Electrolytes in Bovine Prepartum Mammary Secretions and Their Usefulness for Predicting Parturition

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ABSTRACT

Accurately predicting parturition in cows can be difficult because of the variability in gestation length and uncertainty of the time of parturition. The goal of the present study was to determine the electrolyte concentrations of prepartum mammary secretions of gravid cows and to investigate retrospectively the possible predictive relationships between these concentrations and the time of parturition. Twenty-three cows were sampled once daily for 3 to 12 d before they calved. The concentrations of calcium, inorganic phosphorus, chloride, magnesium, sodium, and potassium were determined by photometric methods. In addition, the concentrations of calcium, magnesium, and inorganic phosphorus were determined using rapid test kits. The correlation between the photometrically measured electrolyte concentrations and the time of parturition was highest for inorganic phosphorus ($r = 0.74$). The inorganic phosphorus concentration was 11.8 to 26.5 mmol/L in cows that calved within 24 h of sample collection. When 11.8 mmol/L was used as a cutoff concentration for inorganic phosphorus, 21.7, 47.8, and 87.0% of cows calved within 24, 48, and 72 h, respectively. Within 12 d prepartum, the time of parturition can be estimated, with an error of ± 32.4 h, by using the results of simultaneous determination of inorganic phosphorus and sodium concentrations in mammary secretions and a formula derived from a multiple regression analysis. The results of a phosphate field test positively and significantly correlated with those of the photometric analysis ($r = 0.69$) and the time of parturition ($r = 0.41$).

Key words: mammary secretion, cow, electrolyte, predicting time of parturition

INTRODUCTION

In large dairy herds, it may be difficult to monitor individual animals closely during the prepartum and

peripartum periods. However, failure to recognize and correct calving-related problems promptly can have catastrophic consequences for the well-being and productivity of the cows. For decades, researchers have investigated possible means of predicting the onset of parturition, ranging from observation of external signs to the determination of physiological parameters (Porterfield and Olson, 1957; Ewbank, 1963; Birgel et al., 1994; Erices and Blaufuss, 1996). Predicting the time of parturition would benefit cow-calf herds in which the percentage of live births is economically important. In bull-bred herds, the time of breeding is often not known, making the monitoring of parturition especially difficult. If one could predict the time of parturition, losses caused by dystocia could be reduced. Recently, 2 studies were published in which the time of parturition was predicted in beef cows (Wehrend and Bostedt, 2004; Aoki et al., 2005). The calving management of dairy cows with a history of dystocia or of cows carrying an expensive calf would be facilitated if the time of parturition were known. Clinical signs such as a decrease in the rectal or vaginal temperature (Porterfield and Olson, 1957; Ewbank, 1963), relaxation of the pelvic ligaments, flexibility of the tip of the tail, reduced active tail movement, vulvar swelling, and filling up of the udder all have a low predictive value (Dufty, 1971; Birgel et al., 1994; Wehrend and Bostedt, 2004). Measuring the progesterone concentration over a period of several days is the only variable with generally accepted usefulness for predicting the time of parturition (Birgel et al., 1994).

In horses, the electrolyte concentrations of prepartum mammary secretions were used to predict the time of foaling (Peaker et al., 1979). Special test kits (Cash et al., 1985; Brook, 1987) or a commercial water hardness test, which determine the total concentration of calcium and magnesium ions semiquantitatively (Cash et al., 1985; Ousey et al., 1989; Erices and Blaufuss, 1996), were used for this purpose.

To the authors' knowledge, there have been no reports on the electrolyte concentrations in prepartum mammary secretions in cattle. The goal of the present study was to investigate electrolyte concentrations in prepartum mammary secretions and to determine

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whether the results can be used to predict parturition. In addition, the accuracy of an inexpensive and simple cow-side test kit was assessed retrospectively.

MATERIALS AND METHODS

Animals and Sample Collection

Twenty-three pregnant and nonlactating cows were used. They included 4 cows from the Vetsuisse-Faculty, University of Zurich, and 19 privately owned cows referred to the Vetsuisse-Faculty, University of Zurich, for monitoring for calving. Eighteen cows were Swiss Braunvieh and 5 were Red Holstein, ranging in age from 2 to 15 yr (mean = 6 yr). Eight cows were primiparous, and the remaining 15 cows had had 1 to 13 previous pregnancies. The calvings occurred over a period of 15 mo. The cows were divided retrospectively based on their parity and into 4 groups based on the time of year when they calved. Collection of mammary secretions began when the mammary gland started to fill. After disinfecting the teats with an alcohol-soaked swab, 3 to 5 mL of secretion was collected from each teat by hand milking between 0700 and 0900 h, once daily, until calving. The udder parenchyma was palpated and the secretion was evaluated grossly to rule out inflammation. A total of 3 to 12 samples were collected from each cow before calving, providing 168 samples. Day 0 was defined as the day when the cow calved within 24 h of collection of the mammary secretions. Immediately after collection, the samples were frozen and stored at -20°C until processing.

Laboratory Methods

For evaluation of electrolyte concentrations, the samples were thawed at room temperature and 6.7% HNO_3 was added at a ratio of 1:1 (wt/wt) for protein precipitation. The samples were then centrifuged at $20,000 \times g$.

The concentrations of calcium (Calcium 125, Ref. A11A0112; ABX Diagnostics, Diatools, Dietikon, Switzerland), inorganic phosphorus (Inorganic Phosphorus, Ref. A11A00098; ABX Diagnostics), chloride (Chloride, Ref. 000010120021; ABX Diagnostics), and magnesium (Magnesium 160, Ref. A11A00096; ABX Diagnostics) in the supernatant were determined photometrically using color tests. The concentrations of sodium and potassium were determined with a flame atomic emission spectrophotometer (IL; Ingold, Urdorf, Switzerland; ISO 4571, International Organization for Standardization, 1981).

Test Kits

In addition to the photometric analyses, 101 samples of mammary secretions from 17 of 23 cows were analyzed using 2 semiquantitative rapid test kits. The results of the photometrically determined values of the first 6 cows were used to calculate the dilution factor of the prepartum mammary secretions for the rapid tests.

A water hardness test (Total Hardness test, 1.10025.0001, Merckoquant; Merck, Darmstadt, Germany) was used to determine the concentrations of calcium and magnesium. In this test, the calcium and magnesium ions form complexes with Tritriplex III. The test strips contain 4 reaction zones, which change from green to red when the total concentrations of calcium and magnesium ions are greater than 0.7, 1.3, 2.5, and 3.8 mmol/L, respectively. Because preliminary measurements showed that the calcium concentration of bovine prepartum mammary secretions can exceed 20 mmol/L, the samples were diluted at a 1:6 ratio with distilled water (1 part mammary secretions, 6 parts water, Aqua B. Braun; B. Braun, Melsungen, Germany; Cash et al., 1985). In addition, samples diluted 1:50 were measured. The test strip was placed in the diluted mammary secretions for 1 s, withdrawn, and shaken to remove any residual fluid. The strips were read after 1 min and the number of red and green areas was counted.

A rapid test for inorganic phosphorus (Phosphate Test, 1.10428.0001, Merckoquant; Merck) was used to measure the concentration of inorganic phosphorus. The test is based on the reduction of inorganic phosphorus in the presence of molybdenum ions; in a solution acidified with sulfuric acid, orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue. With an increase in the inorganic phosphorus concentration, the reaction zone changes from yellow to dark blue. The color of the reaction zone was compared with a color chart to determine semiquantitatively the concentration of inorganic phosphorus. Because preliminary measurements showed that the concentration of inorganic phosphorus in bovine prepartum mammary secretions can reach 20 mmol/L, the samples were diluted at a 1:5 ratio with distilled water (1 part mammary secretions, 5 parts water, Aqua B. Braun; B. Braun). The test strip was placed in the diluted mammary secretions for 15 s, withdrawn, and excess fluid allowed to drain onto a paper towel. A drop of test kit reagent (PO_4 -1 reagent) was placed on the reaction zone. After 15 s, the reagent was removed by draining it onto a paper towel. The test strip was read 1 min later by comparing the color with a color

scale. When the color on the test strip appeared between 2 colors on the scale, the higher value was used. The individual colors were scored by comparison with a color scale as follows: 1 (0.01 to 0.53 mmol/L), 2 (0.54 to 1.31 mmol/L), 3 (1.32 to 2.63 mmol/L), 4 (2.64 to 5.25 mmol/L), 5 (5.26 to 13.13 mmol/L), 6 (13.14 to 26.25 mmol/L). These concentration ranges reflected those that were supplied by the manufacturer after correction for dilution.

Statistical Analyses

The data were analyzed using StatView 5 (SAS Institute, Wagen bei Dübendorf, Switzerland). The results were given as mean $\pm$ standard deviation ($\bar{x} \pm \text{SD}$). The error bars for standard deviation in the ANOVA are pooled errors for each time point. Significant differences were determined by ANOVA, Student's *t*-test, and the χ^2 test for association. A value of $P < 0.05$ was considered statistically significant. For χ^2 comparison of the results of the semiquantitative rapid tests with those of the quantitative photometric tests, the results of the latter were grouped according to the concentrations represented by the different color scores of the former. Regression analysis was used to examine the relationships between the results of the semiquantitative field tests and those of the photometric tests and between the results of the semiquantitative field tests and the number of days prepartum.

To generate a formula to calculate the time period to parturition based on the photometrically measured values, a regression analysis and a residual analysis with the dependent variable (time interval between sampling and parturition) were carried out for each independent variable. The final model of regression for each variable was achieved by visual residual analysis and optimization of the correlation coefficient (*r*) using quadratic, logarithmic, and root functions. The dependent variables, with their respective functions, were then entered in a multiple regression model, with single variables with a $P < 0.20$ entered in the regression model. The final multiple regression model was achieved by a step-back procedure, using a stepwise elimination of the independent variable with the largest *P*-value > 0.05 . The single standard error for the final model was calculated by the propagation function of the error term ε (Altman, 1994). The propagation function of the error term ε follows a binominal distribution and was calculated according to the formula

$$\varepsilon_0 = \sqrt{\frac{\sum_{i=n} (\varepsilon_n^2)}{n-1}},$$

where ε_0 is the propagated error (standard error) and ε_n is the standard error for each parameter.

RESULTS

All 23 cows calved spontaneously and without assistance after a gestation period of 271 to 293 d. The 15 multiparous cows had been dry for 5 to 9 wk before calving. The calves were healthy and showed no signs of prematurity. None of the cows had clinical mastitis, and at the time of calving, all samples of colostrum were macroscopically unremarkable.

The concentrations of all the electrolytes changed (chloride $P < 0.05$, all other electrolytes $P < 0.001$) during the 12 d before parturition (Figure 1). The concentrations of calcium, potassium, magnesium, and inorganic phosphorus increased, whereas those of sodium and chloride decreased. Of all the electrolytes evaluated, inorganic phosphorus showed the greatest correlation with the time of parturition ($r = 0.74$; $P < 0.001$; Table 1). Cows that calved within 24 h after the collection of mammary secretions had inorganic phosphorus concentrations between 11.8 and 26.5 mmol/L. When 11.8 mmol/L was used as the cutoff for the inorganic phosphorus concentration, 21.7% (5) of the animals calved within 24 h, 47.8% (11) within 48 h, and 87.0% (20) within 72 h.

The final multiple regression model showed that the concentrations of inorganic phosphorus and sodium were the major variables for predicting the time of parturition. The time of year had no significant effect on the concentration of either electrolyte. Parity and number of days prepartum were singular in the ANOVA. Therefore, season and parity were omitted from the model. The course of these electrolytes was not linear, but rather logarithmic (to base 10) for inorganic phosphorus and quadratic for sodium. The following formula was calculated for predicting the time of parturition:

$$\begin{aligned} \text{days before parturition} = & -2.855 + 5.544 \\ & \times \log[\text{inorganic phosphorus}] - 0.563 \times \sqrt{[\text{sodium}]}, \end{aligned}$$

with a standard error of 32.4 h. This corresponds to an inaccuracy of 11% for calculations within 12 d before calving.

The water hardness test (combined concentrations of calcium and magnesium) conducted at either dilution had no significant correlation with the time of parturition. Between d 6 and 1 before calving, 80% of the samples had maximum concentrations (all test fields were red). In contrast, there was a correlation between the results of the rapid test for inorganic phos-

Figure 1. (A) Calcium, potassium, magnesium and inorganic phosphorus concentrations ($\bar{x} \pm \text{SD}$) in prepartum mammary secretions of 23 cows during the last 12 d before parturition. (B) Chloride and sodium concentrations ($\bar{x} \pm \text{SD}$) in prepartum mammary secretions of 23 cows during the last 12 d before parturition.

phorus and the time of parturition ($r = 0.41$, $P < 0.001$), with a correlation equation of

$$y = -7.88 + 0.829x.$$

There was also a correlation with the results of the photometric test for inorganic phosphorus ($r = 0.69$, $P < 0.001$), with a correlation equation of

$$y = 2.233 + 3.58x.$$

Table 1. Correlations and their coefficients between the concentrations of various electrolytes determined photometrically and the time of parturition

Electrolyte	Correlation coefficient	P-value	95% confidence interval, lower limit	95% confidence interval, upper limit
Calcium	0.69	<0.0001	0.60	0.77
Potassium	0.61	<0.0001	0.50	0.70
Magnesium	0.52	<0.0001	0.40	0.63
Inorganic phosphorus	0.74	<0.0001	0.66	0.80
Chloride	-0.29	<0.001	-0.43	-0.14
Sodium	-0.65	<0.0001	-0.73	-0.55

The mammary secretions of 82.4% of the cows ($n = 14$), which calved within 24 h after sample collection, had a score of 6 (13.126 to 26.25 mmol/L of inorganic phosphorus), and 94.1% ($n = 16$) had a score 5 or 6 (5.26 to 26.25 mmol/L of inorganic phosphorus).

Samples of mammary secretions with test scores of 1 to 6 were followed by parturition within 24 h in 0, 0, 0, 5.3, 8.7, and 33.3% of cases, respectively. The probability that a test score of 6 (13.126 to 26.25 mmol/L of inorganic phosphorus) would be followed by parturition within 48 h was 57.1% (24 of 42 samples) and that it would be followed by parturition within 72 h was 78.6% (33 of 42 samples). Fifty-one measurements were carried out in the last 3 d before parturition. The frequency with which the 6 test scores were followed by parturition within 24, 48, or 72 h is shown in Figure 2.

DISCUSSION

There were significant differences in the concentrations of all the electrolytes investigated during the 12 d prior to parturition in our study. In contrast, the concentrations of calcium and phosphorus in colostrum did not differ in samples that were collected 32 h before parturition and those collected 2 h after the start of parturition (Bojkovski et al., 2005). As has been shown for the horse (Peaker et al., 1979; Ousey et al., 1984), the concentrations of these electrolytes were correlated with the time of parturition in the cow and are useful for predicting the time of calving. The use of external signs for predicting the exact time of parturition in cattle has yielded such a wide variation that they have been deemed unreliable (Dufty, 1971;

Figure 2. The 6 scores of a phosphate field test ($n = 51$) and the frequency with which they were followed by parturition within 24, 48, or 72 h.

Birgel et al., 1994; Wehrend and Bostedt, 2004). Slightly more useful results were achieved when a decrease in rectal temperature was used for predicting parturition, although the temperature had to be recorded twice daily for several days (Porterfield and Olson, 1957; Ewbank, 1963; Dufty, 1971). Better, but rather impractical, methods of predicting parturition in cattle were by the continuous measurement of vaginal temperature at 1-min intervals over the last 5 d before parturition (Aoki et al., 2005) or by the daily determination of progesterone concentration (Birgel et al., 1994). However, the time of parturition cannot be estimated based on single measurements.

With the multiple regression model established in the present study, the time of parturition can be estimated based on the concentrations of sodium and inorganic phosphorus in prepartum mammary secretions with an accuracy of less than 3 d (SE = 32.4 h) in the last 12 d before parturition. The ability to predict the time of parturition to within a few days has practical implications for many aspects of the management and veterinary care of the dairy cow. For example, prophylaxis of parturient paresis can be achieved more effectively through the administration of vitamin D₃, which is administered 2 to 8 d prepartum when given intramuscularly or during the last 5 d prepartum when given orally (Radostits et al., 2000). When the time between the administration of vitamin D₃ and parturition is considerably longer than this, the opposite effect may occur, resulting in prolonged and treatment-resistant recumbency (Radostits et al., 2000; Horst et al., 2003).

To make the prediction of parturition on the basis of electrolyte concentration more practical, we not only used laboratory methods, but also a field test that has been used in mares to evaluate readiness for birth (Brook, 1987; Ousey et al., 1989; Erices and Blaufuss, 1996) as well as a rapid test for inorganic phosphorus concentration. The water hardness test overestimated calcium and magnesium concentrations. This overestimation was apparent when the mammary secretions were diluted in a 1:50 instead of a 1:6 ratio and no significant correlation between semiquantitative and photometric measurements was found at either dilution. In contrast, the rapid test for inorganic phosphorus was more effective when planning the monitoring period because it can predict the days on which parturition will not occur. Parturition is not expected within 24 h after a score of 1 to 3, and only 5.3% of cows with a score of 4 calved within 24 h. The probability of calving was so low in cows with a score of <5 that intensive monitoring was not indicated. Thus, close monitoring for parturition can be restricted to a few days.

We consider the semiquantitative phosphate test the method of choice for estimating the time of parturition in dairy cows because in the final multiple regression model, the concentration of inorganic phosphorus was 1 of 2 major variables used for predicting the time of parturition. The other variable was the concentration of sodium; however, no field test is available for this measurement. The phosphate test is straightforward and easily carried out in the field by veterinarians or lay people. It can help determine when to move a cow to the calving pen, and it makes monitoring the cows more efficient. Ideally, this test could be useful for assessing readiness for birth in cases in which parturition is induced or an elective Cesarean section is undertaken. However, further study is needed to investigate the correlation of electrolyte concentration in prepartum mammary secretions and readiness for birth in cows.

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