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**Abstract**

Multiple methods of pregnancy detection can be used in sheep production. The gestation period for sheep is usually between 144 and 150 days; however, depending on the breed and age of the ewes, this number can vary. A breeding marker, such as a breeding crayon, is used to detect when the ewe is bred by the ram, and the date the ewe will lamb is typically calculated based on an average 145-day gestation. Unfortunately, this method provides only a rough estimate of when the ewe could lamb. In addition, because sheep routinely give birth to multiples (twins, triplets, and, sometimes, quadruplets), ewes are renowned for dystocia; thus, the ability to pinpoint the time of parturition to a 24-hour, rather than a five-day, window would enable sheep producers to be prepared for any difficulties, thereby reducing newborn lamb mortality. In horses, the time of parturition can be detected within 24 to 48 hours by measuring calcium concentrations in prepartum milk using the Chemetrics K-1700 testing system. Therefore, the objective of this research was to test the effectiveness of measuring calcium concentrations in prepartum ewe's milk to predict time of parturition in pregnant ewes. The study was designed to collect 10 to 20 mL of ewe's milk twice daily, beginning four days before the calculated date of parturition until lambing. Because the Chemetrics K-1700 testing system is a rapid-test, milk calcium concentrations will be determined within minutes of hand-milking each ewe. It is standard practice to collect "extra" colostrum for orphaned or sickly lambs roughly 12 hours postpartum and after the newborn lamb(s) have ingested sufficient quantities of their mother's colostrum and establish their maternal bonds. Therefore, at approximately 12 hours postpartum, an approximate 25-mL sample of colostrum was collected to measure the immunoglobulin quality of the colostrum using three methods (Colostrum Refractometer, Antifreeze tester, and Equine Colostrometer. Unfortunately, a large enough sample size was not collected to accurately analyze the milk samples using the Chemetrics K-1700. When we analyzed the calcium levels in the colostrum, we found that the colostrum quality was similar using the Anti-freeze tester to that of the Colostrum Refractometer, and was significantly different than the Equine Colostrometer. The Anti-freeze tester was also more highly correlated to the Colostrum Refractometer than that of the Equine Colostrometer.

*Keywords: sheep, colostrum, immunoglobulin quality, mammary secretions*

## Introduction and Literature Review:

In today's industry, sheep producers could benefit from the use of an accurate method to predict the time of parturition (act of giving birth) within 24 to 48 hours, instead of spending valuable time closely watching a ewe without knowing exactly when she is going to lamb. There are multiple methods of pregnancy detection that can be used in sheep production. The gestation period for sheep is typically between 144 and 150 days depending on the breed and age of the ewe. The traditional method is the use of a breeding crayon as a breeding marker to detect when the ewe is bred by the ram, and then the parturition date can be predicted based on an average 145-day gestation. That method provides only a 72 to 96 hour estimate of when the ewe could lamb. In addition, because sheep routinely give birth to multiples (twins, triplets, and, sometimes, quadruplets) (Berger, 2016; Kerslake, et al., 2005; Johnson, et al., 1982), ewes are renowned for dystocia (birthing difficulties); thus, the ability to pinpoint the time of parturition to a 24-hour, rather than a five-day, window would enable sheep producers to be prepared to assist with lambing should there be difficulties, thereby reducing newborn lamb mortality rates. One potential new method that could help producers to predict parturition within 24 to 48 hours is measuring the calcium levels of the ewe's mammary gland secretions beginning four days before their estimated parturition date. This peak in calcium concentrations are important for determining parturition in sheep.

In mares, the time of parturition can be detected within 24 to 48 hours by measuring calcium concentrations in prepartum milk using the Chemetrics K-1700 testing system. It has been shown in equine that there is a peak in the calcium level in milk 24 to 48 hours before parturition (McCue, 2009). The company Chemetrics sells a FoalWatch K-1700 kit (Chemetrics Inc., Midland, VA). Lay et. al. (1993) suggests testing of prepartum milk 10-14 days prior to foaling and continue to test until calcium concentration exceeds 100 ppm. When calcium levels reach 200 ppm approximately 50% of mares will foal within 24 hours. At levels of 300 to 500 ppm nearly all mares will foal. After a thorough search of the published literature, no articles were found that used pre-partum milk calcium levels to determine parturition in ewes within 24 hours of parturition. The objective of the present study was to use prepartum milk calcium levels to predict ewe parturition within 24 hours.

## Materials & Methods:

### Housing

Gestating ewes (n = 25) were moved from pastures surrounding the Pauline Whitaker Animal Science Arena, Fayetteville, AR, to a fully enclosed lambing barn approximately one week before the earliest calculated due date in January. Prior to lambing, ewes had ad libitum (free) access to water and hay and were fed approximately 2 kg/ewe (portions of feed were divided into morning and evening feeding periods). Each pregnant ewe was allowed approximately 1.9 $m^2$/ewe of space and roughly 42 cm/ewe linear feeder space (ADSA, 2010). The ewes and lambs were housed in an enclosed barn on concrete floors covered with approximately 10 cm of fresh straw that was changed weekly. After lambing, the ewes and their lamb(s) were identified with scourable paint and subsequently moved into a 3.5-$m^2$ lambing jug containing a self-feeder, water bucket, and heating box for the lamb. The ewes were fed 4 to 6 kg/day (depending on the number of lambs born) of a corn-based, high energy diet with ad libitum access to alfalfa hay and water. Ewes and lambs were removed from the lambing jug and put back into a pasture at approximately one week after lambing (time in jug depended largely on the health and thriftiness of the lambs). Temperature of the lambing barn is thermostatically controlled (typically set at $18^0$C); however, ewes were in full fleece and could withstand even colder temperatures. The barn had a large garage door at one end, which affords gestating ewes access to pasture on warmer days, otherwise, the door was closed to control drafts. The barn was also ventilated to decrease ammonia accumulation, especially when the barn was closed tightly during the evening.

**Experimental Design**

Twenty-five ewes were marked by a ram using a marking crayon, and due dates were calculated based on an average 145-day gestation. Beginning four days before each ewe's calculated date of parturition, the ewes were caught, haltered, and restrained to collect no less than 15 ± 5 mL mammary secretions via hand-milking. Milk was collected from each ewe twice daily (between 0800 and 0900 hours and again between 1800 to 1900 hours) until parturition. Time of restraint was limited to 10 minutes, if no milk sample can be obtained within the 10-minute restraint period, the ewe was released and an unsuccessful collection will be noted. Milk was collected into 50-mL, plastic tubes with cheesecloth to filter out any debris. Following the manufacturing instructions, prepartum milk calcium levels was determined within 10 to 15 minutes of collection using the Chemetrics K-1700 testing system. Ewes were observed regularly during parturition, and, after passing all fetal membranes and fluid, ewes were moved to a lambing jug and observed closely to ensure that lamb(s) nurse and obtain adequate quantities of colostrum. At approximately 12 hours postpartum, a sample of 15 ± 5 mL colostrum was collected to measure the immunoglobulin quality of each ewe's colostrum. The immunoglobulin quality was be tested using the Colostrum Refractometer, an Anti-freeze tester, and the Equine Colostrometer.

**Statistical Analysis**

There were not enough mammary secretion samples to be able to run statistical analysis on the data collected from the Chemetrics K-1700 testing system. All colostrum data was analyzed using the relationships of the Antifreeze tester and the Equine Colostrometer to the gold standard measure of colostrum quality (Colostrum Refractometer) was determined using the correlation procedure of SAS (SAS Inst., Inc., Casy, NC). Additionally, equations to predict Colostrum Refractometer were developed using the Regression procedure of SAS.

## Results

**Prepartum Mammary Secretions:**

After collection of mammary secretion samples, we attempted to analyze the samples using the Chemetrics K-1700 testing system without diluting the initial samples. Unfortunately, the mammary secretion samples had calcium concentration levels that were unable to be read using the test. Based on these results, the remaining samples were diluted using a ten to one ratio with distilled water. For the samples that contained an adequate amount mammary secretions the calcium concentration levels were readable using the Chemetrics K-1700 teseting system. However, the number of samples was not adequate enough to effectively analyze the data via SAS.

**Post-partum Mammary Secretions:**

After collecting the colostrum samples, colostrum quality was tested using three different tests, the Colostrum Refractometer (standard test), an Anti-freeze tester, and the Equine Colostrometer to test and determine the colostrum quality for each ewe. When comparing the tests via the Pearson Correlation in SAS, the Colostrum Refractometer was significantly correlated with colostrum quality ($P \leq 0.05$) (Table 1). The Anti-freeze tester was highly correlated ($P \leq 0.001$) and significant when compared to the Colostrum Refractometer, but not with colostrum quality we saw no significant difference ($P > 0.05$) compared to the Colostrum Refractometer or the Equine Colostrometer. The relationship of the Colostrum Refractometer to the Equine Colostrometer, was not significant ($P \leq 0.05$) in ability to determine colostrum quality. Furthermore, when comparing the Anti-freeze tester to the Equine Colostrometer, we found that they were negatively correlated ($P \leq 0.05$).

Equations using the Anti-freeze tester was more successful at predicting the Colostrum Refractometer than those using the Equine Colostrometer (Table 2). At an intercept of approximately 6.18, we found that the Equine Colostrometer was significantly different ($P \leq 0.001$) than that of the Colostrum Refractometer. When looking at the Equine Colostrometer, we found no significant differences ($P > 0.05$) or relationships to the Colostrum Refractometer.

## Discussion/Conclusion

We expected the results of using the FoalWatch K-1700 to show an increase in calcium concentrations in the pre-partum milk within 24 hours prior to lambing. Unfortunately, due to a limited sample size we were unable to get accurate, readable numbers to analyze these data. Once we figured out that we were not going to be able to have readable numbers with straight ewe mammary secretions, we decided to dilute the samples in a ten to one ratio. Unfortunately, we were only able to dilute a few sample so once again our sample size was too small to perform statistical analysis. The samples that we diluted did however result in more accurate, readable numbers to test. Therefore, we recommend that this portion of the study be conducted again with increased amounts of mammary secretions and initially diluting the sample using either a ten to one or twenty to one ratio.

Colostrum samples were collected within 12 hours of lambing to test the quality of colostrum and compare alternative tests with the standard method of testing. We compared the Anti-freeze test and the Equine Colostrometer to the Colostrum Refractometer. After analyzing the data via prediction equations, the Anti-freeze tester was more predictive of colostrum quality when compared to the Colostrum Refractometer (Table 2). We also used statistical analysis to determine correlations between all three methods for measuring colostrum quality. Results show that the Anti-freeze tester was highly correlated to the Colostrum Refractometer (Table 2). When comparing the Equine Colostrometer to the Colostrum Refractometer, we found that the Equine Colostrometer was negatively correlated with the Colostrum Refractometer and not significant when evaluating colostrum quality.

After analyzing the ewe mammary secretion data, we concluded that the Chemetrics K-1700 testing system requires diluted samples using a ten to one or twenty to one ratio and increased sample size. From the Colostrum collection data, we can conclude that by using the Anti-freeze tester the approximately 0.77 of the variation can be accounted for (Table 2). When we looked at the colostrum collection data using the Pearson Correlation, we can see that up to approximately 0.82 of the variation can be accounted for (Table 1). This tells us that the Anti-freeze tester is a more practical approach for sheep producers than that of the Equine

Colostrometer, because it more closely resembles the Colostrum Refractometer (standard technique).

From a practical and industry standpoint, there are times when these findings would be beneficial. Young 4-H or FFA members that are starting a ewe herd for their projects could use the anti-freeze tester (from O'Reilly) to test the colostrum quality of their ewes after lambing, instead of paying the price for the standard Colostrom Refractometer. Even though there are other things, like genetics and the environment, which can affect the growth rate of lambs, colostrum quality to help determine why lambs are not growing to their full potential. Unfortunately, large scale producers are less likely to use these tests because they simply do not have time to go out in the field and milk over one-hundred ewes after they lamb. Along with the young 4-H and FFA members, the key target group for this study was seed stock producers who raise rams to produce show lambs. These producers want to enhance the quality of their lambs, which starts when they get colostrum in the first few hours of life. For this reason, the cheap anti-freeze test could save them time and money. They can also use this test to determine which ewes to bank colostrum from for lambs who mothers die or have poor colostrum.

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**Tables:**

Table 1: Pearson Correlation between the Colostrum Refractometer, the Anti-freeze test, and the Equine Colostrometer

| **Pearson (Simple) Correlation** | | | |
|---|---|---|---|
| | Colostrum Quality | Anti-Freeze test | Equine Colostrometer |
| Colostrum Refractometer | 0.5517* | 0.82832*** | -0.43199NS |
| Colostrum Quality | | 0.41662NS | 0.47178* |
| Anti-Freeze test | | | -0.31518* |

NS = P > 0.05

* P ≤ 0.05

** P ≤ 0.01

*** P ≤ 0.001

Table 2: Equations to predict Colostrum Refractometer

| Equation Prediction Table | | | | | | | |
|---|---|---|---|---|---|---|---|
| Equation Number | Intercept | AFR | $AFR^2$ | EQC | $EQC^2$ | $R^2$ | RMSE |
| 1 | 3.53489 | 4.77402*** | --- | --- | --- | 0.69 | 5.181 |
| 2 | -1.14218 | 10.05707*** | 0.97736* | --- | --- | 0.77 | 4.561 |
| 3 | 19.47853 | --- | --- | 0.02712NS | --- | 0.19 | 8.340 |
| 4 | 6.17949 | --- | --- | 14.97069*** | 0.02333*** | 0.60 | 6.044 |
| 5 | 19.45793 | --- | --- | --- | 0.00004236NS | 0.19 | 8.332 |
| 6 | 5.01420 | 4.42929*** | --- | 0.01191NS | --- | 0.72 | 5.048 |
| 7 | 5.01389 | 4.42639*** | --- | --- | 0.00001859NS | 0.72 | 5.047 |
| 8 | 11.25083 | --- | 0.64001* | -0.01546NS | --- | 0.55 | 6.394 |
| 9 | 11.24730 | --- | 0.63935* | --- | 0.00002415NS | 0.55 | 6.391 |

NS = P > 0.05

* P ≤ 0.05

** P ≤ 0.01

*** P ≤ 0.001

AFR = Anti-freeze tester

EQC = Equine Colostrometer