greater lactation. Summer months were considered June to September, inclusive.

Subclinical hypocalcemia was defined in a cow with a corrected serum Ca level of < 7.5 mg/dl and without clinical hypocalcemia 12-24 h postpartum. Somatic cell counts (cells/mL) were grouped in 4 categories: ≤ 100,000; 101,000-200,000; 201,000-400,000 and > 400,000. Farms were modeled as a fixed effect and the correlation matrix used for R was autoregressive. The model we used was:

$$Y = \text{farm (4 index variables)} + \text{summer (2 index variables)} + \text{lactation (4 index variables)} + \text{MIM (10 index variables)} + \text{HCOR (2 index variables)} + \text{MIM} + \text{HCOR} * \text{MIM} + \text{SCCL (4 index variables)} + \text{CEM (2 index variables)} + e.$$

Y was test-day fat percentage, summer represents test days occurring in the summer months, lactation was lactation group, MIM was month in milk, HCOR was sub-clinical hypocalcemia, SCCL was somatic cell count level, and "e" a complex error term representing the within-cow correlation of test-day fat percentage and the residual error. Significance of the fixed effects was determined using the *F*-test (21).

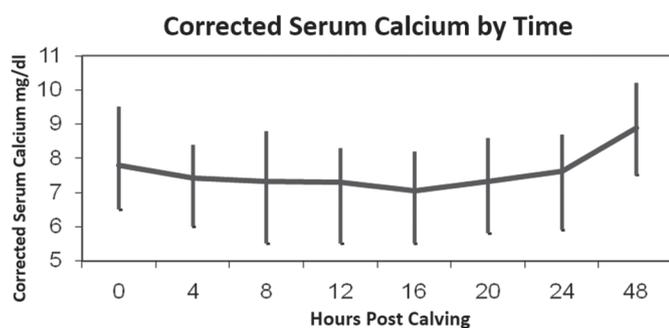
## RESULTS

### Preliminary study

Corrected serum calcium levels obtained from all 11 cows were combined into an average level for each 4 hour interval. These average levels were plotted by time from calving. Calcium levels decreased between calving and reached a nadir at 8 hours-calving and stayed low until 20 hours post-calving. Although not statistically significant, based on these results, 8-20 hours post-calving was chosen as the period of the nadir of serum calcium levels for the main study. (Figure 1).

### Main study

Data sets included measurements from 634 cows. 247, 186, 103 and 97 cows from second, third, fourth, fifth or greater lactations, respectively. There was missing data on calving disease incidence for one cow and 16 cows suffered from clinical



**Figure 1:** Corrected serum calcium (mg/dl) by time from calving (hours)

milk fever. Therefore a total of 617 cows were included in the final data set.

Of all cows 18.9% suffered from subclinical hypocalcaemia. Milk fever incidence was 7.63% and 1.36% for the subclinical hypocalcemic and normocalcemic cows respectively. The probability for development of clinical milk fever was found to be statistically higher in the subclinical hypocalcemic group as opposed to the normocalcemic group of cows. ( $P < 0.0007$ ) (Table 2).

The probability for a cow developing subclinical hypocalcaemia increased with lactation number ( $P < 0.0001$ ) (Table 3).

**Table 2:** Milk Fever by Subclinical hypocalcaemia

Subclinical Hypocalcaemia	Milk Fever		Total
	No	Yes	
No	508	7	515
%	98.64	1.36	
Yes	109	9	118
%	92.37	7.63	
Total	617	16	633
%	97.47	2.53	100

**Table 3:** Subclinical hypocalcaemia by lactation

Lactation Number	Subclinical Hypocalcaemia		Total
	No	Yes	
2	235	12	247
%	95.14	4.86	
3	164	22	186
%	88.17	11.83	
4	63	40	103
%	61.17	38.83	
>=5	53	44	97
%	54.64	45.36	
Total	515	118	633
%	81.36	18.64	100

## Calving diseases

Of the normocalcemic cows, 12% versus 11% of subclinical hypocalcemic cows suffered from retained placenta. There was no statistical difference between normocalcemic and subclinical hypocalcemic cows ( $P < 0.897$ ). Of the normocalcemic 27.6% cows versus 19.3% of subclinical normocalcemic cows suffered from metritis however there was no statistical difference in the probability of a subclinical hypocalcemic cow suffering from metritis ( $P < 0.087$ ). 21.3% of normocalcemic cows versus 25.7% of subclinical normocalcemic cows suffered from ketosis however there was no statistical difference in the probability of a subclinical hypocalcemic cow suffering from ketosis ( $P < 0.376$ ). Only 2 cows from the normocalcemic group suffered from an LDA. 5.7% of normocalcemic cows versus 3.7% of subclinical hypocalcemic cows had stillbirths. There was no statistical difference in the probability of a subclinical normocalcemic cow to have stillbirths ( $P < 0.533$ ).

Although subclinical milk fever (defined as corrected serum calcium below 7.5 mg/dl) was associated with milk fever incidence no association was found between serum calcium and calving diseases.

## Reproduction

Thirty-two cows received a “do not breed” code or were culled before first insemination and therefore 585 cows were included in the reproduction analysis study.

There was no statistical difference ( $P < 0.755$ ) between groups for the first artificial insemination (AI) conception rate. 29.7% vs. 27.6% for the normocalcemic and subclinical hypocalcemic cows respectively. There was no statistical difference ( $P < 0.453$ ) between groups for cows not showing heat. 33.7% vs. 38.1% for the normocalcemic and subclinical hypocalcemic cows respectively. There was no statistical difference ( $P < 0.958$ ) in the cumulative conception until 180 days in milk between both groups (Figure 2).

No associations were found between subclinical milk fever (defined as corrected calcium below 7.5 mg/dl) and reproduction parameters.

## Milk production

Subclinical hypocalcemic cows produced significantly more milk when compared with normocalcemic cows. 3.17, 2.71 and 1.90 kg more milk was produced on the first, second and third test days, respectively (Table 4).

**Table 4:** Milk production by calcium group.

Test day	Subclinical Hypocalcemia	Estimate Kg	Standard error	P-value
1 <sup>st</sup> test day	No	-3.1673	1.1607	0.0064
1 <sup>st</sup> test day	Yes	0		
2 <sup>nd</sup> test day	No	-2.7079	1.1508	0.0187
2 <sup>nd</sup> test day	Yes	0		
3 <sup>rd</sup> test day	No	-1.8951	1.1368	0.0956
3 <sup>rd</sup> test day	Yes	0		

Subclinical hypocalcemic cows produced more milk in the first 6 milk test days (Figure 3).

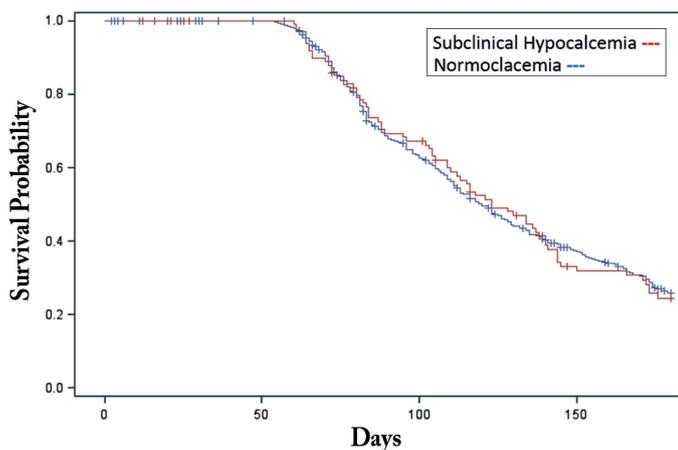
### DISCUSSION AND CONCLUSIONS

Many physiological pathways are dependent on blood ionized calcium levels. Decreased ionized calcium levels have been found to be associated with increased fat mobilization around calving (14) and these decreased levels could presumably influence the gastrointestinal track motility (22) leading to decreased feed intake and as a result an increased prevalence of metabolic disorders and other post-parturient diseases. It has also been shown that decreased levels of calcium stores in peripheral blood mononuclear cells precedes measurable hypocalcemia and that hypocalcemia at parturition further exacerbates the ability of these cells to release intracellular calcium in response to intracellular signals therefore impairing these cells' ability to be activated (8). These changes collectively could probably contribute to the impaired immune system of the periparturient cow and its increased susceptibility to infectious diseases (5, 6, 7, 8).

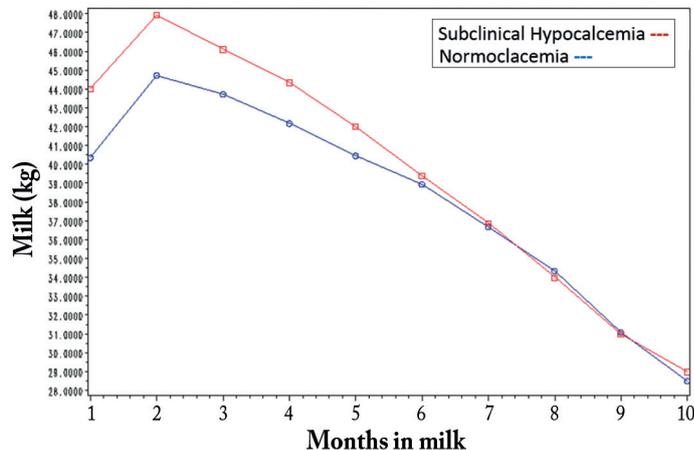
It would be reasonable to assume that these cows would

show impaired reproductive performance and decreased milk production later in their lactation. However, our results demonstrate that in the Israeli high producing cow this subclinical hypocalcemia is not a risk factor for the development of postpartum diseases, furthermore we have demonstrated that subclinical hypocalcemic cows produce more milk. These results agree with previous studies which showed that hypocalcemia at calving is not a significant risk factor for decreased milk yield (14, 23). Our results of higher milk production for the hypocalcemic cows could be related to a higher genetic merit causing these cows to produce more milk and subsequently exhibit lower circulating calcium levels. On the other hand it must be pointed out that these milk parameters do not rule out the possible damage caused by these low circulating blood calcium levels. It has been demonstrated that lame cows produce more milk in comparison to their non-lame herd mates (25, 26). Despite these findings it is clear that lameness is a risk factor for decreased milk production. When comparing the impact of these two diseases it could be that the hypocalcemic cows in our study, although higher in milk than their normoclaemic herd mates could have produced even more milk had we corrected their circulating blood calcium levels. Furthermore, we did not analyze the quality of milk and whether calcium level had any effect on the immunoglobulins or other parameters of milk quality.

Our study had several limitations. First, due to the number of cows in the first study we made a subjective decision on the time of sampling which could have influenced the number of cows being defined as subclinical hypocalcemic. Second, we could not measure ionized calcium and therefore had to



**Figure 2:** Cumulative conception until 180 days in milk



**Figure 3:** Milk production (Kg) by month

use the correction equation according to albumin levels. It is possible that this calculation of corrected calcium resulted in an underestimation of the true level of ionized calcium in the blood and that some cows in the subclinical calcium group should have actually been included as normocalcemic cows. On the other hand our study was done on several farms feeding from different rations, feeds and feed suppliers which gave substantial power to our results.

Taking into consideration other research done in this field, our results should be interpreted with caution and ideally should be validated by future large scale studies and further research.

In conclusion, subclinical hypocalcemia in the Israeli dairy herd does not seem to impair production and reproduction parameters. Therefore the authors do not find it reasonable or necessary to incorporate feed additives that reduce the incidence of this disorder as long as milk fever incidence remains in the normal range for Israeli dairy herds.

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