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Mammary gland secretion pH and electrolytes in prepartum Mangalarga Marchador mares

[*Concentrações de pH e eletrólitos na secreção da glândula mamária de éguas Mangalarga Marchador no período pré-parto*]

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ABSTRACT

The objective of this study was to determine pH and the concentrations of Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Chloride (Cl^-), Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{2+}) in the mammary gland secretion in pre-foaling Mangalarga Marchador (MM) mares to determine the best method to predict parturition in the breed. Forty-two pregnant MM mares were evaluated once daily from 310 days of gestation until parturition. Mammary gland secretion pH, measured by pH meter and pH paper strip, Mg^{2+}, Ca^{2+} and Cl^- levels significantly changed during the last 10 days prior to parturition ($p<0.05$). The lowest pH mean value (pH meter=6.67±0.42; pH strip=6.23±0.53) and significantly higher mean concentrations of Ca^{2+} (12.6±5.8mmol/L) and Mg^{2+} (10.93±3.66mmol/L) were obtained 24 hours prior to foaling ($p<0.0001$). Chloride levels reduced significantly 8 days prior to parturition ($p<0.0001$) but remained unchanged until the due date. Mammary gland pH secretion, measured by pH meter and pH paper strip, was effective to predict parturition. Quantitative pH values obtained with the pH meter had a greater accuracy in comparison to pH paper strips, but a strong correlation was found between both methods. Calcium and magnesium were the only electrolytes that changed in concentration immediately prior to parturition and are better indicators of when not to expect foaling.

Keywords: pH meter, foaling, gestation

RESUMO

O objetivo deste estudo foi determinar o pH e as concentrações de cálcio (Ca^{2+}), magnésio (Mg^{2+}), cloro (Cl^-), sódio (Na^+) e potássio (K^+) na secreção da glândula mamária de éguas Mangalarga Marchador (MM) no período pré-parto, a fim de determinar o melhor método para predizer proximidade do parto na raça. Quarenta e duas éguas MM foram avaliadas diariamente a partir dos 310 dias de gestação até o parto. O pH, aferido pelo pHmetro e por fitas reagentes, e os eletrólitos Ca^{2+}, Mg^{2+} e Cl^- apresentaram diferenças significativas em suas concentrações nos últimos 10 dias pré-parto ($P<0,05$). A diminuição significativa dos valores médios de pH (pHmetro= 6,67±0,42; fitas reagentes= 6,23±0,53) e o aumento significativo nas concentrações médias de Ca^{2+} (12,6±5,8mmol/L) e Mg^{2+} (10,93±3,66mmol/L) foram observados 24 horas pré-parto ($P<0,0001$). As concentrações de cloro diminuíram significativamente oito dias antes do parto ($P<0,0001$), porém continuaram sem alteração até o momento da parição. O pH da secreção foi eficaz para predizer o momento do parto. Valores quantitativos obtidos por meio do pHmetro apresentaram maior acurácia em comparação às fitas de pH, porém obteve-se forte correlação entre os dois métodos. Cálcio e magnésio foram os únicos eletrólitos que apresentaram alterações significativas no pré-parto imediato e são indicadores mais precisos de quando não esperar o parto.

Palavras-chave: pHmetro, parto, gestação

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INTRODUCTION

To ensure the delivery of healthy foals it is important to attend foaling and intervene in a timely manner if abnormalities are detected (Bain and Howey, 1975; Santschi and Vaala, 2011; Canisso *et al.,* 2013). While most foalings require no intervention, when foaling problems arise, such as a delay in the second stage of labor, the consequences may result in the death of the neonate. Therefore, early interventions may improve mare and foal outcome and decrease economical losses (Christensen, 2011; Rosales *et al.,* 2017; Cheong *et al.,* 2019; Nagel *et al.,* 2020). Several methods to predict parturition in mares have been proposed, with different results. Gestation length is variable among mares (320-365 days) and considered an unreliable method to predict parturition (Ley, 2011). Changes in body conformation and evaluation of mammary gland secretion have been used to predict foaling date (Peaker *et al.,* 1979; Ousey *et al.,* 1984). Physical signs of readiness for parturition, such as udder development, relaxation of tailhead, pelvic ligaments and vulva may be subtle and variable among mares and are not considered reliable methods to predict the day of parturition (Peaker *et al.,* 1979; Korosue *et al.,* 2013). Changes in mammary gland concentrations of magnesium (Mg^{2+}), chloride (Cl^-), sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) on serial evaluations have been tested with variable results, with the last being the most used (Ley *et al.,* 1993; Canisso *et al.,* 2013; Korosue *et al.,* 2013). Lately, changes in mammary gland secretion pH were described to predict parturition with good results in Thoroughbred (TB) and light horse mares (Canisso *et al.,* 2013; Korosue *et al.,* 2013).

Mangalarga Marchador (MM) is the largest Brazilian horse breed. They are well known for their march gait and have a significant economic and social impact nationally and now internationally, with the export of horses and embryos to other countries (MAPA, 2016; Criação…, 2018. Mammary gland secretion to predict parturition have been evaluated in several breeds (Ley *et al.,* 1993; Canisso *et al.,* 2013; Korosue *et al.,* 2013, Amorim *et al.,* 2019), but to the best of our knowledge there are not studies in MM mares. It has been previously demonstrated that MM mares have different reproductive parameters in comparison to other breeds, such as reference ranges for the combined utero-placental thickness (Campos *et al.,* 2017), and this might apply for mammary gland secretion as well.

The objective of this study was to determine pH and the concentrations of magnesium, chloride, sodium, potassium, and calcium in the mammary gland secretion in pre foaling Mangalarga Marchador mares to determine the best method to predict parturition in the breed.

MATERIAL AND METHODS

This study was approved by the Ethics Committee for the use of animals of the Universidade Federal de Minas Gerais (CEUA/UFMG) under the protocol 374/2019.

Forty-two pregnant Mangalarga Marchador mares, with ages between 4 and 15 years old (mean 9.16), were used in the study, from 2020 to 2021. The mares were privately owned by a registered MM farm in the state of Minas Gerais, Brazil. They were kept in paddocks with access to free choice water, hay, and mineral salt during the entire experiment. The mares were evaluated once daily in the evening (from 5 pm to 6 pm), from 310 days of gestation until the day of parturition. The mares foaled from November to February.

All mares were inspected daily for physical changes (relaxation of the tailhead and pelvic ligaments, softening and lengthening of the vulva) and udder development and secretion in the mammary gland. T1 was defined as ≤ 24hours prior to foaling; T2: 2 days prior to foaling; T3: 3 days prior to foaling; T4:4 days prior to foaling; T5: 5 days prior to foaling; T6: 6 days prior to foaling; T7: 7 days prior to foaling; T8: 8 days prior to foaling; T9: 9 days prior to foaling; T10: 10 days prior to foaling. All findings were recorded daily in an individual chart. When mammary gland secretion was present, the udder was washed with water and soap, dried with paper towel and between 1 and 2 ml of secretion were collected in a conical tube. Mammary gland secretion pH and water hardness were immediately evaluated. The samples were then frozen at -20°C and stored for further evaluation of electrolyte concentrations.

The pH of the prepartum mammary secretion was analyzed with a portable pH meter with automatic temperature compensation (Kasvi®, Model K39-0014PA, São José dos Pinhais, PR, Brazil) and with a pH indicator paper strip with ranges from 5.5 to 8.0 units with a 0.2 pH incremental interval (Hydrion®, Micro Essential Laboratory, New York, NY, USA) (Canisso *et al.*, 2013). The portable pH meter was calibrated daily prior to its first reading using a buffer solution provided by the manufacturer. The electrode was then placed in the mammary gland secretion and reading was performed as soon as the pH value stabilized. The electrode was rinsed with distilled water between samples and was submerged in KCL solution at the end of the day, according to the manufacturer recommendation. The pH paper strip was immersed in the mammary gland secretion and immediately removed. Results were immediately interpreted by comparing the observed strip color with the color standard chart provided by the manufacturer.

Water hardness was measured by semi-quantitative estimation of calcium carbonate ($CaCO_3$) using a commercial paper strip (Aquadur®, Macherey-Nagel GmbH & Co.KG, Düren, NW, Germany) (Ousey *et al.,* 1984). The paper strip was immersed in the mammary gland secretion for 1 second and left in room temperature for 1 minute prior to reading. Results were classified according to a score system provided by the manufacturer with 5 squares, based on changes in color (0 square: <55ppm of $CaCO_3$; 1 square: >90ppm of $CaCO_3$; 2 squares: >180ppm of $CaCO_3$; 3 squares: >270ppm of $CaCO_3$; 4 squares: >360ppm of $CaCO_3$; 5 squares: >445ppm of $CaCO_3$).

Electrolyte concentrations were analyzed using a chemistry analyzer (Cobas Mira Plus®, Roche Ltd, Basel, Switzerland) (Peaker *et al.,* 1979) and reagents manufactured by Biotécnica® (Varginha, MG, Brazil). Calcium was analyzed by o-cresolphthalein complexone method, magnesium by xylidyl blue method, sodium by β-galactosidase and o-nitrophenol-b-D-galactoside (ONPG) enzymatic method, potassium by pyruvate kinase and phosphoenolpyruvate enzymatic method and chloride by mercury thiocyanate method.

Mares diagnosed with placentitis during the experimental phase or post-partum were excluded from the experiment.

Results of pH and electrolytes were described as mean and standard deviation or absolute and relative frequencies. Pearson correlation was obtained to describe associations between pH measured by pH meter and pH strip and between pH and electrolytes. A Bland-Altman plot was constructed to evaluate the difference in pH measurements with different mean values. Mixed regressions were modeled to evaluate the effect of age, weight, body condition score, gestation length and days to parturition on pH and electrolytes. In the model, animal effect was considered as random to accommodate repeated measures. The best model was chosen by likelihood ratio test. Predictions by the days to parturition were obtained for final models. A logistic regression considering the categorical value foaled or did not foal as response variable was performed as a function of the former prediction. A threshold of 0.5 probability for classification of foaled or did not foal was set to compare with the observed data. ROC curves were constructed, and the best threshold was obtained as the closest top left point. Sensitivity, specificity, accuracy, negative predictive value, and positive predictive value were calculated for the best threshold and the closest highest and lowest values to visualize changes in classification quality parameters. Evaluations were performed considering less than 24 hours to foal as response variables for classification. R software (R Core Team, 2020) was used for all analyzes.

RESULTS

Three mares were diagnosed with placentitis and were excluded from the experiment. One mare did not show udder development and mammary gland secretion and 2 mares did not have enough secretion for the analysis. Therefore, a total of 36 mares were included in the final analysis. Mean gestational length was 333 days ±12.88. None of the mares required assistance during parturition and all delivered healthy foals.

Age, weight, body condition score and gestation length did not affect mammary gland secretion pH and electrolytes. Mammary gland secretion pH, Mg^{2+}, Ca^{2+} and Cl^- levels significantly changed during the prepartum period (Tab. 1). The pH, determined by pH meter and pH paper strip, significantly decreased as mares approached foaling, with the lowest values detected 24 hours prior to parturition (pH meter= mean of 6.67 ±0.42; pH strip= mean of 6.23 ±0.53) ($p<0.0001$) (Fig.1). A strong correlation was found between pH values obtained with the pH meter and pH paper strip (r= 0.89) and a moderate to strong negative correlation was found between pH and Mg^{2+} (pH meter: r= -0.62; pH strip: r= -0.69) and pH and Ca^{2+} (pH meter: r= -0.71; pH strip: r= -0.72). Although a strong correlation was found between pH values obtained by pH meter and pH paper strip, a higher discrepancy between values was noted when lower pH values were observed (Fig.2).

Table 1. Means and standard deviation (SD) for pH and electrolytes during the last 10 days prior to parturition in the mammary gland secretion of Mangalarga Marchador mares

	pH		Electrolytes mmol/L				
	pH meter	pH strip	Ca^{2+}	Mg^{2+}	Cl^{-}	K^{+}	Na^{+}
T1	6.67±0.42^{a}	6.23±0.53^{a}	12.6±5.8^{a}	10.93±3.66^{a}	96.8±26.25^{a}	17.26±1.88^{a}	69.71±31.36^{a}
T2	6.98±0.45^{b}	6.59±0.59^{b}	8.07±4.14^{b}	7.97±3.0^{b}	86.4±11.7^{a}	18.52±5.31^{a}	70.57±45.74^{a}
T3	7.2±0.37^{c}	6.84±0.54^{c}	4.2±2.77^{c}	7.04±3.14^{b}	88.8±11.89^{a}	17.38±5.94^{a}	81±36.62^{a}
T4	7.3±0.3^{c}	7±0.49^{c}	4.92±3.51^{c}	6.54±2.93^{b}	105.5±44.41^{a}	16.18±3.76^{a}	84.5±36.29^{a}
T5	7.3±0.31^{c}	7.09±0.44^{c}	4.1±3.19^{c}	6.71±3.12^{b}	85.3±8.07^{a}	15.57±4.19^{a}	81.81±34.35^{a}
T6	7.27±0.41^{c}	7.17±0.4^{d}	4.12±3.59^{c}	5.84±3.0^{b}	92.9±11.77^{a}	21.94±28.87^{a}	92.36±34.63^{a}
T7	7.4±0.33^{c}	7.28±0.29^{d}	3.92±3.35^{c}	6.11±2.59^{b}	93.1±11.74^{a}	13.14±4.87^{a}	99.92±39.05^{a}
T8	7.52±0.26^{c}	7.29±0.3^{d}	2.52±1.44^{c}	3.59±1.38^{b}	101±17.29^{a}	13.22±3.49^{a}	97.75±38.55^{a}
T9	7.32±0.2^{bc}	7.24±0.23^{d}	2.52±1.94^{c}	5.23±1.87^{b}	161.6±103.35^{b}	11.57±5.32^{a}	89.67±22.48^{a}
T10	7.32±0.18^{bc}	7.28±0.19^{d}	2.92±4.07^{c}	3.73±2.59^{b}	160.4±101.88^{b}	10.5±5.8^{a}	101±32.19^{a}

Means with different letters differ from one another within the same column ($P<0.05$). Mg^{2+}= Magnesium, Ca^{2+}= Calcium, Cl^{-}= Chloride, K^{+}= Potassium, Na^{+}= Sodium. Time points: T1: ≤ 24hours prior to foaling, T2: 2 days prior to foaling, T3: 3 days prior to foaling, T4:4 days prior to foaling, T5: 5 days prior to foaling, T6: 6 days prior to foaling, T7: 7 days prior to foaling, T8: 8 days prior to foaling, T9: 9 days prior to foaling, T10: 10 days prior to foaling.

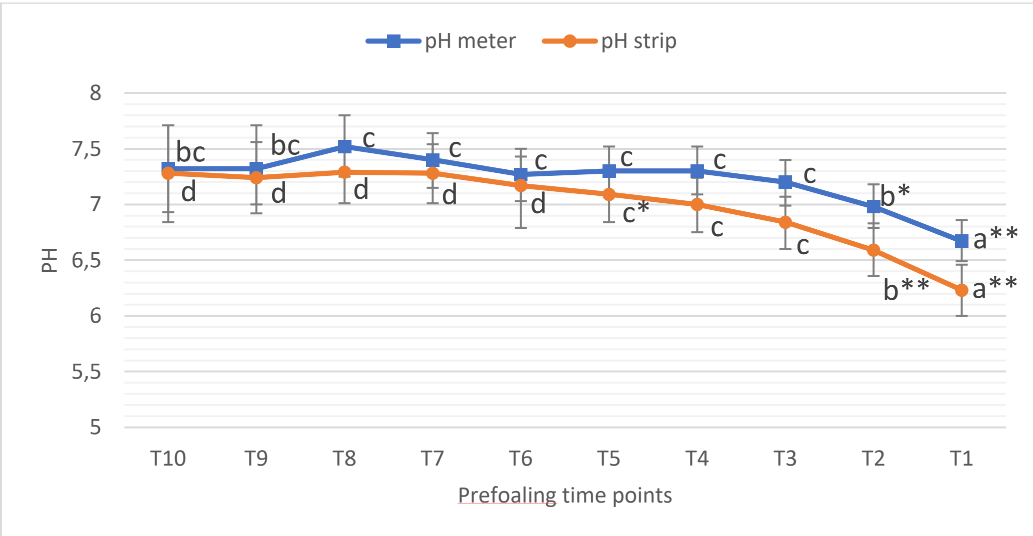

Figure 1. pH mammary gland secretion obtained by pH meter and pH paper strip during the last 10 days prior to parturition in Mangalarga Marchador mares. Different letters represent significant statistical difference between time points within the same method of analysis. Time points: T1: ≤ 24hours prior to foaling, T2: 2 days prior to foaling, T3: 3 days prior to foaling, T4:4 days prior to foaling, T5: 5 days prior to foaling, T6: 6 days prior to foaling, T7: 7 days prior to foaling, T8: 8 days prior to foaling, T9: 9 days prior to foaling, T10: 10 days prior to foaling (*$p<0.05$; ** $p<0.0001$).

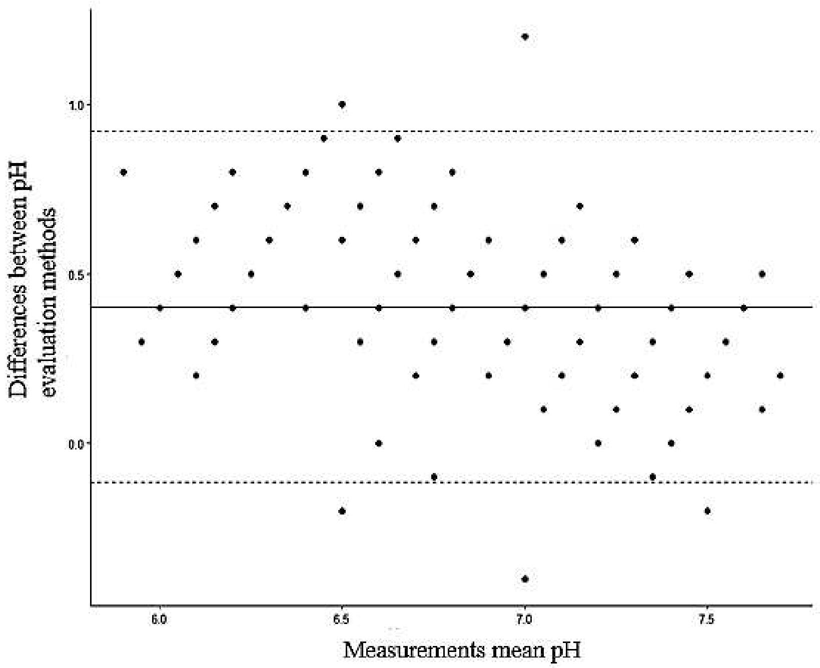

Figure 2. Bland-Altman graphic for pH measurements obtained by pH meter and pH paper strip in the mammary gland secretion of Mangalarga Marchador mares in the last 10 days prior to parturition. A higher discrepancy between values was noted when lower pH values were observed

Significantly higher concentrations of Ca^{2+} (mean 12.6mmol/L ±5.8) and Mg^{2+} (mean 10.93mmol/L±3.66) were obtained 24 hours prior to foaling ($p<0.0001$) (Fig.3). Chloride levels reduced significantly 8 days prior to parturition ($p<0.0001$) and remained unchanged until the day of parturition (mean 96.8 mmol/L ±26.25, 24 hours pre foaling) (Fig.3). No significant changes in K^+ and Na^+ were observed during the experiment.

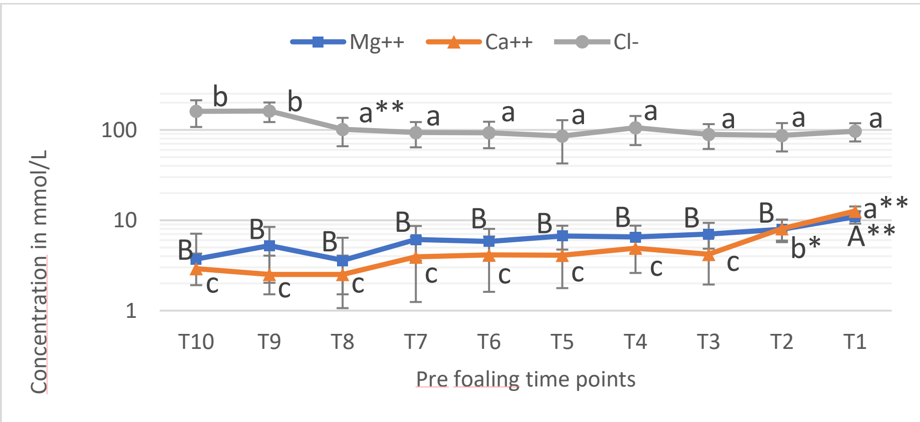

Figure 3. Magnesium, Calcium and Chloride concentrations in the mammary gland secretion of Mangalarga Marchador mares within 10 days prior to foaling. Different letters represent significant statistical difference between time points within the same method of analysis. Mg^{2+}= Magnesium, Ca^{2+}= Calcium, Cl^-= Chloride. Time points: T1: ≤ 24hours prior to foaling, T2: 2 days prior to foaling, T3: 3 days prior to foaling, T4:4 days prior to foaling, T5: 5 days prior to foaling, T6: 6 days prior to foaling, T7: 7 days prior to foaling, T8: 8 days prior to foaling, T9: 9 days prior to foaling, T10: 10 days prior to foaling (*$p<0.05$; ** $p<0.0001$).

The accuracy to predict foaling within 24 hours was 87% after pH decreased to 6.8 with the pH meter and 78% after pH decreased to 6.6 with the pH strip. The optimal predicted threshold for Mg^{2+} and Ca^{2+} were 8.40 mmol/L (accuracy of 87%) and 9.79 mmol/L (accuracy of 94%), respectively. The threshold value with better combination of sensitivity and specificity for each variable analyzed and the calculated positive and negative predict values and accuracy are described in Table 2.

Table 2. Sensitivity, Specificity, Positive Predict Value (PPV), Negative Predict Value (NPV) and Accuracy for pH, Magnesium (Mg^{2+}) and Calcium (Ca^{2+}) in the mammary gland secretion of MM mares 24 hours before parturition, considering different threshold values

	pH						Electrolytes (mmol/L)	
	pH meter			pH strip			Mg^{2+}	Ca^{2+}
VALUES	6.6	6.8*	7	6.2	6.6*	6.8	8.40*	9.79*
Sensitivity (%)	50	65	90	44	86	94	88	90
Specificity (%)	97	93	72	94	76	63	86	95
PPV (%)	84	70	43	64	84	35	65	81
NPV (%)	89	91	96	89	97	98	96	97
Accuracy (%)	88	87	75	85	78	69	87	94

*Estimated values determined as the closest top left point of the relative operating characteristics curve graphic (ROC).

The water hardness test revealed concentrations > 445 ppm of $CaCO_3$ (5 squares) 24 hours prior to foaling, but no significant difference was observed in the last 4 days preceding parturition ($p>0.05$). Between 4 and 10 days prior to foaling significant changes in $CaCO_3$ concentration were noted ($p<0.001$). Mean concentrations above 270 ppm (3 squares) were observed in all experimental time points. The water hardness test had a moderate and negative correlation with pH (pH meter: $r = -0.38$; pH strip: $r = -0.56$) and a moderate and positive correlation with calcium ($r = 0.65$).

All mares showed relaxation of the tailhead and pelvic ligaments, softening and lengthening of the vulva in variable times within the last two weeks prior to parturition.

DISCUSSION

Mares with placentitis were excluded from the study since mammary gland electrolytes are considered an unreliable method of screening in this scenario (Rossdale, 1991). Determining mammary gland secretion pH is practical and economically viable and was effective in predicting parturition in MM mares. Quantitative pH values obtained with the pH meter had a greater accuracy in comparison to pH paper strip, but since a strong correlation was found between methods both can be used, and selection should be based on availability and cost. The appropriate optimal predicted threshold varied according to the method of analysis. Measurements performed once daily were effective in predicting parturition in the present study, as previously demonstrated by Canisso *et al.* (2013).

The optimal pH threshold to predict parturition within 24 hours, considering sensitivity and specificity, were 6.8 and 6.6 for the pH meter and pH paper strip, respectively. Those findings are consistent with previous reports in light horse mares, where a pH <7 predicted foaling within 24 hours (Canisso *et al.*, 2013). Based on findings from the present study, pH values above 6.6 (measured by pH paper strip) have a NPV for foaling within 24 hours of 97% in MM mares. In TB mares, values above 6.4 were previously correlated with a NPV of 99% to predict parturition within 24 hours (Korosue *et al.*, 2013). MM mares showed a fast reduction in pH prior to foaling, similarly to that reported in the literature in mares from other breeds. Contrary to horses, most jennies tend to have a slow drop in pH observed within the last 5 days preceding parturition (Magalhaes *et al.,* 2021).

The mechanism responsible for changes in pH prior to parturition is not completely understood, but appears to involve carbonic anhydrases, that have been previously found in mammary gland secretion and are important to maintain acid-base balance. Carbonic anhydrases catalyze the rapid hydration of CO_2 and dehydration of H_2CO_3 with consequent release of H^+ (Lindskog and Coleman, 1973; Sly and Hu, 1995, Karhumaa *et al.,* 2001; Kitade *et al.,* 2003). The pH analysis was performed soon after the mammary gland secretion was collected, therefore a possible influence of the room temperature, presence of bacteria and oxygenation in the results were not considered in the present study (Amorim *et al.,* 2017).

A moderate to strong negative correlation between mammary gland secretion pH and the electrolytes Mg^{2+} and Ca^{2+} was observed in the present study and previously demonstrated by Canisso *et al*. 2013. Magnesium and Calcium were the only mammary gland secretion electrolytes that increased significantly during the 24 hours period prior to parturition (Fig.3). The NPV of an individual mare foaling within 24 hours after the magnesium in the mammary gland secretion first reaches 8.40mmol/L was 96% and the PPV was 65%, therefore it is a better predictor of when the mare will not foal than when she will foal. Calcium concentration ≥ 9.79 mmol/L were significantly correlated with foaling within 24 hours in the present study, as previously demonstrated (Peaker *et al.,* 1979; Ousey *et al.,* 1984; Ley *et al.,* 1993). Calcium concentrations above 10mmol/L indicate fetal maturity and readiness to birth and can be used as an indicator for induction of parturition (Leadon *et al.,* 1984; Ousey *et al.,* 1984; Peaker *et al.,* 1979; Sgorbini *et al.,* 2020). According to previous reports, the mammary gland concentration of calcium can reach levels significantly higher than 10mmol/L and can remain high for a few days prior to parturition (Ousey *et al.,* 1984), which may be a misleading indicator of imminent foaling. In the present study, calcium concentration ≥ 9.79mmol/L had a specificity of 95% and NPV of 97%, therefore, mammary gland secretion of calcium is also a better predictor of when the mare is not ready to foal than a predictor of the exact moment of parturition. Mammary gland pH and Ca^{2+} were also reportedly used to guide the timing of augmentation in mares with impending signs of parturition with good results (Cheong *et al.,* 2019). The physiologic process responsible for changes in electrolyte concentration in the mammary gland secretion is unknown and does not seem to be influenced by the concentration of serum calcium and parathyroid hormone (PTH) (Martin *et al*., 1996), suggesting that hormones involved in parturition and lactation (cortisol and estrogen) might be involved (Martin *et al.,* 1996).

Chloride levels decreased 8 days prior to parturition but did not change significantly during the last week of gestation (Fig. 3), therefore were not considered a reliable method to predict parturition in the present study. A decrease in mammary gland secretion of chloride was observed immediately pre foaling in light horse mares (Canisso *et al*., 2013) and TB mares (Peaker *et al.,* 1979), although not all light horse mares demonstrated a reduction in chloride levels prior to foaling (Canisso *et al*., 2013).

Although a significant decrease in sodium and a significant increase in potassium levels were previously reported, they were not observed during the prepartum period and were not considered good indicators of foaling in the present study. A Na^+/K^+ ratio inversion has been previously demonstrated in prepartum mares (Ley, 2011; Canisso *et al.* 2013), but was not detected in the MM mares from this study. Light horses mares and TB mares have also previously failed to demonstrate a Na^+/K^+ ratio inversion prior to parturition (Ousey *et al.* (1984) and Canisso *et al.* (2013).

Although a moderate correlation was found between the water hardness test and mammary gland secretion pH and calcium, this is not a useful test to predict parturition in MM mares since values did not change significantly within the last four days prior to foaling. The maximum scale detected by the water hardness kit is >445ppm of $CaCO_3$, therefore the exact maximum concentration could not be precisely determined and might have interfered with our ability to detect significant changes. A previous study in Standardbred mares showed that concentrations >225ppm of $CaCO_3$ should be used as threshold to start closely monitoring the mares for foaling (Amorim *et al*., 2019). In our study $CaCO_3$ concentrations >270ppm were detected in all time points, confirming that this was not a good predictor of foaling in MM mares. The water harness test can be influenced by other electrolytes, such as magnesium (Cash *et al.,* 1985), which explains why higher concentrations were observed prior to an increase in the concentration of calcium was detected in the biochemical analysis.

Although changes in body conformation were visualized within the last 2 weeks prior to parturition, the exact day of appearance varied from mare to mare and therefore were not considered a reliable method to predict the exact day of foaling (Brinsko *et al.,* 2011). Mammary gland usually develops in the last weeks of gestation due to growing of the parenchyma and the presence of colostrum (Peaker *et al.*, 1979). The prepartum maturation of the mammary gland involves an increase in blood flow and changes in the overall volume and composition of the secretion and is associated with a rise in estrogen and a fall in progesterone levels (Purohit, 2010). One mare in the present study did not show udder development prior to parturition and 2 mares did not have enough mammary gland secretion despite having developed their udder. It has been previously demonstrated that nulliparous mares might develop the udder and have a physiologic stimulus for production of mammary gland secretion only a few hours prior to parturition, which might explain our findings (Peaker *et al.,* 1979; Starbuck, 2006; Espy, 2016).

CONCLUSION

Mammary gland pH secretion measured by pH meter and pH paper strip was effective to predict parturition in MM mares, inexpensive and provided immediate results. Quantitative pH values obtained with the pH meter had greater accuracy in comparison to pH paper strip, but a strong correlation was found between both methods. Calcium and magnesium were the only mammary gland secretion electrolytes that changed in concentration immediately prior to parturition but are not very practical to analyze in the field and are better indicators of when not to expect foaling rather than a predictor of when mares will foal. The concentrations of Na^+, K^+, Cl^-, Na^+/K^+ ratio inversion and water hardness were not good predictors of foaling in MM mares.

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