Associations of subclinical hypocalcemia with fertility in a herd of grazing dairy cows

Sebastián Umaña Sedó,* Diana Rosa,† Guillermo Mattioli,† R. Luzbel de la Sota,‡§ and Mauricio J. Giuliodori†[1]
*Private practice, Cañuelas, B1814, Argentina
†Cátedra de Fisiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV-UNLP), B1900AVW, La Plata, Argentina
‡Cátedra y Servicio de Reproducción Animal, FCV-UNLP, B1900AVW, La Plata, Argentina
§Comisión Nacional de Investigaciones Científicas y Tecnológicas (CONICET), C1033AAJ, Buenos Aires, Argentina

ABSTRACT

The main objective was to assess the associations of subclinical hypocalcemia (SCH), diagnosed at parturition (SCH-0) and 7 d in milk (SCH-7), with fertility in a herd of grazing dairy cows. Additional objectives were to characterize Ca concentration on 0 and 7 d in milk (DIM), assessing the risk factors for SCH-0 and SCH-7 and also the relationship with health status (metritis, endometritis, subclinical ketosis, and culling). A prospective observational study was carried out in a dairy farm in Argentina. Holstein cows (n = 126) were body condition scored (BCS, 1–5) on -21 ± 3, 0, 7 ± 3, and 28 ± 7 DIM and blood was collected on 0 and 7 ± 3 DIM to determine Ca and β-hydroxybutyrate concentrations. Calcium concentrations <2.0 and <2.14 mmol/L were used to define SCH-0 and SCH-7, respectively. The associations of SCH with (1) the odds for pregnancy to first service (P1AI) and pregnancy by 100 DIM (P100) were evaluated by logistic models, (2) the services per pregnancy was evaluated by a Poisson regression model, and (3) the hazards of insemination and pregnancy were evaluated with proportional hazards regression models whereas median days from calving to first insemination and to pregnancy were estimated by Kaplan-Meier method. Additionally, Ca concentration was assessed by linear regression models, and the associations of SCH-0 and SCH-7 with the odds for metritis, endometritis, subclinical ketosis, and culling were evaluated by logistic models. Calcium concentrations were similar at 0 and 7 DIM (2.40 vs. 2.41 mmol/L, respectively); they were higher in cows calving in fall than in summer (2.58 vs. 2.24 mmol/L), and they also were higher in primiparous than in multiparous cows (2.53 vs. 2.28 mmol/L, respectively). The proportion of cows having SCH-0 and SCH-7 was 27.3 and 39.3%, respectively. Fall-calving cows had lower odds for SCH-0 [odds ratio (OR) = 0.31, 95% confidence interval (CI) = 0.12–0.86] than summer-calving cows, multiparous cows had higher odds for SCH-0 (OR = 3.96, 95% CI = 1.09–14.39) than primiparous cows, and cows with prepartum BCS ≥3.00 had higher odds for SCH-0 (OR = 4.03, 95% CI = 1.17–13.89) than in cows with BCS <3.00. Conversely, parity and prepartum BCS were not important predictors for SCH-7. Surprisingly, SCH-0 was not a risk factor for SCH-7. Cows with SCH-0 had lower odds for P1AI (OR = 0.26, 95% CI = 0.07–0.99) than normocalcemic cows, given that P1AI was 14 versus 38%, respectively. The hazard of first service was not associated with SCH-0 (hazard ratio = 1.03, 95% CI = 0.63–1.70) but cows with SCH-0 had lower hazard of pregnancy (hazard ratio = 0.39, 95% CI = 0.16–0.98) and took 32 d longer to get pregnant (105 vs. 73) than normocalcemic cows. Conversely, SCH-7 was not associated with fertility. Finally, SCH-0 and SCH-7 were associated with the odds for subclinical ketosis and metritis, respectively. In conclusion, SCH-0 but not SCH-7 is associated with reduced fertility in a herd of grazing dairy cows, but both were associated with health status.

Key words: subclinical hypocalcemia, pregnancy risk, fertility, grazing dairy cow

INTRODUCTION

Before calving, dairy cows require approximately 30 g of Ca/d (15 g for fetuses) that is mainly provided by passive gastrointestinal absorption (DeGaris and Lean, 2008). However, at parturition, as the concentration of Ca in colostrum and milk is 30- and 10-fold higher than in blood (62–75 vs. 25–30 vs. 2.2–2.5 mmol/L, respectively), the requirement may exceed 50 g/d (Horst et al., 2005). This increased Ca demand must be satisfied by active gastrointestinal absorption and bone resorption (DeGaris and Lean, 2008). As the homeo-

static mechanisms involving active gastrointestinal Ca absorption and active bone Ca mobilization have a lag time to take effect (>48 h; Martín-Tereso and Martens, 2014), most cows experience some degree of hypocalcemia at calving (DeGaris and Lean, 2008). Depending on the severity of the blood Ca decrease, some cows (approximately 5%) can develop clinical hypocalcemia, whereas most of them (30 up to 70%) show no clinical signs despite having hypocalcemia [i.e., subclinical hypocalcemia (**SCH**); Reinhardt et al., 2011]. The associations of clinical hypocalcemia with milk production, reproductive performance, and health status of dairy cows have extensively been studied (Curtis et al., 1983, 1984, 1985), whereas the associations of subclinical cases with performance have received less attention (Goff, 2008; Murray et al., 2008).

Recently, studies have focused on this topic with inconsistent results. Some found negative associations with indicators of reproductive performance (Martinez et al., 2012, 2016; Caixeta et al., 2017), whereas others reported no association (Chamberlin et al., 2013; Gild et al., 2015; Rodríguez et al., 2017). These discrepancies could partly be the result of differences in the study design (i.e., timing of diagnosis and cut-point used; Neves et al., 2017). No consensus exists as to when and how to objectively assess SCH, and this lack of standardization of SCH classification may affect our ability to establish associations between SCH and fertility (Neves et al., 2018). Likewise, most recent studies have been performed in confinement herds in North America and Europe, and information about the associations of SCH with fertility of dairy cows under grazing production systems is lacking (Ribeiro et al., 2013).

Our main objective was to assess the associations of SCH at 0 (**SCH-0**) and 7 DIM (**SCH-7**) with fertility in a herd of grazing dairy cows (i.e., odds for pregnancy and hazard of pregnancy). Additional objectives were to characterize Ca concentration at 0 and 7 DIM, assess the risk factors for SCH-0 and SCH-7, and, finally, evaluate the associations of SCH-0 and SCH-7 with health status.

MATERIALS AND METHODS

Farm Management

The current study was conducted on a commercial dairy farm located in Cañuelas, Buenos Aires Province, Argentina (35°05′S and 58°75′W). The herd consisted of Holstein cows (n = 2,000) that were milked in 4 milking parlors (i.e., 500 cows each). Prepartum transition cows that were within 3 wk of expected calving date were maintained on dry lots, fed 15 kg of corn silage, 5 kg of wheat hay, 7 kg of distilled barley, and 5.5 kg of commercial balanced feed (feed composition = 30% corn, 25% wheat bran, 38% soybean meal, and 7% anionic salts; chemical composition = 88% DM, 17% CP, 2.7 Mcal of ME/kg DM, 2.65% ether extract, 12.80% ash, 2.25% Ca, 0.85% P, 1.26% Mg, 0.05% Na, and 2.01% Cl). The target DCAD was −10 to −15 mEq/100 g of DM. Cows were monitored for signs of calving by farm employees trained to assist parturition. After calving, cows were sent for 20 d to the fresh herd at pasture. Lactating cows were at pasture in a rotational system (different paddock in the morning and afternoon). Feed was composed of mixed pastures (lucerne, red clover, ryegrass, tall fescue) and winter annual grasses (oats) and supplemented with TMR diets (16 kg of corn silage, 3 kg of lucerne hay, and 3 kg of wheat bran) formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 650 kg and producing 30 kg of milk (3.5% fat). Finally, 2 kg of concentrates (40% soybean pellets and 60% corn meal) were offered twice daily during milking. Average milk yield was 8,000 kg/lactation. Calving occurred year round, except for the hot summer months (January and February). Lactating cows were milked twice daily at 0200 and 1400 h. Farm veterinarians visited the farm routinely every month to conduct reproductive examinations. After a voluntary waiting period of 40 d, cows were checked for estrus twice daily and AI by the same technician with the am/pm rule. Estrus detection was facilitated by tail painting. Cows were considered in estrus when over 50% of tail paint was removed. An Ovsynch protocol was used for cows not bred within 100 DIM. Pregnancy was diagnosed by ultrasonography between 28 and 32 d postinsemination. Pregnant cows were rechecked by ultrasonography at approximately 90 d of gestation. Relevant data were managed with herd management software Protambo Master 3.0 (DIRSA SA, Gonnet, Argentina).

Study Design, Study Population, Clinical Examination, and Sampling

No protocol of animal use was required, but the experiment was conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and sample and data collection techniques. A prospective observational study was carried out in 1 milking parlor by including all cows calving on Monday to Wednesday between December 21, 2016, and June 21, 2017. Sample size (n = 126) was estimated as the number of cows needed to detect a difference of 15 d (with a pooled SD of 30 d) between means in the interval from calving to pregnancy, with

80% power and 95% confidence, assuming a prevalence of SCH of 50% in the study population (Dhand and Khatkar, 2014).

Cows were BCS on a scale of 1 to 5 (Ferguson et al., 1994) on -21 ± 3, 0 (parturition), 7 ± 3, and 28 ± 7 DIM and tail bled on 0 and 7 ± 3 DIM by the first author. Blood samples were collected in 10-mL polystyrene tubes without an anticoagulant, allowed to clot at room temperature for up to 6 h, centrifuged at $2,200 \times g$ for 10 min at room temperature, and serum was stored at $-20°$C until analyzed for total Ca concentration in 1 batch. Total Ca concentration was measured by using an atomic absorption spectrophotometer (AAnalyst 200; Perkin-Elmer Inc., Shelton, CT) with the flame technique and acetylene gas. After dilution 1:50 of serum in 0.1% lanthanum, the sample was mixed thoroughly and aspirated by the machine. A hollow cathode lamp with a wavelength of 422.7 was used to detect Ca. The BHB concentration was determined cow side with a hand-held device (FreeStyle Precision, Abbott GmbH & Co. KG, Wiesbaden, Germany; Iwersen et al., 2013). Vaginal discharge (**VD**) was assessed by direct inspection of the mucus obtained with Metricheck (Simcro, Hamilton, New Zealand) on 7 ± 3 and 28 ± 7 DIM (McDougall et al., 2007) and categorized as VD0 (clear normal mucus), VD1 (mucus with flecks of pus), VD2 (mucus with purulent content but not fetid), and VD3 (mucus with fetid odor; Giuliodori et al., 2013b, 2017).

Disease Definitions

Calcium concentration <2.0 mmol/L (<8.0 mg/dL) within 24 h after parturition was used to define SCH-0 (Reinhardt et al., 2011), and Ca <2.14 mmol/L (<8.59 mg/dL) at 7 ± 3 DIM was used to define SCH-7 (Martinez et al., 2012). Subclinical ketosis was set at BHB concentration $\geq$1.2 mmol/L at 7 ± 3 DIM (LeBlanc, 2010). Cows with SCH or subclinical ketosis were not treated. Metritis was defined as VD3 at 7 ± 3 DIM and clinical endometritis as VD1, 2, or 3 at 28 ± 7 DIM (Giuliodori et al., 2013a,b, 2017).

Statistical Analysis

Characterization of Ca Concentration. The fixed effect of sampling day postpartum (day: 0 vs. 7 DIM), calving season (summer vs. fall), parity (1 vs. $\geq$2), and their interaction, controlled by prepartum BCS (as continuous predictor), on Ca concentration was evaluated with a linear regression model with normal distribution and identity link function in Proc Glimmix (SAS/STAT ver. 9.4, SAS Institute Inc., Cary, NC). Sampling day was considered as a repeated measure by using a covariance structure of compound symmetry.

Statistical significance was set at $P < 0.05$ and a tendency was declared at $P \leq 0.10$.

Risk Factors for SCH. The odds for SCH-0 and SCH-7 were assessed with binomial distribution and logit link function in Proc Glimmix. These logistic models included the fixed effects of calving season (summer vs. fall), parity (1 vs. $\geq$2), and BCS (<3.00 vs. $\geq$3.00) at 21 ± 3 d prepartum and at parturition. The fixed effect of SCH-0 was also included in the model assessing the odds for SCH-7. Modeling was performed by the backward elimination method with an exclusion criteria set at $P > 0.1$.

SCH and Fertility. The associations of SCH-0 and SCH-7 with the odds for pregnancy to first service (**P1AI**) and for pregnancy by 100 (**P100**) were evaluated by logistic models with binomial distribution and logit link function in Proc Glimmix. Their relationship with the number of services per pregnancy (**SPP**) was evaluated by Poisson regression model with Poisson distribution and log link function in Proc Glimmix, and, finally, their associations with the hazards of being inseminated and of becoming pregnant were evaluated with proportional hazard regression models in Proc PHReg of SAS. All these models included SCH-0, SCH-7, and their interaction, calving season (summer vs. fall), parity (1 vs. $\geq$2), and diseases [subclinical ketosis, metritis, and endometritis (no vs. yes)] as fixed terms. Modeling was performed by the backward elimination method with an exclusion criterion set at $P > 0.1$ (with the exception of SCH-0 and SCH-7 that were forced to remain in the model). Median days from calving to insemination and to pregnancy were estimated via a Kaplan-Meier model by using Proc Lifetest of SAS. Finally, a sensitivity analysis was performed to test the assumption of nonindependent censoring, supposing 2 extreme situations: first, assuming a complete positive correlation between right-censoring and pregnancy, so that all the right-censored cows were assumed as becoming pregnant at censoring time; and second, assuming a complete negative correlation between right-censoring and pregnancy, so that all the right-censored cows before 150 DIM were recorded as if they were right-censored at 150 DIM.

SCH and Health Status. Logistic models assessing the odds for metritis, endometritis, subclinical ketosis, and culling included the fixed effect of SCH-0, SCH-7, and their interaction. They also included the fixed effect of calving season (summer vs. fall) and parity (1 vs. $\geq$2). The model assessing the odds for endometritis also included the fixed effect of metritis and subclinical ketosis, and the model assessing the odds for culling also included the fixed effect of metritis, subclinical ketosis, and endometritis. Modeling was performed by the backward elimination method with an exclusion

criteria set at $P > 0.1$ (with the exception of SCH that was forced to remain in the model).

RESULTS

Descriptive Statistics

Subclinical hypocalcemia was detected in 27.3% (34/126) and in 39.3% (48 / 122) of cows at 0 and 7 ± 3 DIM, respectively. No cow was diagnosed with clinical hypocalcemia. Subclinical ketosis was identified in 9.1% (11/122) of cows. Metritis and endometritis were diagnosed in 20.4% (25/122) and 26.3% (31/118) of cows, respectively. Finally, 20.6% (26/126) of cows were culled (4 cows were culled between 0 and 7 DIM, 4 cows between 7 and 28 DIM, 14 cows between 28 and 100 DIM, and 4 cows were culled between 100 and 150 DIM).

The odds for SCH-0 was evaluated on 126 cows (98 multiparous and 28 primiparous). The odds for SCH-7, subclinical ketosis, and metritis at 7 ± 3 DIM were assessed on 122 cows, the odds for endometritis and P1AI were evaluated on 118 cows, and the odds for P100 was assessed on 104 cows. A total of 26 cows were left-censored and another 29 cows were right-censored at 150 DIM (end of study period) in the analysis of the hazard of pregnancy.

Characterization of Ca Concentration

Sampling day was not associated with Ca concentration (2.40 vs. 2.41 mmol/L on 0 and 7 DIM, respectively; $P = 0.85$; Table 1). Conversely, calving season was associated with Ca concentration ($P < 0.01$), given that fall-calving cows had higher concentrations (2.58 vs. 2.24 mmol/L) than summer-calving cows (Table 1). Parity was also associated with Ca concentration ($P = 0.01$), given that primiparous cows had higher concentrations (2.53 vs. 2.28 mmol/L) than multiparous ones (Table 1). Interactions of sampling day by calving season, sampling day by parity, and calving season by parity were not associated with Ca concentration ($P = 0.38, 0.30,$ and 0.29, respectively). Finally, prepartum BCS tended to be associated with Ca concentration ($P = 0.09$), given that Ca levels decreased 0.29 mmol/L (1.16 mg/dL) per every 1 point of BCS over the mean (Table 1).

Risk Factors for SCH

Important predictors in final model assessing the odds for SCH-0 were parity, calving season, and BCS, whereas only calving season remained in the model evaluating the odds for SCH-7. The odds for SCH-0 were 4.0 times higher in multiparous cows [odds ratio (**OR**) = 3.96, 95% CI = 1.09–14.39, $P = 0.04$] than in primiparous cows (32 vs. 11%, respectively). The odds was lower for cows calving in fall (OR = 0.31, 95% CI = 0.12–0.86, $P = 0.02$) than in summer (16 vs. 38%, respectively). Also, the odds were 4.0 times higher in cows with prepartum BCS ≥3.00 (OR = 4.03, 95%CI = 1.17–13.89, $P = 0.03$) than in cows with BCS <3.00 (44 vs. 19%, respectively). On the other hand, the odds for SCH-7 were also lower in fall-calving cows (OR = 0.31, 95% CI = 0.11–0.84, $P = 0.02$) than in summer-calving ones (24 vs. 50.6%, respectively). Conversely, the odds for SCH-7 was neither associated with parity (37.5 vs. 30.9% for parity ≥2 vs. 1; $P = 0.36$) nor BCS at any time point ($P > 0.1$). Surprisingly, SCH-0 was not associated with the odds for SCH-7 (OR = 0.78, 95% CI = 0.26–2.35, $P = 0.65$) given that the proportion of affected cows were 33.5 and 39.2% for hypocalcemic cows and normocalcemic cows, respectively, at parturition.

SCH and Fertility

Cows with SCH-0 had lower odds for P1AI (OR = 0.26; $P = 0.04$; Table 2) and lower hazard of pregnancy [hazard ratio (**HR**) = 0.39; $P = 0.04$; Table 2] than normocalcemic cows and took 32 d longer to get pregnant (Figure 1), but they had similar hazard of insemination (HR = 1.03; $P = 0.90$; Table 2) and similar SPP (2.5 vs. 2.0; $P = 0.21$; Table 2) to normocalcemic cows. On the other hand, SCH-7 was neither associated with the odds for P1AI (OR = 1.26; $P = 0.71$; Table 2) and P100 (OR = 1.20; $P = 0.77$; Table 2) nor with the hazard of insemination (HR = 1.27; $P = 0.36$; Table 2) and pregnancy (HR = 0.63; $P = 0.21$; Table 2). Cows

Table 1. Multivariable linear regression model evaluating the associations of sampling day relative to parturition, calving season, and parity with total Ca concentration in blood in a herd of grazing dairy cows (n = 126)

Item	Total Ca concentration[1] (mmol/L)		
	LSM	SE	P-value
Sampling day (DIM)			0.85
0	2.40	0.05	
7	2.41	0.06	
Calving season			<0.01
Summer	2.24	0.06	
Fall	2.58	0.07	
Parity			0.01
Primiparous	2.53	0.08	
Multiparous	2.28	0.05	

[1]Calcium concentration decreased 0.29 mmol/L (1.16 mg/dL) per every increase of 1 point in BCS over the mean prepartum BCS ($P = 0.09$). Sampling day by calving season, sampling day by parity, and calving season by parity had P-values of 0.38, 0.30, and 0.29, respectively.

Table 2. Multivariable logistic (pregnancy to first AI and pregnancy by 100 DIM), Poisson (services per pregnancy), and proportional hazard (hazard of 1st AI and hazard of pregnancy) regression models evaluating the associations of subclinical hypocalcemia (SCH) with fertility in a herd of grazing dairy cows (n = 126)[1]

Item	Presence	LSM (%)	M[2]	OR[3]	HR[4]	95% CI	P-value
Pregnancy at 1st AI (P1AI)							
SCH-0[5]	No	37.7		Referent			
	Yes	16.7		0.26		0.07-0.99	0.04
SCH-7[6]	No	30.7		Referent			
	Yes	33.3		1.26		0.37-4.32	0.71
Pregnancy by 100 DIM (P100)							
SCH-0[5]	No	80.9		Referent			
	Yes	67.3		0.49		0.13-1.76	0.26
SCH-7[6]	No	65.9		Referent			
	Yes	69.9		1.20		0.33-4.40	0.77
Services per pregnancy (SPP)							
SCH-0[5]	No	2.03		—		—	
	Yes	2.51		—		—	0.21
SCH-7[6]	No	2.17		—		—	
	Yes	2.34		—		—	0.45
Hazard of 1st AI							
SCH-0[5]	No		60.5		Referent		
	Yes		61		1.03	0.63-1.70	0.90
SCH-7[6]	No		62.5		Referent		
	Yes		60		1.27	0.76–2.10	0.36
Hazard of pregnancy							
SCH-0[5]	No		73		Referent		
	Yes		105		0.39	0.16-0.98	0.04
SCH-7[6]	No		86		Referent		
	Yes		98		0.63	0.31–1.30	0.21

[1]Multivariable models were offered with the effect of SCH-0, SCH-7, SCH-0 by SCH-7, calving season, parity, and disease (subclinical ketosis, metritis, and endometritis) and manually removed if $P > 0.1$. Predictors other than SCH not shown in the table. Interaction SCH-0 by SCH-7 had $P > 0.1$ in final models. The only predictor that remained in models assessing P100 and hazard of pregnancy was calving season ($P < 0.05$ and $P = 0.04$, respectively).

[2]M: median time (d) interval from calving to event (first AI or pregnancy).

[3]OR: odds ratio.

[4]HR: hazard rate.

[5]SCH-0: subclinical hypocalcemia defined as cows having total blood Ca concentration <2.0 mmol/L (<8.0 mg/dL) within 24 h after parturition.

[6]SCH-7: subclinical hypocalcemia defined as cows having total blood Ca concentration <2.14 mmol/L (<8.59 mg/dL) 7 d after parturition.

with or without SCH-7 had similar SPP (2.36 vs. 2.18; $P = 0.45$; Table 2). Finally, the interaction of SCH-0 by SCH-7 was not associated with any of the assessed reproductive parameters ($P > 0.1$, Table 2).

Final multivariable models also showed that calving season was associated with the odds for P100 and with the hazard of pregnancy, given that cows calving in fall had higher odds for P100 (OR = 9.10, 95% CI = 1.02–81.19; $P < 0.05$) and higher daily risk for pregnancy (HR = 3.26, 95% CI = 1.04–10.19; $P = 0.04$) than those calving in summer. The remaining predictors were not associated with any of the evaluated odds ($P > 0.1$).

Sensitivity analysis showed that cows with SCH had a hazard of pregnancy of 0.48 (95% CI = 0.23–1.03) when assuming a complete positive correlation between right-censoring and pregnancy, whereas the analysis had a hazard of pregnancy of 0.38 (95% CI = 0.02–2.27) when assuming a complete negative correlation between right-censoring and pregnancy. We speculated that if the censoring would be influenced by SCH, the

association between SCH and the hazard of pregnancy could be biased. From sensitivity analysis results, we can see that under both hypothetical scenarios the association of SCH with the hazard of pregnancy was similar and remained in the same direction, suggesting that the violation of nonindependent censoring is not a large concern.

SCH and Health Status

Predictors in final models were SCH-0 when measuring the odds for subclinical ketosis (Table 3), SCH-7 when evaluating the odds for metritis (Table 3), metritis when assessing the odds for endometritis, and, finally, endometritis when estimating the odds for culling. Cows with SCH-0 tended to have higher odds for subclinical ketosis (OR = 6.21; $P = 0.10$; Table 3) compared with normocalcemic cows. Conversely, having SCH-0 was neither associated with the odds for metritis (OR = 0.34; $P = 0.14$; Table 3), endometritis (OR = 0.28; $P = 0.12$; Table 3), and culling (OR =
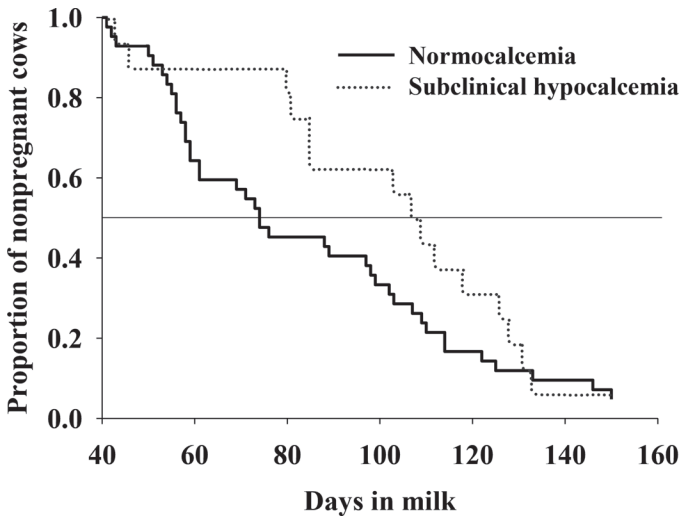
Figure 1. Subclinical hypocalcemia at parturition and the interval from calving to pregnancy in a herd of grazing dairy cows (n = 126). Cows having subclinical hypocalcemia at parturition (defined as <2.0 mmol/L) had lower hazard of pregnancy (hazard ratio = 0.36, 95% CI = 0.14–0.88, $P = 0.03$) and longer calving to pregnancy interval [median (95% CI) = 105 (80–125)] than normocalcemic cows [73 (58–97)].

2.47; $P = 0.27$; Table 3) nor to the hazard of culling (HR = 2.47, 95% CI = 0.43–17.02; $P = 0.27$).

On the other hand, SCH-7 was associated with the odds for metritis (OR = 5.76; $P = 0.01$; Table 3), but it was associated neither with the odds for endometritis (OR = 0.92; $P = 0.88$; Table 3) and culling (OR = 2.32; $P = 0.39$; Table 3) nor the hazard of culling (HR = 0.47, 95% CI = 0.13–1.65; $P = 0.24$). Finally, the interaction SCH-0 by SCH-7 was not associated with any of the assessed odds ($P > 0.1$; Table 3). Multivariable logistic models also showed that metritis was associated with the odds for endometritis (OR = 3.20, 95% CI = 0.99–10.26; $P = 0.05$) and that endometritis was associated with the odds for culling (OR = 5.00, 95% CI = 1.09–22.80; $P = 0.04$). The remaining predictors had $P > 0.1$.

DISCUSSION

The main objective of the present paper was to assess the associations of SCH-0 and SCH-7 with reproductive performance in a herd of grazing dairy cows. Our main finding was that cows having SCH-0, but not SCH-7, had approximately 3-fold lower odds for P1AI, 3-fold lower hazard of pregnancy, and also a calving to pregnancy interval approximately 1 mo longer than normocalcemic cows. Our results are similar to reports that found that cows housed in confinement with SCH have lower odds for P1AI (Caixeta et al., 2017), or tendencies for reduced P1AI (Martinez et al., 2016)

Table 3. Multivariable logistic regression models evaluating the associations of subclinical hypocalcemia (SCH) with health status in a herd of grazing dairy cows (n = 126)[1]

Item	Metritis[2]			Endometritis[3]			Subclinical ketosis[4]			Culling[5]		
	Incidence (%)	OR[6] (95% CI)	P-value	Incidence (%)	OR (95% CI)	P-value	Incidence (%)	OR (95% CI)	P-value	Incidence (%)	OR (95% CI)	P-value
SCH-0[7]												
No	36.3	Referent	0.14	36.5	Referent	0.12	4.7	Referent	0.10	8.9	Referent	0.27
Yes	16.2	0.34 (0.08–1.46)		15.2	0.28 (0.06–1.40)		23.6	6.21 (0.68–56.43)		19.5	2.47 (0.49–12.64)	
SCH-7[8]												
No	16.7	Referent	0.01	34.3	Referent	0.88	3.5	Referent	0.33	5.6	Referent	0.39
Yes	53.6	5.76 (1.43–23.29)		32.3	0.92 (0.29–2.92)		8.0	3.54 (0.27–45.96)		12.0	2.32 (0.34–16.13)	

[1] Multivariable logistic models were offered with the fixed effect of SCH-0 and SCH-7, SCH-0 by SCH-7, calving season (summer vs. fall), parity (1 vs. 2+), and diseases (subclinical ketosis, metritis, and endometritis) and manually removed if $P > 0.1$ (with the exception of SCH that was forced to remain in models). Predictors other than SCH-0 and SCH-7 are not shown in Table 3. The interaction SCH-0 by SCH-7 had $P > 0.1$. Metritis remained in the final model assessing the odds for endometritis (OR = 3.20, 95% CI = 0.99 – 10.26, $P = 0.05$), and endometritis in the final model assessing the odds for culling (OR = 5.00, 95% CI = 1.09 – 22.80; $P = 0.04$).
[2] Metritis was defined as vaginal discharge with fetid odor at 7 ± 3 DIM.
[3] Clinical endometritis was defined as vaginal discharge with pus at 28 ± 7 DIM.
[4] Subclinical ketosis was defined as BHB concentration ≥1.2 mmol/L at 7 ± 3 DIM.
[5] Culling: cows that were dead or sold by 150 DIM.
[6] OR: odds ratio.
[7] SCH-0: cows having total blood Ca concentration <2.0 mmol/L (<8.0 mg/dL) within 24 h after parturition.
[8] SCH-7: cows having total blood Ca concentration <2.14 mmol/L (<8.59 mg/dL) at 7 ± 3 DIM.

and 10 to 15 d longer calving to pregnancy intervals (Martinez et al., 2012, 2016). Conversely, other studies found no association with P1AI (Martinez et al., 2012; Ribeiro et al., 2013; Gild et al., 2015), calving to conception interval (Chamberlin et al., 2013; Gild et al., 2015), or the number of SPP (Chamberlin et al., 2013; Ribeiro et al., 2013; Rodríguez et al., 2017). These discrepancies could be the result of differences in the timing of diagnosis of SCH (0 through 7 DIM) and in the cut-point used to classify the cows (from 1.9–2.2 mmol/L), in the indicator of reproductive performance used (i.e., odds for pregnancy at first service, return to cyclicity, days open, and so on), and, finally, in the production system, given that most of the published works have been performed on confinement dairy herds; our study, however, was carried out in grazing dairy cows. Therefore, the lack of standardization of SCH classification makes the comparison among studies even harder (Neves et al., 2018).

Other sources of variation could be periparturient diseases because they have different incidences, different consequences on a cow's subsequent fertility, and their effects seem to be additive (Borsberry and Dobson, 1989). In fact, these additive negative effects on reproduction have also been reported for clinical and subclinical diseases (Ribeiro et al., 2013). These diseases thus have the potential to negatively affect fertility of dairy cows; therefore, they should be considered in statistical models assessing cow fertility when possible. It has been proposed that SCH is negatively associated with reproductive performance indirectly through delaying uterine involution (Murray et al., 2008) and impairing immunity (Kimura et al., 2006), both leading to increased risk for uterine diseases (Martinez et al., 2012) that, in turn, are associated with reduced fertility (Giuliodori et al., 2013a,b, 2017). In addition, hypocalcemia may reduce DMI postpartum (Jawor et al., 2012; Martinez et al., 2014), compromising nutrient balances including worsening the negative energy balance (Martinez et al., 2012; Chamberlin et al., 2013), which, in turn, it is associated with poorer immune function and fertility. Finally, as Ca plays a critical role in many physiological functions, it is likely that SCH could also exert direct detrimental effect on fertility.

In fact, evidence exists of a cross-talk between skeleton and energy metabolism involving osteocalcin, insulin, adiponectin, and leptin, among other metabolic signals (Lee et al., 2007). Osteocalcin, produced by osteoblasts and released into blood circulation by osteoclasts during bone resorption, may play an important role in homeorhetic adaptation to lactation (Lean et al., 2014). Thus, it is likely that after parturition, when osteocalcin concentration increases, the associations of bone tissue with energy metabolism, and likely with cow health, production, and reproduction, could go beyond the effect of Ca concentration alone, as previously speculated.

Regarding our additional objectives, we found that Ca concentrations were similar at 0 and 7 DIM, that they decreased with parity and with increasing prepartum BCS, and, finally, that they varied with calving season (i.e., lower in cows calving in summer than in those calving in fall). Our findings are in line with the following previous reports. Martinez et al. (2012) found similar Ca concentrations when comparing 0 and 7 DIM in normocalcemic cows and in cows with SCH. Kamgarpour et al. (1999) reported that cows, once recovered from the initial Ca nadir at parturition, experience a subsequent Ca nadir at 6 and 9 DIM when calving in winter and summer, respectively. In addition, Eldon et al. (1988) reported that Ca concentration decreased from autumn to summer season (2.5 vs. 2.3 mmol/L) in lactating dairy cows. Kamgarpour et al. (1999) found higher Ca concentrations in summer-calving cows than in winter-calving cows (2.26 vs. 2.17 mmol/L). Conversely, Jonsson et al. (1997) found no differences in Ca concentration between cows calving in summer and winter months (2.34 vs. 2.32 mmol/L).

In our study, 27.3 and 39.3% of cows had SCH-0 and SCH-7, respectively. Reported incidences of SCH in grazing dairy cows were 20% (cut-point: <2.0 mmol/L, 3–7 DIM; Sepúlveda-Varas et al., 2015) and 43% (cut-point: <2.14 mmol/L, 7 DIM; Ribeiro et al., 2013). Other studies with cows in confinement, mostly in the United States and Europe, have found an incidence of SCH ranging from 18.9% (<1.87 mmol/L, 0 DIM; Gild et al., 2015) to 78.0% (<2.14 mmol/L, 1–3 DIM; Caixeta et al., 2017). The main reason for this high variability is likely due to the cut-point used, given that the higher the cut-point the higher the proportion of affected cows. Other possible sources of variation could be timing of diagnosis that varied from 0 to 7 DIM and those associated with management of cows (i.e., production system, nutrition, milk yield, and so on). We found that calving season was associated with the odds for SCH-0 and SCH-7 (i.e., higher odds in summer-calving cows). We also found that parity was associated with the odds for SCH-0 but not with the odds for SCH-7, consistent with Neves et al. (2017), who reported that parity is an important predictor for SCH at calving but not at 2 DIM, and with Martinez et al. (2012), who found that primiparous cows had higher Ca concentration than multiparous cows at calving but similar concentrations between parities from 1 to 12 DIM. A negative correlation between age and Ca (r = −0.23) has been reported (Eldon et al., 1988), with bone and intestine vitamin D receptors declining with aging (Horst et al., 1990). Therefore, increased age is

expected to play a role in the development of SCH, but why that association is only seen at parturition remains unanswered. Finally, and surprisingly, SCH-0 was not associated with the odds for SCH-7. As far as we know, this is the first report of this lack of association between SCH-0 and SCH-7. Taken together all the above information would suggest that risk factors for SCH could change with timing of diagnosis.

Our results regarding the associations of SCH-0 and -7 with health status should be interpreted with caution, because sample size estimation was not based on detecting differences between proportions (it was based on detecting differences in the calving to pregnancy interval). Therefore, the power for these analyses was poor and the possibility of type II error occurrence was present. We found that SCH-0 was associated with the odds for subclinical ketosis and that SCH-7 was associated with the odds for metritis, with other analyses showing fairly large nonsignificant associations. Other reports similarly found greater concentrations of BHB (Martinez et al., 2012), higher incidence of subclinical ketosis (Ribeiro et al., 2013), and even higher odds for clinical ketosis (Rodríguez et al., 2017) in cows with SCH than in normocalcemic cows. In some studies SCH was found to be predictive of metritis (Martinez et al., 2012; Ribeiro et al., 2013; Rodríguez et al., 2017), whereas in others such association was not found (Chamberlin et al., 2013; Neves et al., 2018) or even it acted as a protective factor (Gild et al., 2015).

Other limitations were that our study was run in only 1 herd and herd has been identified as a risk factor for SCH (Neves et al., 2017). We also lack data regarding individual milk yield because the study herd stopped running official monthly milk tests at the beginning of the experiment. A final consideration could be that time windows for Ca assessment were different (1 d at 0 DIM vs. 7 d at 7 ± 3 DIM), thus a wider window could be another reason why SCH-7 was not associated with certain outcomes.

CONCLUSIONS

Subclinical hypocalcemia at parturition, but not at 7 DIM, was associated with lower odds for pregnancy, lower hazard of pregnancy, and longer interval from calving to pregnancy; therefore, it is associated with poor fertility in a herd of grazing dairy cows. We suggest that it would be better to test the cows for SCH the day of calving than testing 7 d later.

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