Association of low serum calcium concentration after calving with productive and reproductive performance in multiparous Jersey cows

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ABSTRACT

Consequences of postpartum low blood calcium (Ca) concentration are still under study and literature describing this condition in Jersey cows is scarce. A prospective cohort study was conducted to evaluate the association of low serum Ca concentration shortly after calving with milk and energy-corrected milk yields, somatic cell count linear score, and pregnancy to first service and within 150 d in milk in multiparous Jersey cows from 2 commercial herds. Blood samples for serum Ca determination were collected on average at 3 h 10 min postpartum from 352 multiparous Jersey cows. Productive data up to the 10th monthly test were obtained from the Dairy Herd Improvement Association and reproductive data were obtained from herd records. Multiple linear, log-binomial, and Cox's proportional hazards regressions were used to evaluate the association of low serum Ca concentration with productive and reproductive outcomes. Serum Ca concentration ≤ 2.18 mmol/L was associated with 1.43 and 1.85 kg/d more milk and energy-corrected milk. However, lower serum Ca concentrations were associated with a 0.28-unit-higher somatic cell count linear score per monthly test (Ca ≤ 2.00 mmol/L), and decreased pregnancy risk at first service (risk ratio = 0.64; Ca ≤ 1.94 mmol/L) and hazard of pregnancy within 150 d in milk (hazard ratio = 0.40; Ca ≤ 1.90 mmol/L). The present study is based on a convenience sample of multiparous Jersey cows from 2 commercial herds; further research including more herds and additional blood Ca determinations is needed to describe postpartum blood Ca dynamics and its association with productive and reproductive outcomes for the Jersey breed.

Key words: Jersey cow, calcium, subclinical hypocalcemia, milk production, reproduction

INTRODUCTION

Subclinical health disorders during the transition period have been associated with negative consequences (Chapinal et al., 2012; McArt et al., 2012). Accordingly, herd monitorization of these conditions is recommended to assess their incidence and to supervise the efficacy of prophylactic management strategies implemented during the transition period (Oetzel, 2004; Mulligan et al., 2006; Goff, 2008). Among these health disorders, subclinical hypocalcemia, which is described as blood calcium (Ca) concentration lower than normal (2.25 to 2.50 mmol/L) with or without clinical signs of hypocalcemia (Oetzel, 2004; Goff, 2018), has a notable prevalence among periparturient multiparous Holstein (Ca < 2.00 mmol/L: 47%; Reinhardt et al., 2011) and Jersey cows (Ca < 2.00 mmol/L: 40%; Valdecabres et al., 2019) from commercial dairy herds. However, the most appropriate definition for subclinical hypocalcemia is still being studied, and the available literature is largely based on Holstein cows. Currently, the blood Ca concentration threshold suggested for the definition of subclinical hypocalcemia varies depending on the observed outcome and the time of Ca determination relative to parturition. For instance, based on specific health and performance outcomes, different authors define subclinical hypocalcemia with thresholds ranging from 1.80 to 2.20 mmol/L of blood Ca within 4 d postpartum (Rodríguez et al., 2017; Neves et al., 2018a; Venjakob et al., 2018).

The associations between subclinical hypocalcemia and productive and reproductive outcomes are still not established. Studies that evaluated the association between low blood Ca concentration and milk yield either did not detect an association (Rodríguez et al., 2017; Wilhelm et al., 2017) or found that it was associated with higher (Jawor et al., 2012; Neves et al., 2018a; Gobikrushanth et al., 2020) or lower milk yield (Chapinal et al., 2012; Neves et al., 2018b; Venjakob et al., 2018). The literature that evaluates the association between subclinical hypocalcemia and milk components is scarce and reflects lack of consensus (Chamberlin et al., 2013; Wilhelm et al., 2017; Rodrigues et al., 2020).

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Similarly, a few studies found that subclinical hypocalcemia was associated (Rodrigues et al., 2020) or was not associated with increased milk SCC (Chamberlin et al., 2013; Wilhelm et al., 2017). Last, studies that evaluated the association between low blood Ca concentration and reproductive outcomes did not detect an association (Chamberlin et al., 2013; Wilhelm et al., 2017; Neves et al., 2018b) or found it to be associated with impaired reproduction (Caixeta et al., 2017; Rodríguez et al., 2017; Umaña Sedó et al., 2018). In addition to the different definitions of subclinical hypocalcemia used in the aforementioned studies (blood Ca threshold and time relative to parturition), differences in the experimental design (sample size, cow breed, observed calcemia percentile distribution in the population, or analytical systems) may explain the variability of results across studies. A detailed description of the studies and approaches followed to evaluate the associations of subclinical hypocalcemia with productive and reproductive performance can be found in a recent review by Couto Serrenho et al. (2021).

Breed differences have been observed in Ca metabolism and prevalence of hypocalcemia. Higher risks of clinical hypocalcemia have been reported for Jersey than for Holstein cows (Roche and Berry, 2006; Saborío-Montero et al., 2017), and of subclinical hypocalcemia for Jersey $\times$ Holstein crossbreed than for Jersey cows (Valdecabres et al., 2019). Unpublished data referenced by Goff et al. (1995) suggest a lower number of vitamin D receptors in the colon in Jersey than in Holstein cows. Furthermore, breed differences have been found in productive (i.e., milk yield and fat, protein, and mineral concentrations; Cerbulis and Farrell, 1976; Prendiville et al., 2011) and reproductive performance (i.e., days from calving to first service, pregnancy rates, days open; Fonseca et al., 1983; Silva et al., 1992; Washburn et al., 2002). Thus, associations observed between low serum Ca concentration and productive and reproductive performance in Holsteins may not be transferable to Jersey cows. Jersey cows are the second most popular dairy breed in the United States (USDA-NAHMS, 2014). Nevertheless, to our knowledge, only one recent study has evaluated the association between postpartum blood Ca concentration and productive (milk yield) and reproductive outcomes (pregnancy by 150 DIM) specifically in Jersey cows (Menta et al., 2021).

We hypothesized that in multiparous Jersey cows, low serum Ca concentration shortly after calving was associated with productive and reproductive performance. Thus, the objective of this prospective cohort study was to evaluate the association of low serum Ca concentration shortly after calving with productive (milk and ECM yields, and SCS) and reproductive out-

comes (pregnancy to first service and within 150 DIM) in multiparous Jersey cows from 2 commercial herds fed an acidifying prepartum diet.

MATERIALS AND METHODS

The prospective cohort study described herein was conducted as part of larger studies approved by the University of California Davis Institutional Animal Care and Use Committee (#18846).

Study Population

Multiparous Jersey cows from 2 commercial dairy herds in California were enrolled as part of larger studies from January to May 2016 (herd A) and from August to October 2016 (herd B). Herd A, a dry-lot facility, milked 2,789 Jersey cows twice a day in a double herringbone parlor with an average 305-d mature-equivalent milk yield of 8,748 kg. Herd B, a freestall facility, milked 9,660 Jersey and Jersey $\times$ Holstein crossbred cows twice a day in a double herringbone parlor with an average 305-d mature equivalent milk yield of 9,697 kg. Only Jersey cows were included in the present study. A description of transition cow and reproductive management can be found elsewhere (Valdecabres and Silva-del-Río, 2021).

Experimental Design and Data Collection

A prospective cohort study was conducted to evaluate the association of low serum Ca concentration shortly after calving with productive and reproductive outcomes. A convenience sample of 352 multiparous Jersey cows from 2 commercial herds [herd A ($n = 228$); herd B ($n = 124$)] with serum Ca concentration assessed at calving was considered for the analyses. Enrolled cows were part of larger studies where all multiparous cows calving during the study period were sequentially assigned to control or oral Ca supplementation postpartum without any further selection or exclusion criteria (50 to 60 g of Ca as bolus at 0 and 1 DIM; Valdecabres et al., 2018; Valdecabres and Silva-del-Río, 2021); only cows not receiving oral Ca supplementation were included in the present study.

Serum total Ca concentration was determined from coccygeal blood samples collected by the researchers using evacuated tubes without anticoagulant (BD Vacutainer, Trace Element Serum; BD) at 3 h 10 min postpartum (± 2 h 17 min; mean $\pm$ SD). Immediately after drawing blood, samples were placed into a portable refrigerator set at 4°C and transported to the laboratory within 3 h after collection. Samples were centrifuged at $1,430 \times g$ for 15 min at 15°C and serum

was stored at -20°C until analysis. Total serum Ca was determined by inductively coupled plasma-optical emission spectrometry (Melton et al., 1990) at the California Animal Health and Food Safety Laboratory (University of California, Davis). The intraassay and interassay coefficients of variation were 2.0 and 4.4%, respectively.

Body condition and locomotion scores were assigned to all cows by A.V. at the time of blood sample collection, using a 1 to 5 scale with 0.25 and 1 unit increments for BCS and locomotion, respectively (Ferguson et al., 1994; Sprecher et al., 1997). Monthly milk yield, fat and protein concentrations, and SCC information up to the 10th monthly test were obtained from the DHIA (AgriTech Analytics). Herd records were used to obtain previous lactation (305-d mature-equivalent milk yield, lactation length, and dry period length), calving-related (parity and calving ease and time), and reproductive information (breeding code, breeding date, and pregnancy diagnosis; DairyComp305; Valley Agricultural Software). Descriptions of the acidified close-up (herd A: DCAD = -176 mEq/kg of DM; herd B: DCAD = -168 mEq/kg of DM) and fresh cows' diets, TMR sample collection, processing, and analyses can be found in Valdecabres and Silva-del-Río (2021).

Statistical Analyses

All statistical analyses were performed with SAS (version 9.4; SAS Institute Inc.). Descriptive statistics were generated using the MEANS and FREQ procedures. Briefly, first the association between serum Ca concentration on a continuous scale and the study outcomes was assessed as a decision step to evaluate its association with serum Ca as a categorical variable. Second, the categorical variable that better predicted each outcome was identified. Third, the association between low serum Ca concentration and the study outcomes was quantified (objective of the study).

Study Outcomes. Evaluated study outcomes included milk yield, ECM yield $[(\text{kg of milk} \times 0.327) + (\text{kg of milk fat} \times 12.86) + (\text{kg of milk protein} \times 7.65)]$; Siverson et al., 2014], and SCS $[\log_2 (\text{SCC}/100) + 3]$ up to the 10th monthly test, and pregnancy to first service and within 150 DIM.

Explanatory Variables. Considered covariates for all outcomes were herd, parity (categorized as second, third, and $\geq$ fourth), previous lactation length, previous lactation 305-d mature-equivalent milk yield, dry period length, BCS (categorized as ≤ 2.50 , 2.75 to 3.25, and ≥ 3.50), and locomotion score at calving (categorized as 1 and ≥ 2), and calving ease (categorized as assisted and not assisted calving). Previous lactation information was available for 339 cows; BCS and locomotion score

at calving were available for 328 and 329 cows, respectively; and calving ease information was available for 264 cows. Additional variables considered in the models were milk SCS (models for milk and ECM yields), milk yield (model for milk SCS), current lactation peak milk yield (models for pregnancy to first service and within 150 DIM), DIM at first service (model for pregnancy to first service), and breeding code (timed AI and heat breeding; model for pregnancy to first service).

Health records were only available for herd A; thus, sick cows that were not removed from the herd remained in the data set, and disease incidence was not considered as a covariate in the statistical analyses. Given the limited variation on blood sampling time relative to parturition, and its low correlation with serum Ca concentration ($r = 0.08$), sampling time and its interaction with serum Ca concentration were not considered in the statistical models. Plots generated with the SGPLOT procedure were used to evaluate linearity on the continuous dependent and independent variables relationship; nonlinear relationships were not detected. Univariable models as described in the following paragraphs were used to identify potential explanatory variables for the multivariable models.

Productive Outcomes. Multiple linear regression using a 2-piece linear function for DIM, was used to model the shape of the lactation curve for milk and ECM yields up to the 10th monthly test based on Aly et al. (2010) using the MIXED procedure. Monthly test DIM were transformed into 2 variables: DIM pre-lactation peak $[\text{DIM pre-peak} = (\text{DIM} - \text{DIM at peak})/\text{DIM at peak}]$ and DIM postlactation peak $[\text{DIM postpeak} = (\text{DIM} - \text{DIM at peak})/(\text{total DIM} - \text{DIM at peak})]$. For monthly tests DIM pre- and at lactation peak, DIM postpeak = 0; and for monthly tests DIM postlactation peak, DIM pre-peak = 0. The 2-piece linear function for DIM resulted in 2 splines that met at a knot equivalent to each cow lactation peak, representing an increase up to the lactation peak and a subsequent decrease in milk and ECM yields (Silvestre et al., 2009). Additionally, quadratic effects and random coefficients for DIM pre- and postpeak were considered to account for the curvilinear shape of each spline and the variability between cows in pre- and postpeak milk and ECM yields. Multiple linear regression was also used to model monthly test SCS, using monthly test number as the time variable instead of the 2-piece linear function for DIM. The Kenward-Roger method was used to estimate the denominator degrees of freedom. Models included a REPEATED statement with cow as the subject and the variance-covariance structure selected based on lowest Akaike's information criterion at the beginning of the model building processes and re-evaluated in the final models (unstructured, compound

symmetry, autoregressive 1, heterogeneous autoregressive 1, Toeplitz, and Toeplitz heterogeneous were the variance-covariance structures evaluated).

Reproductive Outcomes. Log-binomial regression was used to model pregnancy to first service using the GENMOD procedure with the log link function. Cox proportional hazards regression was used to model pregnancy within 150 DIM using the PHREG procedure. Right censoring was applied to cows present in the herd without a pregnancy diagnosis date, dead, or sold by 150 DIM. The proportional hazards assumption was assessed with plots of the logarithm of the hazard function against the logarithm of the time (categorical predictors; LIFETEST procedure) and plots of the scaled Schoenfeld residuals against the logarithm of the time (continuous predictors; LOESS procedure). Median days to pregnancy and their associated 95% confidence intervals were estimated using the LIFETEST procedure.

Serum Ca as Continuous Variable. Multivariable models initially included all explanatory variables unconditionally associated with the outcome of interest at $P < 0.15$ (Dohoo et al., 2012), as well as serum Ca on a continuous scale (variable of interest), herd, parity (possible confounder), and time variables for repeated measures models (DIM pre- and postpeak or monthly test number); after collinearity assessment (VIF tool; REG procedure). Reduced multivariable models were formed via manual backward elimination. Serum Ca concentration, herd, parity, and the time variables were retained in all models regardless of the P -value. All other independent variables were treated as fixed effects and removed from the statistical models when $P > 0.10$ for type 3 tests of fixed effects, or at $0.05 < P \leq 0.10$ if they caused a $<20\%$ change in the estimates for the serum Ca concentration effect. Then, pairwise interactions of serum Ca concentration and parity with the time variables were individually evaluated. Finally, interactions with $P \leq 0.10$ were collectively offered to the multivariable model and removed at $P > 0.05$ to form the final multivariable models.

Serum Ca as Categorical Variable. If serum Ca concentration on a continuous scale was associated with the outcome at $P \leq 0.10$ for the type 3 test of fixed effects, a serum Ca concentration categorical variable replaced the continuous variable in the multivariable models. Serum Ca was categorized using thresholds that ranged from 1.80 to 2.20 mmol/L at 0.02 mmol/L intervals (21 thresholds), including those previously reported in the literature (Chapinal et al., 2012; Martinez et al., 2012; Venjakob et al., 2018) and embracing $>20\%$ of the observations on each side. The aforementioned arbitrary criteria were selected to allow for an adequate number of observations on each side of

the threshold to proceed with the analysis, as well as to limit the number of evaluated serum Ca categorical variables and reduce the probability of type I errors.

Separate multivariable models were built for each of the outcomes and serum Ca categorical variable. After the replacement of the serum Ca continuous variable with the categorical variable, the significance of remaining variables was reassessed as described for the models with serum Ca on a continuous scale. The serum Ca categorical variable was forced into all multivariable models as the variable of interest; and the one with the lowest type I error rate (smallest P -value) for the solution of fixed effects (continuous outcomes) or maximized sensitivity and specificity (largest area under the curve; categorical outcomes) was chosen as the optimal categorization in our study. For pregnancy to first service and within 150 DIM receiver operating characteristic curve analyses were built using the LOGISTIC procedure or enabling the receiver operating characteristic curve from the PHREG procedure PLOT option, respectively.

The association between the optimal serum Ca categorical variable and the respective study outcome was quantified using the LSMEANS statement. The statement included the EXP option for the reproductive outcomes. The risk of pregnancy to first service represents the ratio of the probability of pregnancy from first breeding for cows below the selected serum Ca threshold, to the probability for cows above the threshold. The hazard ratio of pregnancy within 150 DIM represents the ratio of the hazard of pregnancy within 150 DIM for cows below the selected serum Ca threshold, to the hazard for cows above the threshold.

Overall model fit was assessed by graphical evaluation of residuals for homoscedasticity and independence of errors (productive outcomes), goodness-of-fit chi-squared test (pregnancy at first service), and deviance residuals plots (pregnancy within 150 DIM). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$ for all results. The accompanying figures were created with SigmaPlot (version 14.0; Systat Software Inc.), using locally weighted smoothing (loess) with tricube weighting and polynomial regression to plot the predicted values from the described models for milk and ECM yields.

RESULTS

Cows eligible for the cohort study are described in Table 1. A total of 7 cows were sold ($n = 2$) or died ($n = 5$) before the first monthly milk test, 21 cows were sold ($n = 12$) or died ($n = 10$) before the end of the end of the voluntary waiting period, and reproductive information was not available for 8 cows. Thus, the

Figure 1. Range of serum Ca thresholds for which serum Ca $\leq$ shortly after calving was associated with higher milk yield, ECM yield, and SCS up to the 10th monthly test, and lower risk of pregnancy to first service and hazard of pregnancy within 150 DIM at $P < 0.10$. Serum Ca concentration was determined within 5 h postpartum from 345 multiparous Jersey cows from 2 commercial herds enrolled in a prospective cohort study. Gray bars indicate thresholds associated with the study outcome at $P < 0.10$. Black bars indicate the threshold with lowest type I error rate (continuous outcomes) or maximized sensitivity and specificity (categorical outcomes). No bars indicate no association of the threshold with the study outcome.

final data set for productive outcomes comprised 345 [herd A ($n = 223$); herd B ($n = 122$)] and for pregnancy to first service comprised 316 multiparous Jersey cows [herd A ($n = 203$); herd B ($n = 113$)]. Average serum Ca concentration was 2.00 ± 0.26 mmol/L ($\pm$ SD). Early lactation disease herd records were only available for herd A; retained placenta, metritis, and milk fever were recorded respectively for 3.4, 5.4, and 1.1% of cows. Incidence of clinical mastitis within 60 DIM was on average 4.9% for herd A and 7.6% for herd B.

Table 1. Description of categorical (proportions) and continuous (mean $\pm$ SD) cow-level variables from the multiparous Jersey cows eligible for inclusion in a cohort study

Item	Herd A	Herd B
Cows (n)	228	124
Parity (%)		
2	34.5	44.4
3	26.0	27.1
≥ 4	39.5	28.8
BCS at calving (%)		
≤ 2.5	6.2	6.8
2.75–3.25	87.1	86.4
≥ 3.5	6.7	6.8
Lame ¹ (%)	12.7	28.4
Assisted calving (%)	6.2	19.5
Serum Ca ² (mmol/L)	1.96 ± 0.28	2.07 ± 0.18
Days dry (d)	62 ± 30	79 ± 29
Previous lactation length (d)	300 ± 32	327 ± 70
Previous lactation 305-d MEM ³ (kg)	$8,548 \pm 1,074$	$9,289 \pm 1,504$

¹Cows scored ≥ 2 for locomotion at calving.

²Serum Ca was assessed at 3 h 10 min $\pm$ 2 h 17 min postpartum (mean $\pm$ SD).

³MEM = mature-equivalent milk yield.

Productive Performance

Milk Yield. Serum Ca concentration on a continuous scale was inversely associated with milk yield. Serum Ca concentration less than or equal to thresholds ranging from 2.02 to 2.20 mmol/L were or tended to be associated with 0.8 to 1.4 kg/d higher milk yield (Figure 1). Serum Ca concentration >2.18 mmol/L and ≤ 2.18 mmol/L predicted the outcome best; cows below this threshold produced on average 1.40 kg/d more milk (27.05 ± 0.25 kg/d; $n = 231$) compared with cows above the threshold (25.65 ± 0.42 kg/d; $n = 81$; $P = 0.004$; Figure 2). The full model is described in Table 2.

ECM Yield. Serum Ca concentration on a continuous scale tended to be inversely associated with ECM yield. Serum Ca concentration less than or equal to thresholds from 2.02 to 2.08 mmol/L and 2.12 to 2.20 mmol/L were or tended to be associated with 0.9 to 1.8 kg/d higher ECM yield (Figure 1). Serum Ca concentration >2.18 mmol/L and ≤ 2.18 mmol/L predicted the outcome best; cows below this threshold produced on average 1.80 kg/d more ECM (33.39 ± 0.29 kg/d; $n = 231$) compared with cows above the threshold (31.59 ± 0.49 kg/d; $n = 78$; $P < 0.001$; Figure 2). The full model is described in Table 2.

Linear SCS. Serum Ca concentration on a continuous scale tended to be and was inversely associated with SCS. Serum Ca concentration less than or equal to thresholds ranging from 1.80 to 1.84 and 1.98 to 2.00 mmol/L were or tended to be associated with 0.26 to 0.31 higher SCS units per monthly test (Figure 1). Serum Ca concentration >2.00 mmol/L and ≤ 2.00 mmol/L predicted the outcome best; cows below this threshold had an average SCS 0.28 unit higher per monthly test (3.31 ± 0.10 units of SCS/monthly test; n

= 155) compared with cows above the threshold (3.03 ± 0.09 units of SCS/monthly test; $n = 177$; $P < 0.001$). The full model is described in Table 3.

Reproductive Performance

Pregnancy to First Service. Overall, the proportion of cows that were diagnosed pregnant at first service was 35.4%. Serum Ca concentration on a continuous scale was positively associated with pregnancy to first service. Models including serum Ca concentration >1.80 to 1.94 mmol/L or ≤ 1.80 to 1.94 mmol/L were discriminant for the outcome (area under the curve ≥ 0.60 ; Figure 1) with the associated risk ratios ranging from 0.62 to 0.74. Serum Ca concentration as >1.94 or

≤ 1.94 mmol/L had maximized sensitivity and specificity to predict pregnancy to first service (area under the curve = 0.61); cows below this threshold had a 36% decreased pregnancy risk to first service ($n = 114$) compared with cows above the threshold ($n = 202$; risk ratio = 0.64; $P = 0.03$; Table 4). The proportion of cows pregnant at first service was 24.9 and 38.6% for low ($\text{Ca} \leq 1.94$ mmol/L) and high serum Ca concentration cows, respectively. The average DIM at first service was 59 ± 8 d for low and for 58 ± 7 d high serum Ca concentration cows ($\pm$ SD). Additional effects included in the final model are described in Table 4.

Pregnancy Within 150 DIM. Overall, the proportion of cows that were diagnosed as pregnant within 150 DIM was 53.6%. Serum Ca concentration on a continuous scale was positively associated with pregnancy within 150 DIM. Models including serum Ca concentration defined as >1.80 to 2.20 or ≤ 1.80 to 2.20 mmol/L were discriminant for the outcome (area under the curve >0.66 ; Figure 1) with the associated hazard ratios ranging from 0.40 to 0.83. Serum Ca concentration as >1.90 or ≤ 1.90 mmol/L had maximized sensitivity and specificity to predict pregnancy within 150 DIM (area under the curve = 0.69); cows below this threshold had a 60% decreased hazard of pregnancy within 150 DIM ($n = 99$) compared with cows above the threshold ($n = 226$; hazard ratio = 0.40; $P < 0.001$; Figure 3; Table 5). The proportion of cows that conceived within 150 DIM was 33.4 and 64.8% for cows below and above the 1.90 mmol/L threshold, respectively. Median days to pregnancy were 265 d (95% CI, 223–272 d) and 101 d (95% CI, 88–113 d) for cows below and above the 1.90 mmol/L threshold, respectively ($P < 0.001$). Additional effects included in the final model are described in Table 5.

DISCUSSION

The association between blood Ca concentration and productive and reproductive outcomes in dairy cows has been evaluated in earlier studies; nevertheless, findings are inconclusive and only one recent study used Jersey cows (Menta et al., 2021). We conducted a prospective cohort study to evaluate the association of serum Ca concentration shortly after calving with productive (milk and ECM yields and SCS) and reproductive (pregnancy to first service and within 150 DIM) outcomes. Overall, we observed that serum Ca concentration below various thresholds was associated with higher milk yield and impaired reproduction. However, as pointed out by Couto Serrenho et al. (2021), the interactions across multiple parameters during the transition period make the evaluation of associations between serum Ca concentration and productive and

Figure 2. Locally weighted scatterplot smoothing (loess) of the magnitude of association between serum Ca concentration shortly after calving and milk (A) and ECM yields (B) up to the 10th monthly test as predicted by model in Table 2 for multiparous Jersey cows with serum Ca concentration $>$ and ≤ 2.18 mmol/L ($n = 97$ and 243 , respectively) enrolled in a cohort study.

Table 2. Association of serum Ca concentration ≤ 2.18 mmol/L shortly after calving with milk and ECM yields up to the 10th monthly test for 324 multiparous Jersey cows from 2 commercial herds enrolled in a cohort study

Item ¹	n	Milk			ECM		
		Estimated milk (kg)	SE	P-value	Estimated ECM (kg)	SE	P-value
Intercept	324	16.10	1.55	<0.001	24.43	1.86	<0.001
Ca ≤ 2.18 mmol/L							
No	81	Referent	—	—	Referent	—	—
Yes	243	1.43	0.47	0.003	1.85	0.57	0.001
Parity							
2	124	Referent	—	—	Referent	—	—
3	88	3.63	0.61	<0.001	3.98	0.74	<0.001
≥ 4	112	4.52	0.59	<0.001	3.95	0.71	<0.001
Herd							
A	212	Referent	—	—	Referent	—	—
B	112	−2.47	0.44	<0.001	−4.34	0.52	<0.001
DIM pre-peak ²	324	8.01	0.46	<0.001	15.89	2.20	<0.001
DIM pre-peak $\times$ DIM pre-peak		—	—	—	9.68	2.81	0.001
DIM postpeak ²	324	−18.17	1.11	<0.001	−16.75	1.50	<0.001
DIM postpeak $\times$ DIM postpeak	324	4.90	0.98	<0.001	3.41	1.33	0.01
Previous lactation 305-d MEM ³ (kg)	324	0.002	0.0002	<0.001	0.002	0.0002	<0.001
Linear SCS ⁴	324	−0.44	0.05	<0.001	−0.43	0.07	<0.001
Parity $\times$ DIM postpeak							
Parity 2 and DIM postpeak	124	Referent	—	—	Referent	—	—
Parity 3 and DIM postpeak	88	−3.74	0.86	<0.001	−4.73	1.11	<0.001
Parity ≥ 4 and DIM postpeak	112	−3.80	0.81	<0.001	−4.91	1.04	<0.001

¹Models also included the random effect of DIM postpeak by cow.²DIM pre- and postpeak are 2-piece linear function for DIM pre- and postlactation peak, respectively.³MEM = mature-equivalent milk yield.⁴Calculated as $\log_2(\text{SCC}/100) + 3$ for each monthly test.

reproductive outcomes difficult. To account for some of these complex interactions, we included parity in all productive and reproductive performance multivariable models. Indicators of productive performance (previous lactation 305-d mature equivalent and current lactation peak milk yield) were considered in the reproductive

performance models. Nevertheless, limitations in study design prevented us from accounting for the potential effects of health events on blood Ca concentration, and productive and reproductive outcomes (van Werven et al., 1992; Pinedo et al., 2017; Rodríguez et al., 2017). Last, herd effect was accounted for and considered in

Table 3. Association of serum Ca concentration ≤ 2.00 mmol/L shortly after calving with linear SCS up to the 10th monthly test for 332 multiparous Jersey cows from 2 commercial herds enrolled in a cohort study

Item	n	Estimated SCS ¹	SE	P-value
Intercept	332	3.484	0.26	<0.001
Ca ≤ 2.00 mmol/L				0.05
No	177	Referent	—	—
Yes	155	0.284	0.14	0.05
Parity				<0.001
2	126	Referent	—	—
3	88	0.984	0.27	<0.001
≥ 4	118	1.584	0.26	<0.001
Herd				<0.001
A	215	Referent	—	—
B	117	0.491	0.13	<0.001
Monthly test milk yield (kg)	332	−0.05	0.004	<0.001
Monthly test number	332	0.026	0.02	0.30
Parity $\times$ monthly test number				0.002
Parity 2 and monthly test number	126	Referent	—	—
Parity 3 and monthly test number	88	−0.084	0.04	0.02
Parity ≥ 4 and monthly test number	118	−0.12	0.04	<0.001

¹Linear SCS for each monthly test was calculated as $\log_2(\text{SCC}/100) + 3$.

Table 4. Log-binomial regression model for the association of serum Ca ≤ 1.94 mmol/L shortly after calving with risk of pregnancy to first service for 316 multiparous Jersey cows from 2 commercial herds enrolled in a cohort study

Item	n	Estimate	SE	Risk ratio	95% CI	P-value
Intercept	316	-0.73	0.12	—	—	<0.001
Ca ≤ 1.94 mmol/L						0.03
No	202	Referent	—	—	—	—
Yes	114	-0.44	0.20	0.64	0.43–0.96	0.03
Parity						0.53
2	124	Referent	—	—	—	—
3	86	0.06	0.18	0.95	0.67–1.33	0.95
≥ 4	106	-0.23	0.21	0.79	0.53–1.20	0.52
Herd						0.12
A	203	Referent	—	—	—	—
B	113	-0.25	0.16	0.76	0.56–1.07	0.12

our models as an overall herd effect variable and in variables that typically differ by herd (parity, previous lactation length, previous lactation 305-d mature-equivalent milk yield, dry period length, BCS, locomotion score, and calving ease). Nevertheless, as our study included only 2 herds, the external applicability of our results is limited and research including a larger number of herds is needed. Furthermore, recent research suggests that postpartum blood Ca dynamics (persistent, transitory, delayed) is what determines future productive performance rather than a single blood Ca assessment as performed in the present study (McArt and Neves, 2020; Seely et al., 2021).

Figure 3. Survival curve for the association of serum Ca concentration $>$ and ≤ 1.90 mmol/L ($n = 227$ and 101 , respectively) shortly after calving with time to pregnancy within 150 DIM as predicted by model in Table 5 for multiparous Jersey cows enrolled in a cohort study.

Similar to our observations, Venjakob et al. (2018) found that serum Ca concentration below various thresholds shortly after calving was associated with overall higher milk yield but also with impaired reproductive performance in Holstein cows. However, Menta et al. (2021) observed higher milk yield for Jersey cows with plasma Ca concentration ≤ 1.84 mmol/L within 24 h postpartum but did not detect an association between plasma Ca concentration and pregnancy within 150 DIM.

Higher milk yield has been previously associated with a blood Ca concentration below various thresholds within 24 h postpartum in Holstein [Jawor et al., 2012 (≤ 1.80 mmol/L); Neves et al., 2018b (≤ 1.95 mmol/L); Venjakob et al., 2018 (< 2.10 mmol/L)] and Jersey cows [Menta et al., 2021 (≤ 1.84 mmol/L)]. Nevertheless, the threshold for optimal serum Ca categorization to predict milk yield was higher in our study than those previously reported by other authors. In contrast, Wilhelm et al. (2017) did not observe this association using as threshold ≤ 2.00 mmol/L within 2 h postpartum in a younger population of cows (58% primiparous). Nevertheless, in that study the threshold was defined based on optimal sensitivity and specificity to predict metritis or calf diarrhea. Differences in the study population (blood Ca percentile distribution, size, breed) or analytical system (method, equipment, reagents, analyst) may explain the discrepancies across studies. Interestingly, the studies that evaluated blood Ca concentration after 24 h postpartum reported no association [Rodríguez et al., 2017 (≤ 2.14 mmol/L at 24 to 48 h postpartum); Menta et al., 2021 (at 3 DIM)] or lower milk yield [Neves et al., 2018a (≤ 2.20 mmol/L at 4 DIM); Gobikrushanth et al., 2020 (≤ 2.10 mmol/L at 2 to 7 DIM)] for cows with low blood Ca concentration. Low serum Ca concentration within 24 h postpartum may reflect the abrupt increase in Ca demands associated with the synthesis of colostrum and milk, as evidenced by Goff et al. (2002) which compared the calcemia of mastectomized and in-

tact cows. But later, as proposed by McArt and Neves (2020) and Couto Serrenho et al. (2021), low blood Ca concentration could indicate cows' inability to adapt to increased Ca demands, or reflect lower feed intake, inflammation, or disease. In a recent study, Seely et al. (2021) observed that cows with persistent ($\text{Ca} \leq 1.95$ at 1 DIM and ≤ 2.20 mmol/L at 4 DIM) and delayed ($\text{Ca} \leq 2.2$ mmol/L at 4 DIM) subclinical hypocalcemia had statistically and numerically lower DMI, respectively, compared with normocalcemic cows. Only Østergaard and Larsen (2000) reported ECM yield, which, contrary to our findings, was not associated with plasma Ca concentration assessed within 12 h postpartum.

Consistent with the negative association observed between serum Ca and SCS in our study, Rodrigues et al. (2020) reported higher SCC during the first 14 DIM in Holstein $\times$ Girolando cows with serum $\text{Ca} \leq 2.125$ mmol/L within the first 5 DIM. However, other studies that categorized blood Ca concentration using pre-established Ca thresholds or based on other outcomes did not find this association (Chamberlin et al., 2013; Rodríguez et al., 2017; Wilhelm et al., 2017). Blood Ca concentration has been associated with intracellular Ca stores, which are necessary for peripheral blood mononuclear cells activation (Kimura et al., 2006) and neutrophil function (Martinez et al., 2014). Because macrophages are the predominant type of immune cells in the normal udder, and neutrophils are the predominant type during mammary gland infection (Sordillo and Streicher, 2002), blood Ca concentration may be important to support mammary gland health. In addition, delayed teat canal closure after milking has been associated with serum Ca concentration < 2.00 mmol/L (Barragan et al., 2018). Thus, calcemia may be associated with mammary gland health through immune cell function and teat sphincter contraction. However, it is

uncertain whether serum Ca concentration within 24 h postpartum interfered with SCS later into lactation. For instance, a higher SCC is generally associated with a decreased milk yield (Dohoo et al., 1984; Hagness-Nielsen et al., 2009; Gonçalves et al., 2018), but higher milk yields have also been associated with a higher SCC and risk of clinical mastitis (Jamali et al., 2018).

Negative associations between postpartum blood Ca concentration below various thresholds and reproductive outcomes are commonly reported in Holstein cows (Caixeta et al., 2017; Umaña Sedó et al., 2018; Venjakob et al., 2018); however, Menta et al. (2021) did not detect an association between postpartum plasma Ca concentration and pregnancy within 150 DIM in Jersey cows. Overall, we observed an impaired reproductive performance for cows with low serum Ca concentration shortly after calving, compared with previous research. Nevertheless, differences in study design including breed, parity, sample size, sampling time relative to parturition, and approaches for blood Ca categorization (pre-established thresholds or identified based on different outcomes), make it challenging to compare our results with prior studies.

In agreement with our findings, previous studies have reported lower odds of pregnancy to first service for cows with blood Ca concentration below various thresholds [Chapinal et al., 2012 (< 2.20 to 2.40 mmol/L within 3 wk postpartum); Umaña Sedó et al., 2018 (≤ 2.00 mmol/L within 24 h postpartum); Gobikrushanth et al., 2020 (≤ 2.10 mmol/L at 2 to 7 DIM)]. The same optimal threshold for serum Ca categorization and prediction of pregnancy to first service identified in our study (1.90 mmol/L within 24 h postpartum) was reported by Venjakob et al. (2018). This study included 1,238 cows from 125 herds and reported a 40% decrease in the odds of pregnancy to first service for cows with

Table 5. Cox hazard regression model for the association of serum $\text{Ca} \leq 1.90$ mmol/L shortly after calving with hazard of pregnancy within 150 DIM for 328 multiparous Jersey cows from 2 commercial herds enrolled in a cohort study

Item	n	Estimate	SE	Hazard ratio	95% CI	P-value
Ca ≤ 1.90 mmol/L						< 0.001
No	227	Referent	—	—	—	—
Yes	101	−0.92	0.22	0.40	0.26–0.61	< 0.001
Parity						0.33
2	127	Referent	—	—	—	—
3	85	−0.17	0.18	0.85	0.59–1.21	0.35
≥ 4	116	−0.30	0.20	0.75	0.50–1.11	0.11
Herd						0.99
A	210	Referent	—	—	—	—
B	118	−0.01	0.16	0.99	0.73–1.36	0.99
BCS at calving (%)						0.02
2.75–3.25	285	Referent	—	—	—	—
≤ 2.5	21	−0.43	0.34	0.67	0.33–1.28	0.22
≥ 3.5	22	0.68	0.28	1.98	1.15–3.40	0.01

serum Ca concentration <1.90 mmol/L. In contrast, other studies assessing blood Ca concentration within 24 h postpartum did not observe an association between blood Ca concentration and pregnancy to first service [Gild et al., 2015 (<1.87 mmol/L); Wilhelm et al., 2017 (≤ 2.00 mmol/L); Neves et al., 2018b].

Similarly, lower hazard of pregnancy within 150 DIM and longer days to pregnancy have been reported for cows with blood Ca concentration below various thresholds within 24 h postpartum in some studies [Umaña Sedó et al., 2018 (≤ 2.00 mmol/L); Venjakob et al., 2018 (<1.80 mmol/L); Braga Paiano et al., 2019 (<2.00 mmol/L)], but no association has been reported in other (Gild et al., 2015; Gobikrushanth et al., 2020; Menta et al., 2021). Other authors have shown detrimental effects of postpartum blood Ca concentration below various thresholds on estrus cyclicity (Ribeiro et al., 2013; Rodríguez et al., 2017; Jeong et al., 2018) and number of services per pregnancy (Ribeiro et al., 2013; Braga Paiano et al., 2019). Although it is unclear how blood Ca postpartum interferes with reproductive performance, it is noteworthy to remark that Ca is an intracellular signal that leads to oocytes maturation (Homa et al., 1993). Hypocalcemia has been associated with a higher incidence of retained placenta, metritis, subclinical endometritis, and clinical endometritis (Martínez et al., 2012; Pinedo et al., 2017; Rodríguez et al., 2017), which in turn are associated with impaired reproduction (van Werven et al., 1992; Giuliadori et al., 2013a,b). Thus, it is possible that the association between serum Ca concentration and reproduction was influenced by these conditions, which were not accounted for in our models. Plausible indirect associations between serum Ca concentration and these conditions include the role of Ca in muscle contraction and immune system function (Ebashi and Endo, 1968; Ducusin et al., 2003; Kimura et al., 2006).

Interestingly, we found that the optimal serum Ca categorizations associated with positive outcomes (higher milk and ECM yields) were higher than those associated with impaired reproduction. The optimal serum Ca categorization associated with undesirable outcomes varied across outcomes. In our field, blood Ca thresholds are described to facilitate the applicability of a subclinical hypocalcemia definition; however, they have some limitations. First, cows just above and below the thresholds may not be physiologically different, and second, analytical differences (different laboratory procedures and techniques) might influence these thresholds by providing varying readings for same blood Ca concentration. We consider that limiting the definition of subclinical hypocalcemia to a single Ca threshold and to individual outcomes may restrain its value in herd monitoring programs and its incorpora-

tion into prophylactic management strategies. Instead, the description of blood Ca concentration ranges based on its association with multiple outcomes (productive, reproductive, and health), and the consideration of the dynamics of these associations according to time relative to parturition may facilitate the identification of a valuable herd-level definition for subclinical hypocalcemia, and the incorporation of this definition into prophylactic management programs.

CONCLUSIONS

Our study conducted in 2 Jersey dairy herds indicates that serum Ca concentration below multiple thresholds was associated with higher milk and ECM yields, but also with a higher milk SCS, and lower risk of pregnancy to first service and hazard of pregnancy within 150 DIM in multiparous Jersey cows. However, the optimal serum Ca categorization associated with higher milk and ECM yields ($\text{Ca} > \text{or} \leq 2.18$ mmol/L) was higher than those associated with impaired reproductive performance ($\text{Ca} > \text{or} \leq 1.90$ to 1.94 mmol/L). Thus, while subclinical hypocalcemia within 24 h postpartum should not be defined based on a serum Ca threshold derived from its association with milk yield, its definition based on its association with reproductive outcomes might allow for on-farm identification of cows with compromised future reproductive performance. Further studies are necessary to elucidate the role of peripartum serum Ca concentration on productive and reproductive performance, and the applicability of a low serum Ca concentration definition in Jersey cows.

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