**NOTE** *Internal Medicine*

# Relationship between blood calcium level and ST peak interval of electrocardiographic variables in peripartum Holstein cows

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ABSTRACT. The association between blood calcium levels and electrocardiographic variables was compared in 137 normal parturient and 36 peripartum recumbent Holstein cows to determine whether hypocalcemia in peripartum dairy cows can be rapidly diagnosed using electrocardiograph. Inverse of STc (ST peak interval/SS interval$^{0.5}$) and blood ionized calcium or serum calcium concentrations were strongly correlated, and both correlation coefficients were 0.81 ($P<0.001$). The 95% prediction interval indicated that cows with STc >0.385 ± 0.001 sec are very likely to be hypocalcemic (blood ionized or serum calcium concentrations of <0.9 mmol/*l* or <7.5 mg/d*l*, respectively). These findings indicate that hypocalcemia in parturient cows can be non-invasively estimated using the STc.

KEY WORDS: cow, diagnosis, electrocardiograph, hypocalcemia

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Milk fever (parturient paresis) is a common disease of peripartum dairy cows. The most reliable diagnostic method is measurement of the blood calcium (Ca) concentrations, which is difficult to accomplish on site. Fluctuations in blood Ca levels cause changes in electrocardiographic (ECG) variables, such as the QT interval and ST segment [2, 16]. Littledile *et al.* [11] reported that plasma Ca and ionized calcium ($Ca^{++}$) concentrations are linearly related to the corrected QoT interval (QoTc) in cows with hypocalcemia. Daniel *et al.* [3] found in cows with experimentally induced hypocalcemia and hypercalcemia that corrected QT intervals (QTc) of >450 and <400 msec indicate plasma Ca levels of <1.84 and >2.09 mmol/*l*, respectively. Matsuo *et al.* [12] found that both serum Ca and $Ca^{++}$ significantly correlated with QoTc and QTc in cows under treatment for milk fever, but concluded that the margin of error was too large to estimate blood Ca from ECG findings. In contrast, Kvart and Larsson did not find any correlation between the degree of hypocalcemia and the QoTc in cows with parturient paresis [8]. We aimed to define the relationship between ECG findings and blood Ca levels in peripartum dairy cows including those with milk fever and explored the use of ECG as a rapid, non-invasive tool for diagnosing hypocalcemia in dairy cows.

We investigated 137 clinically normal parturient Holstein cows (age 4.5 ± 1.7 years, mean ± S.D.) and 36 peripartum recumbent Holstein cows (age 5.9 ± 2.0 years) at 25 farms. Normal cows were ≤24 hr postpartum, and recumbent cows ranged from 1 day prepartum to 15 days postpartum. Jugular venous blood was collected into 500 $\mu l$ tubes containing heparin lithium (CAPIJECT, Terumo, Tokyo, Japan) and into 5 m*l* plain tubes (VENOJECT II, Terumo). Blood $Ca^{++}$ was measured in the heparinized samples using a blood gas/electrolyte/hematocrit analyzer (Chiron 348, Siemens AG, Munich, Germany). Non-heparinized blood samples were separated by centrifugation at 3,000 rpm for 15 min at 4°C, and serum Ca was analyzed using an automated chemistry analyzer (SYNCHRON CX5, Beckman Coulter, Brea, CA, U.S.A.). A portable ECG system (ES-202, Parama-Tech, Fukuoka, Japan) was used for monitoring in the field, and electrode tips were modified into clips for easier attachment to the animals. The ECG findings were recorded from a base-apex (A-B) lead [14] for about 30 sec and transferred to a personal computer using software (EP-STATION) supplied with the monitor. QT, QoT, ST peak and SS intervals (Fig. 1) measured using software (Lenaraf ver. 2.20, Atelier M&M, Chiba, Japan) are shown as averages. After clarifying the proportional relationship between QT or QoT intervals and ST peak intervals by regression analysis, the ST peak intervals were corrected using Bazett's formula [1]

$$\mathrm{STc} = \frac{\mathrm{ST}}{\sqrt{\mathrm{SS}}}$$

where STc is the corrected ST peak interval (sec), ST is the ST peak interval (sec), and SS is the SS interval (sec).

The correlation between blood $Ca^{++}$ and serum Ca was clarified by regression analysis and calculated from ionization rates. Regression equations, correlation coefficients, *P* values and the 95% prediction intervals between blood Ca levels (blood $Ca^{++}$ and serum Ca) and STc were examined by regression analysis. All data were statistically analyzed

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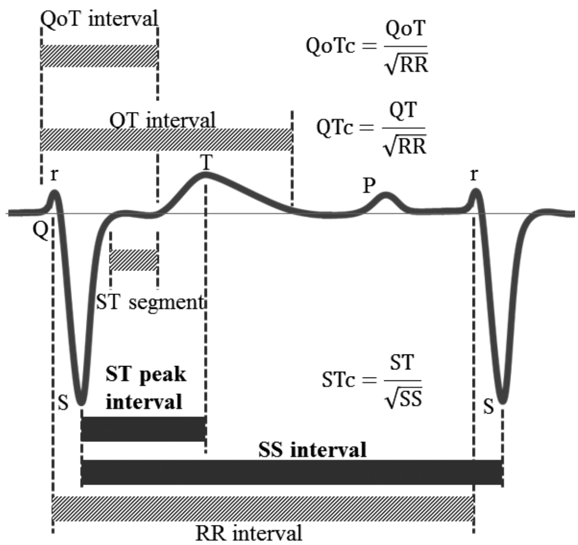


Fig. 1. Normal ECG waves in A-B lead of cows and its components. ST peak interval is the interval between the apex of S wave and the apex of T wave. SS interval is the time interval between two consecutive S waves. QT interval is the interval between the onset of QRS complex and the end of T wave. QoT interval is the interval between the onset of QRS complex and the onset of T wave. RR interval is the time interval between two consecutive R waves. ST segment is the time interval between the end of S wave and the onset of T wave. QTc is the QT interval with a formulated correction by the RR interval. QoTc is the QoT interval with a formulated correction by the RR interval. STc is the ST peak interval with a formulated correction by the SS interval.

using R version 3.0.3 statistical software. Cows with serum Ca <7.5 or <5.0 mg/d*l* were defined as subclinically hypocalcemic and hypocalcemic, respectively [6].

Blood $Ca^{++}$ and serum Ca in all (normal parturient and recumbent) cattle were within the range of 0.22–1.22 mmol/*l* and 2.81–10.91 mg/d*l*, respectively. Serum Ca was <5.0, 5.0–7.5 and >7.5 mg/d*l* in 21 (recumbent, n=16), 59 (recumbent, n=5) and 93 (recumbent, n=15) cows, respectively. The correlation between QT or QoT intervals and ST peak intervals was very strong, both with correlation coefficients of 0.99 and $P<0.001$. Thus, the ST peak interval was used as the ECG variable instead of QT or QoT interval in further analyses. The correlation between blood $Ca^{++}$ and serum Ca was very strong (correlation coefficient=0.95; $P<0.001$). The ionization rate was 47 ± 5% (mean ± S.D.), and the concentration of blood $Ca^{++}$ was about 0.9 mmol/*l* when the serum Ca was 7.5 mg/d*l*. A strong positive correlation shown below was found between blood $Ca^{++}$ or serum Ca and the inverse of the STc ($STc^{-1}$).

$$Ca^{++} = a \times STc^{-1} + c$$

or

$$Ca = a \times STc^{-1} + c$$

where $Ca^{++}$ is the blood ionized calcium concentration (mmol/*l*), Ca is the serum calcium concentration (mg/d*l*),

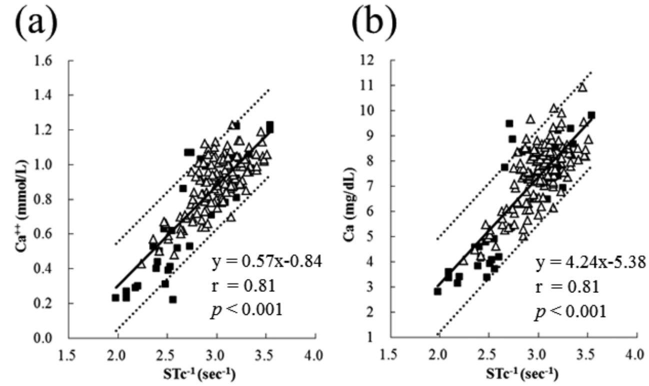


Fig. 2. Scatter plots of inverse STc and (a) blood $Ca^{++}$ and (b) serum Ca concentrations in 173 peripartum dairy cows. Solid and dotted lines represent the regression line and 95% prediction intervals, respectively. The linear regression of y (blood $Ca^{++}$ or serum Ca) against x (inverse of STc), correlation coefficient (r) and *P* values are indicated. Normal postpartum (Δ) and recumbent (■) cows.

STc is the correlated ST peak interval (sec), and "a" and "c" are the regression coefficients. As shown in Fig. 2 (a), the coefficient of regression formula "a" was 0.57 ± 0.03 (mean ± S.E.), "c" was −0.84 ± 0.09, the correlation coefficient was 0.81, and the *P* value was <0.001 for $Ca^{++}$ (mmol/*l*). Figure 2 (b) shows that these values for Ca (mg/d*l*) were 4.24 ± 0.23, −5.38 ± 0.70, 0.81 and <0.001, respectively. The average blood $Ca^{++}$ and serum Ca estimated from the regression equation were <0.9 mmol/*l* and <7.5 mg/d*l* when the STc was ≥0.328 and ≥0.329 sec, respectively. There was 95% likelihood that STc of ≥0.384 and ≥0.386 sec would result in a blood $Ca^{++}$ of <0.9 mmol/*l* and a serum Ca of <7.5 mg/d*l*, based on the 95% prediction interval (dotted line in Fig. 2).

It is well known that low blood Ca levels prolong the QT interval and the ST segment [2, 16]. Some studies have found that the QTc or the QoTc can serve as a diagnostic index of hypocalcemia in humans [13, 15]. Hypocalcemia can easily develop in peripartum dairy cows, but ECG variables have not been used as a diagnostic index of hypocalcemia in these animals because the relationship between the ECG variables and blood Ca was unclear, early treatment was important, and attaching an ECG device to cows was difficult. However, ECG devices have recently become smaller, inexpensive and portable, thus allowing simple measurement of ECG variables on site. Here, we clarified the relationship between ECG findings and blood Ca in peripartum dairy cows using a portable ECG device. The relationship between ECG and blood Ca was previously investigated based on QTc or QoTc [3, 11, 12] that are the respective corrected QT or QoT intervals for the RR interval. The components of the AB lead that are generally used in cows for ECG [4] are characterized by the absence of Q waves, small R waves, and large S and T waves [14]. The ST peak interval and the SS interval are extracted more easily and accurately than the QT and QoT interval. Because artifacts, such as body motion or myoelectricity, can become mixed with ECG variables when measuring cows on site, ECG variables that are easily detectable even in the presence of noise are important. Therefore, we clarified that the results were similar even when the QT or QoT intervals were initially replaced by the ST peak interval. Because they are all heartbeat intervals, we used the SS interval instead of the RR interval. We considered that to use the STc, the ST peak interval should be corrected by the SS interval as an explanatory variable of the regression equation.

Only ionized Ca is physiologically active during skeletal or cardiac muscle contraction [5]. However, serum Ca is generally used to clinically diagnose hypocalcemia in cows. Therefore, we measured not only blood $Ca^{++}$, but also serum Ca and found a strong correlation between them, as has also been found in previous reports [9, 10]. About 45% of serum Ca is usually ionized [6, 10], and the average ionization rate of blood Ca was 47% in our study. Based on regression equations, the blood $Ca^{++}$ and serum Ca concentrations were about 0.9 mmol/*l* and 7.5 mg/d*l*, respectively, which was indicative of subclinical hypocalcemia. These values were consistent with the clinical symptoms of hypocalcemia, because cows with blood $Ca^{++}$ of 0.80–1.05 mmol/*l* had no appetite or rumen activity and were recumbent [9]. We considered that either blood $Ca^{++}$ or serum Ca could serve as an objective variable in the regression equation. Scatter plots showed that the relationship between blood $Ca^{++}$ or serum Ca and the STc was a fractional function in this study. Because the relationship between blood Ca and STc was nonlinear, if a linear regression equation of blood Ca and STc was determined, the deviation occurred in the residual and the correlation coefficient was reduced. Inverse transformation of the STc resulted in an essentially straight line, improved estimation accuracy and the regression equation became simpler. The correlation coefficients between blood $Ca^{++}$ or serum Ca and $STc^{-1}$ were both 0.81, indicating a very strong correlation. The STc appeared sufficient to estimate blood $Ca^{++}$ or serum Ca and thus allow the non-invasive diagnosis of hypocalcemia in dairy cows using ECG. Furthermore, in this study, there were some recumbent cows that were not hypocalcemic. It was considered that they were recumbent due to dystocia or a fall. In these cows, a strong positive correlation was found between blood $Ca^{++}$ or serum Ca concentration and $STc^{-1}$. STc was a useful indicator, despite the cow's situation. Calcium salts should be injected into cows with STc $>0.329 \pm 0.001$ and $>0.385 \pm 0.001$ sec, because they are either likely to be or will almost certainly be hypocalcemic, respectively. However, estimated Ca values tend to be lower than actual values in cows with normal calcium levels. Feed intake and milking might influence this, because it is reported that the cardiac function changes with daily milk yield in healthy cows [7]. Estimation accuracy will probably be further improved, if a cause is found and the regression equation is corrected. We plan to clarify the cause of the calculation error in cows with normal Ca levels and to estimate blood Ca levels in peripartum cows using regression equations in the clinical field and to improve the accuracy of rapid diagnosis of hypocalcemia in dairy cows using ECG.

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