Optimizing the Tracking of Gestation in the Equine Industry:

An analysis of the links between the pH and chemical composition of mare's milk and the onset of parturition

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Abstract

Purpose of Study: The purpose of this study was to determine if pH is a viable and cost effective factor to use in the prediction of parturition in mares.

Background: The tracking of pH levels is a promising indicator of the onset of parturition in mares. As this study was performed on only one mare, more data will need to be collected from a larger sample size in order to know if this would be a cost effective predictor of parturition in mares.

Methods: The milk from a brood mare was collected, tested, and compared using an inexpensive over-the-counter pH test strip, a pH meter, and a Redox SYS oxidative-reductive testing machine. The samples were taken using sterile techniques, while trying to control for as many variables as possible, in order to obtain better comparative data. The samples also were tested for ORP and calcium hardness concentrations.

Results: There was a strong correlation ($r=0.9187$) at ($P<0.01$) and a significant P value of 0.000024 found between the pH testing methods utilized in this study. Furthermore, there was a consistency of calcium hardness concentrations prior to parturition, followed by a significant spike in those values at the time of parturition and continuing after the event.

Conclusion: The results of this study indicate that mare’s milk pH does drop prior to parturition and could be a reliable indicator of parturition occurring within 24–48 hours of such noted drops even when tested with the over-the-counter pH strips. Testing with such over-the-counter pH strips would make tracking such drops cost effective in production settings.

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I. Introduction

Inspiration for this research was provided during a lecture by Dr. Steven Cooper in a course entitled Horse Science, ANSI 4423. In that lecture, Dr. Cooper discussed the physical indicators of the onset of parturition in horses, including restless behavior, relaxing of the muscles around the tail and pelvis, waxing on the teats, enlarged teats, and dripping milk. Dr. Cooper went on to discuss the composition of the mare's colostrum and milk, as well as the theoretical uses of calcium and pH levels as an indicator of the onset of parturition.

The purpose of this study was to determine whether a significant drop in pH value was observed in the milk of pre-partum mares and, if so, whether it could be used effectively and accurately to track the progression of late gestation and whether it was indicative of parturition within a 24–48 hour period. This study was conducted in the hope of identifying a reliable and cost effective method for the prediction of parturition for clinical purposes and/or equine management practices.

II. Methods

A. Mare Selection

Our subject mare was selected from the Oklahoma State University herd of brood mares. We were limited to mares whose disposition and duration of gestation permitted the safe and reliable retrieval of milk; therefore, we worked with one mare,[1] who allowed us reliably to collect samples from her teat directly. This mare also had foaled several times and historically was very predictable in her length of gestation, showed obvious signs of the onset of parturition, and had no history of dystocia. These factors made her a good candidate for purposes of our research.

[1] The precise age and number of previous foals is unknown.

B. Sample Collection

About 25–30 mL of milk was collected each day to ensure that the pH meter probe would be submerged fully in the sample in order to ensure accurate and consistent readings. Sterile techniques were followed throughout the sampling process.

The mare was milked by hand and the milk sample was collected in a plastic collection tube (Figure 1). Each collection was performed at approximately the same time, i.e., mid-afternoon, every day of the testing period to maintain consistency of samples with respect to other variables, such as temperature and elapsed time after feeding, and to limit disruptions to the mare's daily routine. After collection, the collection tube was transported on ice to the laboratory for testing. Samples were tested in the laboratory no more than ten (10) minutes after collection.

Figure 1: Falcon Plastic Collection Tube

C. Laboratory Testing

Several tests were performed on each sample to evaluate certain components of the milk samples. Again, sterile techniques were followed throughout these tests.

Using a RedoxSYS® oxidative-reductive testing machine,[2] a Redox test was run to determine the Static Oxidative-Reduction Potential (ORP) and Capacity Oxidative-Reduction Potential (ORP). A small sample of milk was placed in a centrifuge tube to bring the sample to room temperature. A sensor (Figure 2) was inserted into the machine (Figure 3), and a pipette then was used to transfer a forty (40) microliter (µL) sample of milk from the centrifuge tube onto the sensor. Once the milk sample was absorbed by the sensor, the machine would perform a four (4) minute analysis of the sample and produce the results. This test was performed twice to ensure accuracy, and a third time if values for tests 1 and 2 varied significantly, i.e., by more than a value of 15 units.

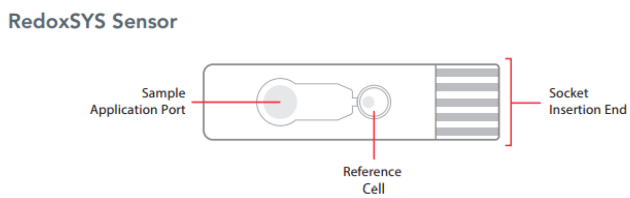

Figure 2: RedoxSYS® sensor (Aytu BioScience, Inc., 2015)

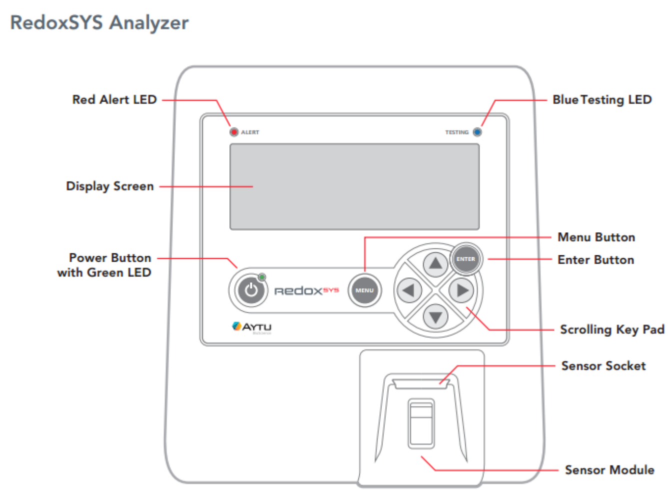

Figure 3: RedoxSYS® machine (Aytu BioScience, Inc., 2015)

[2] Manufactured by Luoxis Diagnostics, Inc., Greenwood Village, CO.

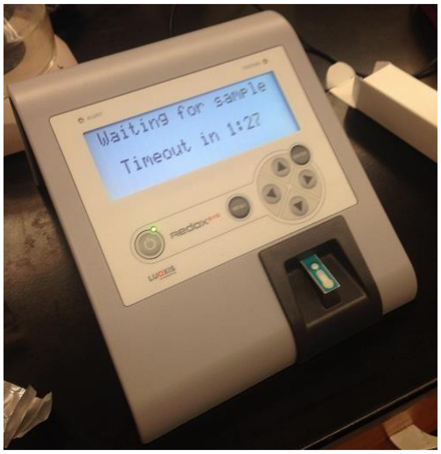

Figure 4: RedoxSYS® machine in use

Two different pH tests were performed on each of the milk samples. The first test was performed using a common, over-the-counter test strip, which evaluated the pH and the total hardness (calcium and magnesium hardness concentration) in parts per million (ppm) of the sample. The purpose of using this test strip was to evaluate its ability to measure accurately the pH of the milk samples, when compared with the measurements obtained from the pH meter in the laboratory, in order possibly to provide an affordable and practical alternative method to measure milk pH outside of a laboratory setting. For these purposes, a test strip was dipped directly into the milk sample for one (1) second, then removed and placed on a flat surface for one (1) minute. Once the test strip colors stabilized, the strip was compared with a color key on the strip box to determine the numerical value of the test.

The second pH test utilized a pH meter in the laboratory. The pH meter was first calibrated using two controlled liquid samples of known pH values. Before and after each application, the probe containing the pH sensor was washed with deionized water. The probe was inserted into each liquid, and the readings were confirmed with the known pH values. The probe then was inserted into the milk sample, so that the sensor was immersed completely, and it was held in place until the reading on the screen stabilized, giving a calibrated measurement of the pH value for each sample.

D. Additional Post-Partum Tests

The mare had a normal parturition that occurred over a period of about thirty (30) minutes and passed the complete, intact placenta within the hour following parturition. One final milk sample was performed two (2) hours after the mare foaled to evaluate the milk post-partum. The same laboratory testing was conducted on the final post-partum milk sample. A SNAP® Foal Immunoglobulin G (IgG) test also was performed on a blood sample from the foal to confirm adequate immunoglobulin absorption from the mare's colostrum. For this test, a blood sample was taken from the foal and deposited into a vacutainer tube; the tube was opened, and an eyelet was used to remove a drop of the whole blood sample. The blood sample then was mixed in an eye dropper with a diluting solution. Five drops were squeezed from the eye dropper containing the diluted sample, one drop then was placed in the sample receptacle on the SNAP® Foal IgG test device. The conjugate solution from the test kit accompanying the device was poured into the larger receptacle and allowed to drain through the opening onto the panel, the device then was snapped down flat and allowed to sit for seven (7) minutes. The indicator spots on the panel were compared with the reference panels on the test kit to determine the level of immunoglobulin absorption of the foal. In Figure 5 below, the results of our SNAP® IgG test are shown, which indicated that the foal had received ≥ 800 mg/dL IgG, the highest and ideal amount from colostrum (Idexx Laboratories, Inc., 2016).

Figure 5: SNAP® Foal IgG Test Results

III. Results

The results are intended to provide a visual representation of the trends for the tested values and their possible correlations with the onset of parturition. The pH data table and graph directly compare the values recorded from the pH test strip and pH meter and also shows the drop in pH prior to parturition. The total hardness results show the consistency of these values prior to parturition followed by a significant spike in those levels at the time of parturition and continuing after parturition. The Capacity ORP and Static ORP values correlate with oxidative stress and antioxidant response. In this case, these values can be used to track periods of pre-partum stress and provide further insight into the period of late gestation.

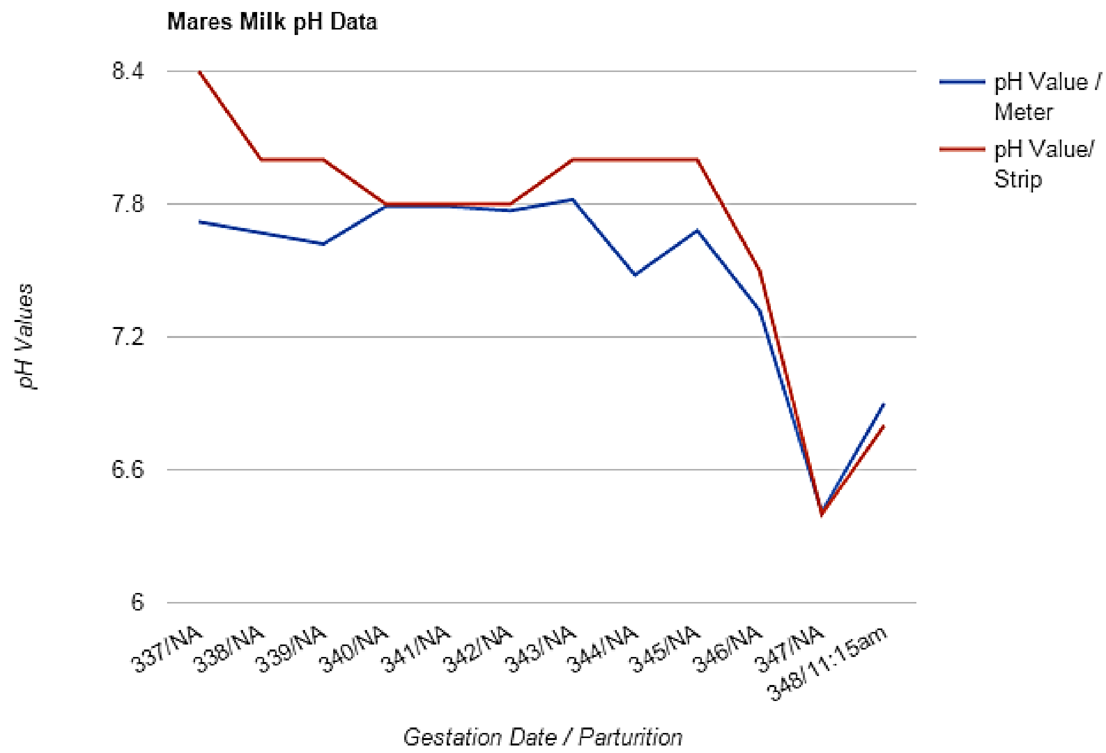

Figure 6: Mare's Milk pH Data comparing the accuracy of the pH test strip compared to the pH meter in the laboratory.

Gestation Date/ Parturition	pH Meter	pH Strip
337/ N/A	7.72	8.4
338/ N/A	7.67	8
339/ N/A	7.62	8
340/ N/A	7.79	7.8
341/ N/A	7.79	7.8
342/ N/A	7.77	7.8
343/ N/A	7.82	8
344/ N/A	7.48	8
345/ N/A	7.68	8
346/ N/A	7.32	7.5
347/ N/A	6.41	6.4
348/ 11:15 am	6.9	6.8

Table 1: pH values for milk samples in late gestation using the pH meter in the laboratory and the pH test strip.

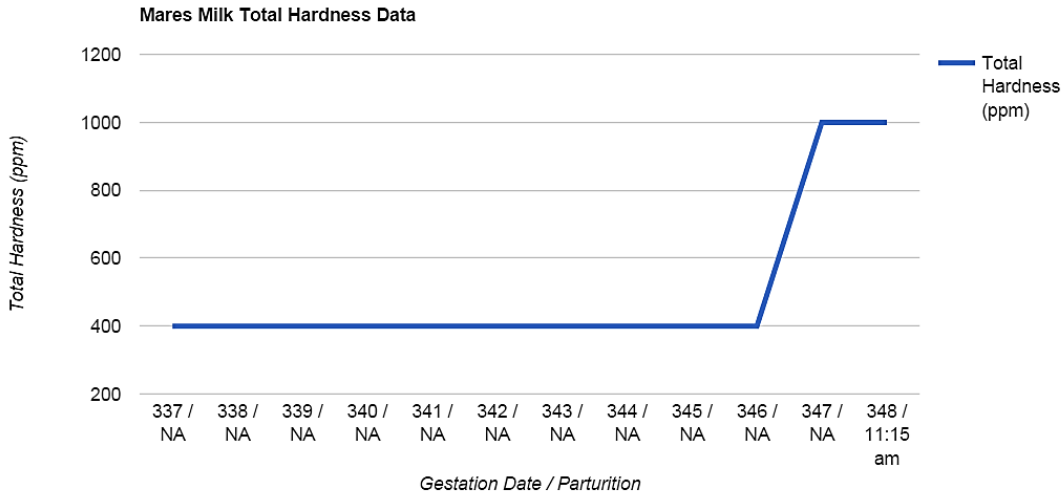

Figure 7: Mare's Milk Total Hardness Data measuring the concentration of calcium in parts per million in the milk samples. This line graph shows a significant increase on day 347 contemporaneous with the onset of parturition and that increase was maintained after foaling.

Gestation Date / Parturition	Total Hardness (ppm)
337 / NA	400
338 / NA	400
339 / NA	400
340 / NA	400
341 / NA	400
342 / NA	400
343 / NA	400
344 / NA	400
345 / NA	400
346 / NA	400
347 / NA	1000
348 / 11:15 am	1000

Table 2: Total Hardness values based on test strip, showing an increase in calcium deposit from 400–1000 ppm.

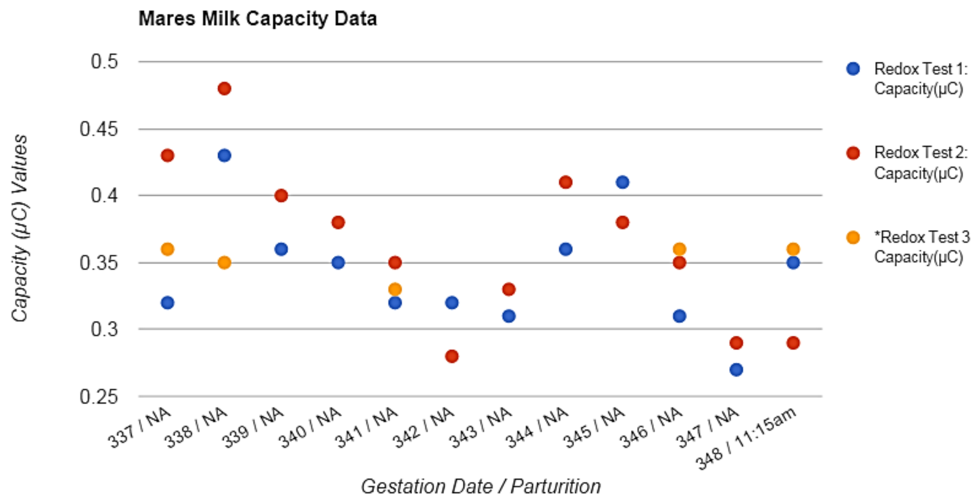

Figure 8: Mare's Milk Capacity ORP Data showing the measurements in microcoulombs (μC) for each Redox test.

*Redox Test 3 was measured only when the values for Tests 1 and 2 showed a significant difference.

Gestation Date/ Parturition	Redox Test 1: Capacity(μC)	Redox Test 2: Capacity(μC)	*Redox Test 3 Capacity(μC)
337 / NA	0.32	0.43	0.36
338 / NA	0.43	0.48	0.35
339 / NA	0.36	0.4	
340 / NA	0.35	0.38	
341 / NA	0.32	0.35	0.33
342 / NA	0.32	0.28	
343 / NA	0.31	0.33	
344 / NA	0.36	0.41	
345 / NA	0.41	0.38	
346 / NA	0.31	0.35	0.36
347 / NA	0.27	0.29	
348 / 11:15am	0.35	0.29	0.36

Table 3: Capacity ORP values from Redox Tests.

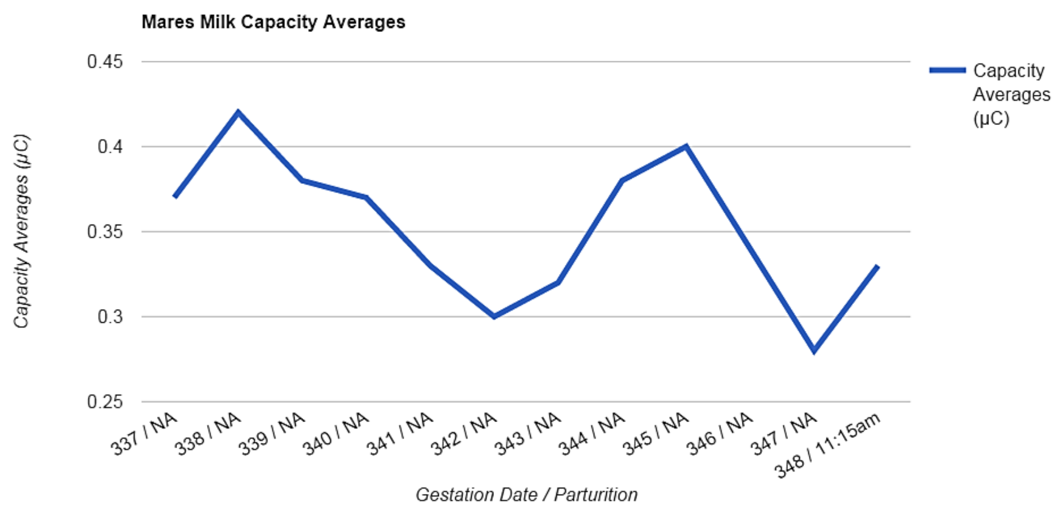

Figure 9: Mare's Milk Capacity ORP Averages from Redox Tests.

Gestation / Parturition	Capacity Averages (µC)
337 / NA	0.37
338 / NA	0.42
339 / NA	0.38
340 / NA	0.37
341 / NA	0.33
342 / NA	0.3
343 / NA	0.32
344 / NA	0.38
345 / NA	0.4
346 / NA	0.34
347 / NA	0.28
348 / 11:15am	0.33

Table 4: Capacity ORP Averages for values measured from Redox Tests.

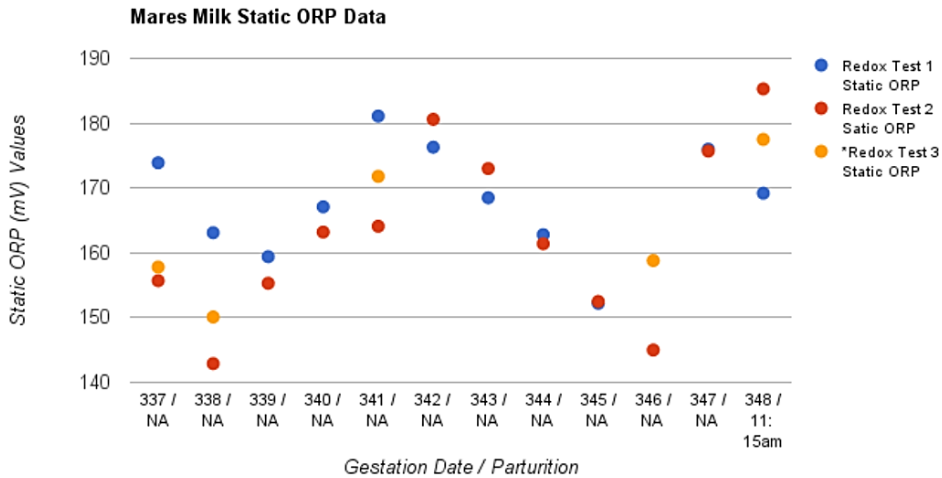

Figure 10: Mare's Milk Static Oxidation-Reduction Potential (ORP) Data displaying the measurements in millivolts (mV) for each Redox test.

*Redox Test 3 was measured only when the values for Tests 1 and 2 showed a significant difference.

Gestation Date/ Parturition	Redox Test 1 Static ORP (mV)	Redox Test 2 Static ORP (mV)	*Redox Test 3 Static ORP (mV)
337 / NA	173.9	155.7	157.8
338 / NA	163.1	142.9	150.1
339 / NA	159.4	155.3	
340 / NA	167.1	163.2	
341 / NA	181.1	164.1	171.8
342 / NA	176.3	180.6	
343 / NA	168.5	173	
344 / NA	162.8	161.4	
345 / NA	152.2	152.5	
346 / NA	117.9	145	158.8
347 / NA	176	175.7	
348 / 11:15am	169.2	185.3	177.5

Table 5: Static ORP values from Redox Tests.

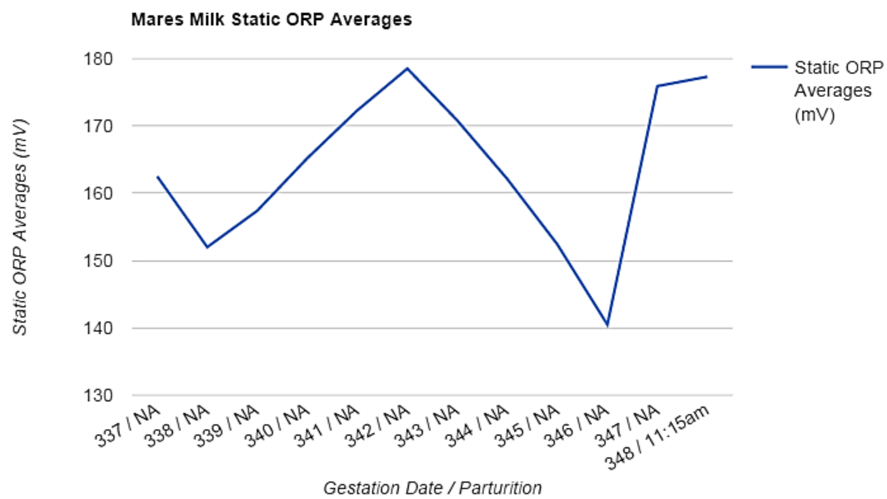

Figure 11: Mare's Milk Static ORP Averages from Redox Tests.

Gestation / Parturition	Static ORP Averages (mV)
337 / NA	162.5
338 / NA	152
339 / NA	157.4
340 / NA	165.2
341 / NA	172.3
342 / NA	178.5
343 / NA	170.8
344 / NA	162.1
345 / NA	152.4
346 / NA	140.5
347 / NA	175.9
348 / 11:15am	177.3

Table 6: Static ORP Averages for values measured from Redox Tests.

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2$$

Figure 12: Formula used to calculate variance in data for Capacity ORP and Static ORP (Arcidiacono, 2014)

Gestation Date/ Parturition	Capacity Mean ($\bar{x}$)	Capacity Variance (s^2)
337/ N/A	0.37	0.0031
338/ N/A	0.42	0.0043
339/ N/A	0.38	0.0008
340/ N/A	0.37	0.00045
341/ N/A	0.33	0.00023
342/ N/A	0.3	0.00079
343/ N/A	0.32	0.0002
344/ N/A	0.39	0.00125
345/ N/A	0.4	0.00044
346/ N/A	0.34	0.00069
347/ N/A	0.28	0.00019
348/ 11:15 am	0.33	0.00143

Table 7: Mean and Variance for Capacity ORP Data from Redox Tests results.

Gestation Date/ Parturition	Static ORP Mean ($\bar{x}$)	Static ORP Variance (s^2)
337/ N/A	162.46	99.143
338/ N/A	152.03	104.81
339/ N/A	157.35	8.4
340/ N/A	165.15	7.61
341/ N/A	172.3	72.463
342/ N/A	178.45	9.245
343/ N/A	170.75	10.125
344/ N/A	162.1	0.979
345/ N/A	152.35	0.045
346/ N/A	140.56	432.943
347/ N/A	175.85	0.045
348/ 11:15 am	177.33	64.823

Table 8: Mean and Variance for Static ORP Data from Redox Tests results.

IV. Discussion

The results of this study showed a clear link between the decrease in pH value (from fluctuating values >7 to 6.4) measured in a pre-partum mare's milk samples and the onset of parturition within a 24–48 hour period. In Figure 6, the graph depicts the trend in pH values and, in particular, a gradual decrease in pH from day 345 until the parturition event on day 348. As indicated above in Table 1, the sample collected at 2:45 PM on day 347 of the mare's gestation period measured a pH of 6.41 using the pH meter and 6.4 using the pH test strip, and foaling began at 11:15 AM on day 348. In the study by Korosue et al., (2013), the researchers established a milk pH decrease to 6.4 as the standard value indicative of the onset of parturition. The results in Table 1 also show a gradual increase in pH (6.9 using the pH meter and 6.8 using the pH test strip) in the colostrum sample collected at 1:00 PM on day 348, after parturition and passage of the placenta, designated as the first phase of lactation by Pecka et al. (2013). In evaluating the trend in pH values, the reliability and accuracy of the pH test strip in comparison with the pH meter also was examined, and the pH test strip was found to be equally reliable and indicative of parturition. There was a strong positive correlation ($r=0.9187$) at ($P<0.01$) between the pH test methods and a significant P value of 0.000024. Given the correlation between the results obtained using the pH test strip and the pH meter, use of the pH test strip would be an accurate, faster, and more affordable option for testing milk pH levels from an equine breeding and/or management prospective.

The results of the total hardness data of the milk samples from the test strip showed similar trends. In Figure 7, the graph depicts a consistent level followed by a marked increase in total hardness, relating mainly to calcium hardness concentrations, as well as correlating magnesium hardness concentrations, from 400 ppm to 1000 ppm (the maximum value on the test strip) on day 347 (the day prior to parturition). Ousey et al. (1984) measuring results of electrolytes show similar trends during the period prior to parturition. This study also showed a strong negative correlation between pH values and total hardness concentrations ($r=-0.9119$) at ($P< 0.01$) and a significant P value of 0.000038, which supports the trends observed in decreasing pH values and concurrent increasing total hardness concentration values. In a later study, Ousey et al. (1989), found that measurements of calcium hardness concentrations, taken using test strips similar to the ones used in this study, showed a significant increase at least twenty-four (24) hours prior to parturition. The colostrum sample taken the afternoon after foaling also maintained the high total hardness values. Ousey et al. (1989), Canisso et al. (2013), and Korosue et al. (2013) speculated that these measured values of total hardness, electrolytes, or calcium and magnesium hardness concentrations would be more useful in designating mares in a breeding operation during late gestation, which did not require diligent observation, rather than in predicting parturition events.

The RedoxSYS® tests produced interesting data that did not directly show indications of the onset of parturition, but the data could be indicative of maternal and fetal health. The machine was designed for testing blood, urine, and other biological samples, but milk was deemed an acceptable medium. The tests evaluated Static ORP and Capacity ORP of the milk samples. According to Aytu BioScience, Inc. (2015) these ORP values strongly correlate with redox balance within the body and can indicate certain levels of health, as well as potential imbalances in the mare’s system. Elevated

Static ORP values could indicate oxidative stress or an imbalance in the mare's system, which would be pertinent during periods of gestation and parturition.

In Figure 11, the graph depicts the average values of Static ORP measured during this study, including the peaks and troughs that could indicate periods of stress or redox balance respectively. Capacity ORP values have been found to correlate strongly with the antioxidant levels and the ability to equalize imbalances in times of injury or stress (Aytu BioScience, Inc., 2015).

In Figure 9, the graph depicts the average values of Capacity ORP and the peaks and troughs that could indicate variations in antioxidant levels. Comparing the figures for Static ORP and Capacity ORP shows points of contrasting peaks and troughs at days 338, 342, and 346. The variance values were calculated for Static ORP and Capacity ORP in each group of redox testing for each day's samples (Tables 7 and 8). This redox testing level still was evaluated; therefore, there is the possibility that, in the future, it could be useful for testing blood or milk samples from pre-partum mares, but, at this time, its uses are only speculative based on the given correlations related to stress and illness.

Many factors affect the gestation and parturition events of mares, including, but not limited to, breed, uterine environment or abnormalities, nutrition, environment, and weather. The mare's age and previous foaling experience also may impact gestation and parturition. Further, it is important to note that, during this study, substantial fluctuations in temperature and barometric pressure occurred, and severe thunderstorms took place, which could have affected the length of gestation of the mare. Nevertheless, the results of this study strongly support those of previous studies showing that pH measurements are an effective and reliable method of indicating the onset of parturition.

Often foaling occurs early in the morning or late at night, at times when levels of activity and staffing on a farm or breeding management facility are lower; therefore, it is important to explore further these strong correlations in order to develop a reliable method for tracking gestation and the timing of parturition. Based on the results of this study, an over-the-counter pH test strip qualifies as a capable alternative to a pH meter, which reduces cost and eliminates the need for laboratory analysis. The implementation of milk pH analysis of pre-partum mares in late gestation could ensure more successful foaling by reducing the number of dystocia events and enabling necessary immediate care to both mare and neonatal foal through the improved management practices.

V. References

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