Relationship Between Blood Calcium Level and Post-milking Teat Canal Closure in Dairy Cows

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Research Article

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Abstract

The teat canal–one of the primary defense mechanisms of the udder–ensures the milk flow during milking in bovines and prevents pathogens from entering the udder by forming a barrier through the elastic muscle and keratin layers tightly closing the surrounding area. The current study investigated the effects of blood calcium status on teat closure in cows after milking. The study covered 200 healthy teats, of which 100 were from normocalcemic (NC) cows and 100 were from subclinical hypocalcemic (SCH) cows. Teat canal length (TCL) and width (TCW) were measured with ultrasonography at 0-min pre-milking and 15- and 30-mins post-milking. Cylindrically shaped teat canal volume (TCV) was calculated by deriving from TCL and TCW. Time-dependent changes in teat canal closure and their relationships with blood calcium levels were analyzed. The results showed that the calcium level did not affect TCL, TCW, and TCV ($P > 0.05$) during the 15 minutes post-milking period. However, TCL ($P < 0.001$), TCW ($P < 0.05$), and TCV ($P < 0.001$) were higher in NC cows than in SCH cows at 30 minutes post-milking. At 15 minutes post-milking, no correlation existed between the teat canal closure (ΔTCL, ΔTCW, and ΔTCV) and the blood calcium level, while significant correlations were available between the teat canal closure and the blood calcium level {ΔTCL (r:-0.288, $P < 0.001$), ΔTCW (r:-0.260, $P < 0.001$), ΔTCV (r:-0.150, $P < 0.05$)} at 30 minutes post-milking. The current study concluded that the blood calcium status significantly impacts the teat canal closure in bovines, and calcium status should be meticulously monitored with the mastitis control program to apply necessary strategic steps.

1. Introduction

The teat canal, a primary anatomical defense system structure of the bovine udder, is surrounded by an elastic musculature extending from the external ostium of the teat to the sinus papillary (Blowey and Edmondson 2010). This unique and functional structure allows milk to flow and then closes tightly after milking, preventing pathogenic bacteria from entering the udder (Risvanlı et al., 2019). Reportedly, delayed closure of the teat canal increases the risk of developing new intramammary infections in dry cows (Dingwell et al., 2003, Martin, 2020), lactating cows (Neijenhuis et al., 2001; Strapák et al., 2017) and heifers (Krömker and Friedrich, 2009). After milking, the teat canal is closed by contraction of the surrounding sphincter muscles and clamping tightly for sealing (Giesecke et al., 1972; Van Der Merwe, 1985; Paulrud, 2005). In addition, with its keratin layer and cationic proteins, the teat canal acts as a critical “gate” that prevents pathogenic bacteria from entering the udder (Notcovich, 2021).

The vacuum effect created by machine milking systems in modern dairy farming adversely affects the teat canal perfusion and interstitial fluid dynamics (Paulrud, 2005). Some previous studies have documented that teat congestion and edema cause the delay of teat canal closure and adversely affect the cellular defense mechanism (Jankus and Baumann, 1986; Besier et al., 2016). Although some studies claimed that teat penetration and teat canal diameter improved two hours post-milking, Neijenhuis et al. (2001) and Wieland et al. (2019) reported that the teat canal did not regain its pre-milking state even after 6–8 hours.

Many studies have reported that subclinical hypocalcemia occurs in almost half of the cows depending on parity in dairy herds (Jawor et al., 2012; Neves et al., 2018; Da Silva et al., 2019). Subclinical hypocalcemia impairs the metabolic and physiological responses that regulate the welfare and productivity of transitional dairy cows (Rodrigues et al., 2020). Calcium mineral is crucial in numerous structural and biochemical processes in udder defense mechanisms, including the contractile activity of the teat sphincter (Paulrud, 2005; Mahjoubi et al., 2017), immune cell functions (Kimura et al., 2006; Zhang et al., 2019), blood coagulation (Libera et al., 2021), and teat canal keratin formation and S100 calcium-binding protein functions (Smolenski et al., 2015; Zhong et al., 2018). Histomorphological studies have shown that the teat canal returns to its original state after milking with the contraction of flexible, smooth muscle cells surrounding the teat canal (Giesecke et al., 1972; Van Der Merwe, 1985; Paulrud, 2005). The findings that calcium influx significantly impacts the smooth muscle cells' contraction (Amberg and Navedo, 2013) and blood circulation to the teat is 2–3 times greater in lactating cows than the dry-period cows (Hamann and Burvenich, 1994a; Melvin et al., 2019) provide evidence that "blood calcium levels" may considerably impact the closure of the teat canal after milking.

The present study hypothesized that post-milking teat canal closure is lower in SCH cows compared to NC cows. In order to test this null hypothesis, this experimental study investigated the relationship between blood calcium and pre- and post-milking teat conditions in dairy cows.

2. Material And Method

Ethical permission was obtained from the Tekirdağ Namık Kemal University Animal Experiments Ethics Committee (2021 – 576/2).

2.1. Animals, Herd management, and Milking procedures

The current study was performed in a commercial dairy farm in the Lüleburgaz district of Kırklareli between February and May 2021. There were 540 Holstein dairy cows on this farm. In the study, cows were grouped according to their milk yield and fed twice daily with a ration tailored to their nutritional requirements (NRC, 2001). The ration included corn silage, sunflower meal, clover, hay, concentrate feed, and vitamin-mineral premix. Milking was carried out twice daily using a 12x2 fish-bone system (DeLaval Milking system). Of the milking machine, the vacuum power was 42 kPa, the pulse count was 65 cycle/min, and the pulse ratio was 65:35. Teat dip (pre-dipping and post-dipping) was a routine application within the mastitis control program on the farm. Herd health, reproduction, and milk yield records were followed with the herd monitoring system (Allflex® Livestock Intelligence™, SCR Engineers, Israel).

The study covered 50 Holstein cows, of which 25 were subclinical hypocalcemic (SCH) and 25 normocalcemic (NC). Cows were selected according to particular criteria to ensure uniformity and minimize variation. The cows in the first or second lactation, between 50–100 DIM and with no postpartum udder disease participated in the study. The cows evaluated for their average 7-day milk yields and milk electrical conductivity data were also examined for their udder and teat diseases by palpation, strip cup test, and California Mastitis Test (CMT). CMT-negative cows with no inflammation and anatomical defects in udder lobes and teats took part in the study.

2.2. Teat canal ultrasonography and measurement of teat canal dimensions

The teat canal closure was measured with teat canal ultrasonography at the 0th-minute pre-milking and 15th and 30th minutes post-milking. The routine pre- and post-dipping treatments were not applied in the study because of the antiseptic solutions' astringent effects. Teat canal sonography was performed using a portable veterinary ultrasound device (Hasvet 838, Hasvet, Turkiye) with an 8 MHz linear probe (CTS-800, Hasvet, Turkiye), as described by Neijenhuis et al. (2001) and Strapak et al. (2017). According to this technique, after the teat was immersed in the thin-wall polyethylene container filled with water, horizontal two-dimensional and real-time ultrasonographic images of the teat were obtained by contacting the probe's scanning surface, which was applied a large amount of ultrasound gel, to the outside of the container.

The images were focused on measuring the teat canal length (TCL) and teat canal width (TCW) with maximum accuracy. While TCW was measured between two opposite points facing the lumen in the middle of the teat canal, TCL was measured from the teat canal opening (ostium papillary) to the Furstenberg rosette. Ultrasonographic measurements of TCL and TCW were recorded at 0th minutes pre-milking and 15th and 30th minutes post-milking. The cylindrical shaped teat canal volume (TCV) was calculated using the standard cylinder volume formula ($V = \pi r^2 h$).

The closure of the teat canal was assessed according to the differences (Δ) in TCL, TCW, and TCV at 15th and 30th minutes post-milking. The differences between teat measurements (Δ) before milking (0th minute) and 15th and 30th minutes after milking were calculated with the following formula: ($\Delta_{2-1} = Measurement_2 - Measurement_1$). Accordingly, the differences in TCL, TCW, and TCV between pre-milking and the 15th-minute post-milking were calculated as ΔTCL_{15-0}, ΔTCW_{15-0}, and ΔTCV_{15-0}, respectively. Similarly, differences in TCL, TCW, and TCV between 15th and 30th minutes post-milking were calculated as ΔTCL_{30-15}, ΔTCW_{30-15}, and ΔTCV_{30-15}, respectively.

2.3. Blood collection and Blood Calcium Tests

Blood samples for measuring calcium (Ca) levels were collected into 10 ml vacuum tubes by puncturing the coccygeal vein before milking. The samples left at room temperature for about two hours were centrifuged at 3500 rpm for 10 minutes. Later, the serums were placed in Eppendorf tubes and stored at -20°C until blood Ca levels were measured with a colorimetric enzymatic reaction (Roche Cobas 8000). The blood Ca ranges of NC and SCH cows were determined according to the studies of Horst et al. (2003) and Goff (2008). The blood Ca ranges were 8.5–11.5 mg/dL (2.1–2.8 mmol/L) in NC cows and 5.5–8.5 mg/dL (1.38–2.1 mmol/L) in SCH cows. In NC and SCH cows, the blood Ca cut-off value was accepted as 8.5 mg/dL (2.1 mmol/L).

2.4. Statistical analysis

In the study, the number of cows was determined with statistical power analysis (G*Power Version 3.1.9.4) and considering the sample sizes of previous similar studies (Neijenhuis et al., 2001; Krömker and Friedrich, 2009; Strapak et al., 2017). According to power analysis, the number of teats in each group (NC and SCH cows) was calculated as 100 teats by taking an effect size of 0.40, Type 1 error of 0.05, and statistical power of 0.8 (Cohen, 1988).

All statistical analyzes were performed using the GraphPad Prism statistical and graphical software program (Version 8.0.2 for Windows). Two-way ANOVA and post hoc Tukey test (2×3 factorial design) were used to evaluate TCL, TCW, and TCV of NC and SCH cows at pre-milking and 15th and 30th minutes post-milking, thus the main factors (Blood-Ca status and time) and their interactions were tested. Pearson correlations were calculated for each blood Ca level corresponding to ΔTCL_{15-0}, ΔTCW_{15-0}, ΔTCV_{15-0}, ΔTCL_{30-15}, ΔTCW_{30-15}, and ΔTCV_{30-15}. For each combination, residual error plots were generated to display the changes of difference between teat canal measurements and blood Ca levels. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant and indicated strong evidence for the alternative hypothesis.

3. Results

The mean ± SD of blood Ca levels of NC and SCH cows were 9,45 ± 0,58 mg/dL (8,80 – 10,50) and 6,61 ± 0,60 mg/dL (5,60 – 7,74), respectively.

3.1. Teat canal measurements at pre- and post-milking

The main factors (Blood Ca status and time) and their interactions were compared using Two-way ANOVA (2×3 factorial design). Table 1 shows the relative changes, the mean ± SEM value, and the post hoc test of Ca status in TCL, TCW, and TCV at pre-milking and post-milking 15th and 30th minutes.

Table 1
Relative changes, mean value ± SEM, and the post hoc test of Blood Ca status on TCL, TCW, and TCV at pre-milking and 15th- and 30th-minute post-milking.

	Normocalcemia (n = 100)	**Subclinical hypocalcemia (n = 100)**	***P***
TCL (cm)			
Pre-milking (0.min)	0.859 ± 0.022[a]	0.889 ± 0.017[a]	ns
15 min. post-milking	1.213 ± 0.030[b] (+ 41%)	1.258 ± 0.025[b] (+ 41%)	ns
30 min. post-milking	0.955 ± 0.022[Ac] (+ 11%)	1.135 ± 0.021[Bc] (+ 27%)	*
P	***	***	
TCW (cm)			
Pre-milking (0.min)	0.246 ± 0.010[a]	0.232 ± 0.006[a]	ns
15 min. post-milking	0.363 ± 0.015[b] (+ 47%)	0.351 ± 0.007[b] (+ 51%)	ns
30 min. post-milking	0.279 ± 0.010[Ac] (+ 13%)	0.311 ± 0.006[Bc] (+ 34%)	*
P	***	***	
TCV (cm^3)			
Pre-milking (0.min)	0.043 ± 0.003[a]	0.041 ± 0.002[a]	ns
15 min. post-milking	0.129 ± 0.008[b] (+ 200%)	0.130 ± 0.007[b] (+ 217%)	ns
30 min. post-milking	0.060 ± 0.003[Ac] (+ 39%)	0.092 ± 0.004[Bc] (+ 124%)	**
P	***	***	
Two-way ANOVA posthoc Bonferroni test for multiple comparisons (P < 0.05)			
TCL: Teat canal length, TCW: Teat canal width, TCV: Teat canal volume			

3.1.1. Effects of Ca level and time on Teat Canal Length (TCL)

Analysis results showed significant effects in Blood Ca status {F (1,198): 8.485, P < 0.05}, time {F (1.375, 272.3): 430.7, P < 0.0001} and their interaction {F (2, 396): 22.30, P < 0.0001}. The NC and SCH cows' mean TCL at the 15 min post-milking increased by 41% compared to the pre-milking. Furthermore, at 30 min post-milking, the NC and SCH cows’ mean TCL decreased by 21% and 9%, respectively, when compared to the 15th-minute mean TCL (Table 1, Fig. 1a).

3.1.2. Effects of Ca level and Time on Teat Canal Width (TCW)

Although time and interaction had significant effects on TCW {for time, $F(1.288, 255.1)$: 370.4, $P < 0.0001$; for interaction, $F(2, 396)$: 17.91, $P < 0.0001$}, blood Ca status had no significant effect {$F(1,198)$: 0.022, $P > 0.05$}. In NC cows, TCW increased by 47% at the 15th-minute post-milking compared to pre-milking and reduced by 23% at the 30th-minute post-milking compared to the 15th-minute post-milking (Table 1, Fig. 1b). On the other hand, TCW in SCH cows increased by 51% at the 15th-minute post-milking compared to pre-milking but reduced by 11% at the 30th-minute post-milking compared to the 15th-minute post-milking. While there was no difference between TCW at the 15th-minute post-milking in NC and SCH cows ($P > 0.05$), the teat canal recovery was lower at the 30th-minute post milking in SCH cows ($P < 0.05$).

3.1.3. Effects of Ca level and time on Teat Canal Volume (TCV)

The time and the interaction had significant effects on TCV {for time $F(1.61, 229.8)$: 329.4, $P < 0.0001$; for interaction $F(2, 396)$: 14.66, $P < 0.0001$}, but calcium status had no effect on TCV {for calcium status, $F(1, 198)$: 3.132, $P = 0.078$}. The mean TCV of NC and SCH cows at the 15th-minute post-milking was $0.129 cm^3$ and $0.130 cm^3$, respectively ($P > 0.05$). At the 30th-minute post-milking, TCV in NC and SCH cows decreased by 53% and 29%, respectively ($P > 0.05$) (Table 1, Fig. 1c).

3.2. Relationship between Teat canal closure and Blood Ca status

Correlation analyzes were used to examine the relationship between blood Ca status and teat canal closure on the 200 teats at the 15th- and 30th-minutes post-milking. Table 2 shows Pearson correlation coefficients and R^2 with a 95% confidence interval, while Fig. 2 shows residual plots. The results showed that the correlations in teat canal closure at the 15th-minute post-milking were not statistically significant {for ΔTCL_{15-0}, $r = -0.06$, $P > 0.05$; for ΔTCW_{15-0}, $r = -0.052$, $P > 0.05$; for ΔTCV_{15-0}, $r = -0.071$, $P > 0.05$}. However, the results showed an inverse relationship between Blood Ca status and teat canal closure at 30th-minute post-milking {for ΔTCL_{30-15}, $r = -0.288$, $P < 0.0001$; for ΔTCW_{30-15}, $r = -0.260$, $P < 0.0001$; for ΔTCV_{30-15}, $r = -0.150$, $P < 0.05$}. These findings showed that teat canal closure was slower at the 15th-minute post-milking in SCH cows than in NC cows (Fig. 2).

Table 2

Pearson correlation coefficients and R^2 with 95% confidence intervals between blood Ca status and the Δ Measurements of the teat canal

Δ Measurements	Blood Ca status			
	r	R^2	95% CI	P
ΔTCL_{15-0}	-0.060	0.0036	-0.197 to 0.079	ns
ΔTCL_{30-15}	-0.288	0.0832	-0.410 to -0.156	***
ΔTCW_{15-0}	-0.052	0.0027	-0.190 to 0.086	ns
ΔTCW_{30-15}	-0.260	0.0679	-0.385 to -0.126	***
ΔTCV_{15-0}	-0.071	0.0051	-0.208 to 0.067	ns
ΔTCV_{30-15}	-0.150	0.0227	-0.283 to -0.012	*
ns: P > 0.05, *: P < 0.05, ***: P < 0.0001				

4. Discussion

Subclinical hypocalcemia causes significant adverse conditions in dairy cows that affect productivity, welfare, and physiology. It plays direct or indirect roles in the pathogenesis of many diseases such as ketosis, abomasal displacement, mastitis, metritis, retained placenta, uterine subinvolution, fatty liver, and suppression of clinically critical immune system functions in the bovine (Rodriguez et al., 2017). Calcium is essential for the normal functioning of both skeletal and smooth muscle cells. Reportedly, normocalcemia is theoretically necessary for the contractility of the teat canal sphincter, which is one of the primary defense mechanisms of the udder during lactation and dry periods in dairy cows (Kimura et al., 2006; Goff, 2008; Libera et al., 2021). Some studies have reported that calcium level does not affect milk leakage in dry-off dairy cows (Mahjoubi et al., 2018). However, to our knowledge, no study has investigated the effect of blood calcium levels on teat canal closure in lactating cows. Therefore, this study is the first to examine the impact of blood calcium status on teat closure in dairy cows post-milking.

Information about the post-milking teat canal closure is controversial. While some researchers (McDonald, 1975; Blowey & Edmondson, 2010; Löfstrand, 2018) claimed that two hours was sufficient for the teat canal closure post-milking, others claimed that the teat canal could not completely close at the 3rd-hour (Stadnik et al., 2010; Stojnovic and Alagic, 2012) and not even at the 8th-hour post-milking (Neijenhuis et al., 2001; Wieland et al., 2019). As a barrier, the teat canal plays a crucial role in preventing intramammary infections. Previous studies have shown that inoculation of pathogens directly into the teat canal significantly increases the likelihood of developing an intramammary infection compared to the inoculation into the sinus papillary (Notcovich, 2021). However, even if the teat canal, a mechanical barrier, is not fully closed, the hydrophobic layer formed by keratin and cationic proteins in the teat canal prevents milk flow in the first 30 minutes post-milking. Therefore, the current study investigated the effect of blood calcium status on teat canal closure in the first 30 minutes post-milking.

The study findings showed that pre-milking teat canal measurements (TCL, TCW, and TCV) were not different between NC and SCH cows ($P > 0.05$). Previous studies (Klein et al., 2005; Çelik et al., 2008; Paulrud and Rasmussen, 2004) reported that the length of the teat canal was around 1 cm (0.3 to 1.8 cm), although it varied according to race, parity, and lactation period. Similar to previous studies, the current study found that pre-milking TCL was almost identical in both NC and SCH cows (0.85 and 0.88, $P > 0.05$). TCL was similar in NC and SCH cows at the 15th minute after milking, and an increase was observed in both groups at the same rate. Moreover, there was no significant relationship between ΔTCL15-0 and blood calcium level. At the 30th minute post-milking, TCL in NC cows was significantly close to the pre-milking state ($P < 0.01$) compared to SCH cows, and there was a significant correlational relationship between ΔTCL30-15 and blood calcium level. This situation suggests the vacuum effect created by the milking system at the 15th minute may suppress the sphincter muscle rhythmic contractions surrounding the teat canal (Paulrud, 2005; Witzel, 1965), or the inability of muscles to perform their normal functions may be caused by acute congestion and edema in the region (Hamann et al., 1994b; Wieland et al., 2020). Therefore, it can be concluded that due to the adverse effects of the milking system, the blood calcium level could not affect the TCL at the 15th minute after milking. However, after the milking system's unfavorable impact gradually disappears at the 30th-minute post-milking, the TCL returns to its pre-milking state more rapidly in NC cows. A recent study (Wieland et al., 2020) investigated blood perfusion of the teat pre- and post-milking and revealed time-dependent different blood flow patterns. In this respect, it can be concluded that machine milking delays teat canal closure as it reduces the blood flow to the teat canal and thus the calcium required for contraction of the teat canal muscles.

The current study found that the mean pre-milking TCW in NC cows was 0.2 cm. While Giesecke et al. (1972) stated that TCW was 0.5 cm on average, Melvin et al. (2019) reported that TCW varied between 0.86 mm and 3.45 mm, with an average of 1.94 mm. Teat canal width, an important parameter that reflects the milk flow rate (Paulrud, 2005), is also a contributing factor in the susceptibility of cows to mastitis (Lacy-Hulbert and Hillerton, 1995). Reportedly, there is a strong positive correlation between TCW and somatic cell numbers (Jorstad et al., 1989). Although the data obtained from studies conducted with different measurement techniques differ, they were almost the same (Giesecke et al., 1972; Hamann, 1987; Neijenhuis et al., 2001; Çelik et al., 2008). However, different cow genotypes might be a reason for these differences. Similar to our TCL findings, no difference occurred in TCW between NC and SCH cows at the pre-milking and the 15th-minute post-milking. The TCW recovery was higher in NC cows than in SCH cows. However, the recovery rate of TCW was less than that of TCL. At the 30th-minute post milking, NC cows' TCW recovery was statistically less insignificant than their TCL recovery, despite their higher pre-milking period return rate than SCH cows. This finding may be related to the elasticity of the muscle fibers surrounding the teat canal because the vertical part of the teat can directly adapt to the mechanical forces caused by machine milking than the longitudinal part (Besier et al., 2016; Löftstrand, 2018).

The current study found no difference in TCV at the 15th-minute post-milking in NC and SCH cows. There was a significant recovery in TCV at 30th-minute post-milking in NC cows compared to SCH cows. For the first time in the literature, the authors of this paper suggest that the volume of the cylindrical teat, depending on the length and width of the teat canal, will give a more reliable result in evaluating teat canal closure. Since no similar explaining model has been found in the literature, the researchers are the first to suggest this model. Net-like muscle fibers surrounding the teat canal (Van Der Merwe, 1985) and the machine milking-related interstitial edema at the teat canal tip rather than the upper Furstenberg rosette regions (Besier et al., 2016; Neijenhuis et al., 2001) imply that using TCV might be more advantageous in evaluating teat canal closure.

The current study has, for the first time, revealed that blood calcium status significantly affects post-milking teat canal closure in machine milking. In the study, it was observed that blood calcium did not affect the teat canal closure at the first 15th-minute post-milking, possibly due to the destructive effect of machine milking. However, at the 30th-minute post-milking, it played a significant role while the teat canal regained its pre-milking state. Within the scope of mastitis control program, in addition to strategic applications including teat dipping, teat film formation, and post-milking feeding. the calcium status of the lactating herd should be periodically scanned and a good feeding management should be implemented.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest.

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Data Availability Statement

The datasets generated during and/or analysed during the current study are available in the personal cloud repository,
docs.google.com/spreadsheets/d/1IJEnw9L7X4c3LFdbXehOMqUG9TGfwqoq/edit#gid=370962030.

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Figures

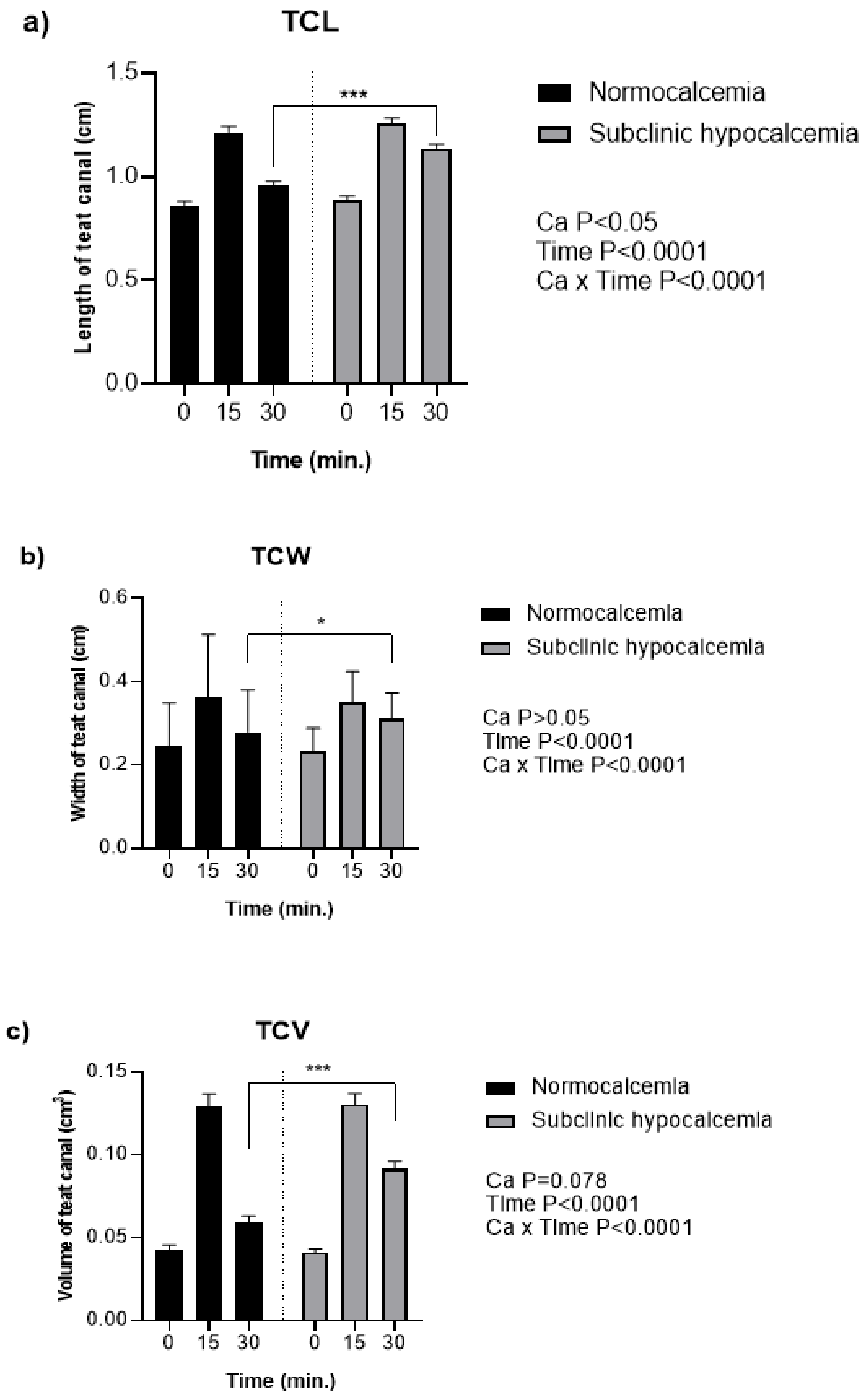

Figure 1

The effects of blood Ca status on changes in teat canal length (TCL), width (TCW), and volume (TCV) between pre-milking and 15th- and 30th-minute post-milking.

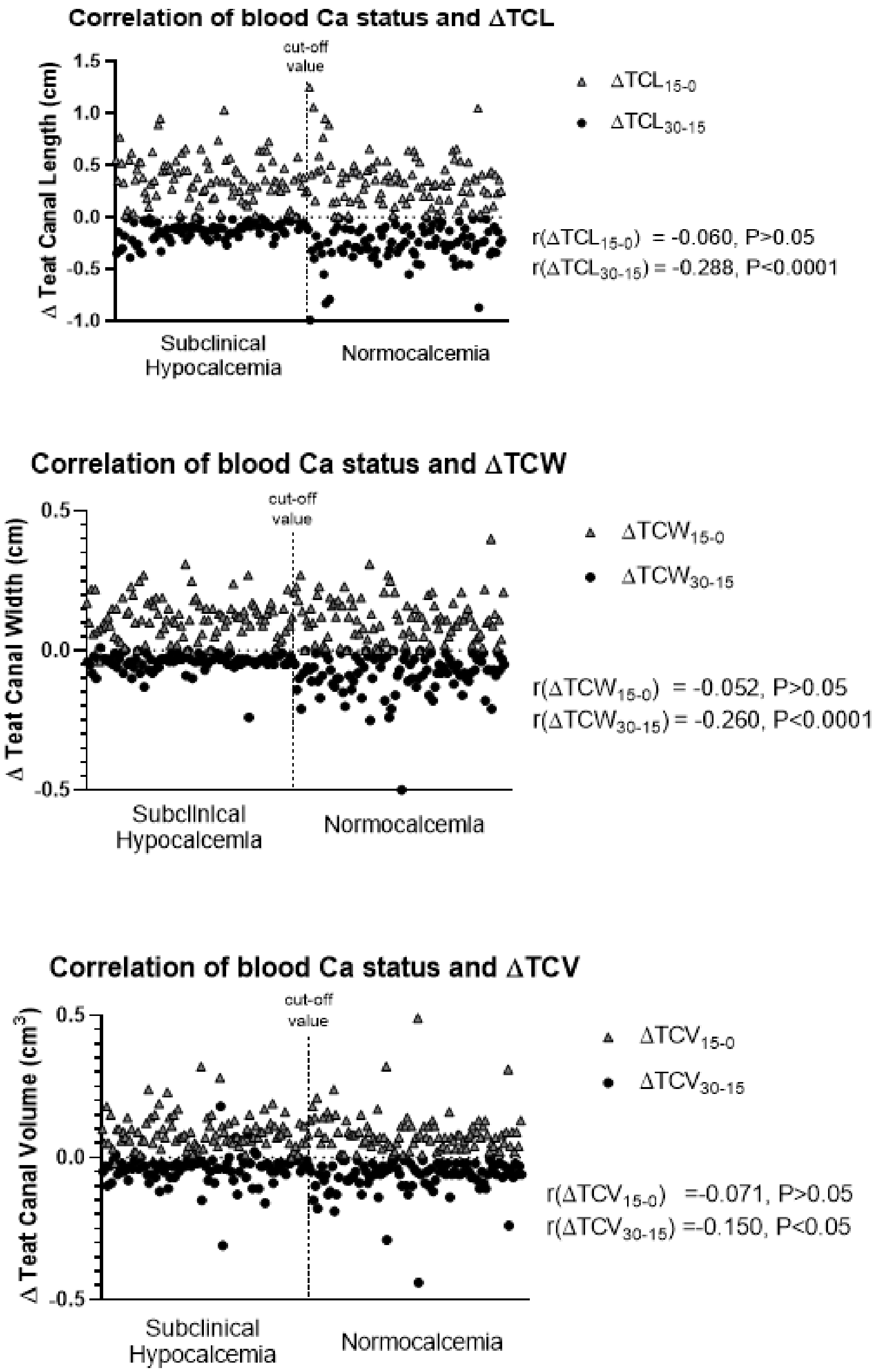

Figure 2

Correlations between blood calcium status and changes in teat canal measurements at different time points