



Refereed

## DAYTIME MANAGEMENT OF THE MARE. 1: Pre-foaling Mammary Secretions Testing

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### SUMMARY

Fifty-six mares of lighthorse breeding were utilized in a controlled management scheme for induced daytime foaling. All mares had pre-foaling mammary secretions sampled for evaluation of water hardness (ppm) or calcium carbonate content (ppm). Sampling began 10 days prior to expected foaling date for each mare and was performed once daily for 3 days followed by twice daily until foaling occurred. Samples were diluted 1:6 in distilled water and tested by each of 3 methods: Sofchek<sup>TM</sup> Test Strips<sup>a</sup>, Predict-A-Foal<sup>TM</sup> Test Kit<sup>b</sup> and Titrets<sup>TM</sup> Calcium Hardness Test Kit<sup>c</sup>. Mares were then either induced (i) to foal (no.= 33) according to a decision point of readiness for birth as indicated by pre-foaling mammary secretion testing ( $\geq 250$  ppm water hardness by Sofchek<sup>TM</sup> test,  $\geq 250$

ppm calcium carbonate content by Titrets test,  $\geq 4$  color bar changes by Predict-A-Foal test), or allowed to foal spontaneously (s) (no.=23). There were no differences ( $p>0.05$ ) in the mean pre-foaling mammary secretion test values ( $\mu_i=293$ ppm,  $\mu_s=329$ ppm;  $\mu_i=4.1$ ,  $\mu_s=4.2$ ; and  $\mu_i=281$ ppm,  $\mu_s=298$ ppm; for Sofchek, Predict-A-Foal and Titrets, respectively) between mare groups at the time of foaling (time=0) for any of the 3 testing methods employed. Mean intervals to foaling after reaching decision points of readiness for birth were different ( $p<0.05$ ) between Sofchek and Titrets test for both mare groups, but only in the induced-to-foal group between Sofchek and Predict-A-Foal tests. Probabilities of 79%, 53% and 59% were calculated for mares foaling spontaneously within 24 hours of reaching the decision points used in this trial of readiness for birth on the initial occasion for Sofchek, Predict-A-Foal and Titrets<sup>TM</sup> tests, respectively. Each test was determined to have the ability to predict readiness of approaching parturition and found to be easily applicable to field use. The Titrets test was found to be least variable in its response to measurement of pre-foaling mammary secretion hardness changes both within and between mares.

<sup>a</sup>Sofchek<sup>TM</sup> Water Hardness Test Strip, Environmental Test Systems, Elkhart IN 46514.

<sup>b</sup>Predict-A-Foal<sup>TM</sup> Mare Foaling Predictor Kit, Animal Healthcare Products, Vernon CA 90058.

<sup>c</sup>Titrets<sup>TM</sup> Calcium Hardness Test Kits, CHEMetrics, Inc., Calverton VA 22016.

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Acknowledgements: Special thanks are given to Syntex Agribusiness, Environmental Test Systems and Animal Healthcare Products for assistance in this project.

Accepted for publication: December 1988.

### INTRODUCTION

Foaling care for the mare includes a wide spectrum of levels of management; systems range from completely

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natural pasture foaling, to intermittent observations during the night by owners or nightwatch personnel, to 24-hour surveillance by closed circuit camera. Since the majority of mares foal at night or in the very early morning hours<sup>1,5</sup>, efforts to ensure a safe and attended delivery of the foal become labor-intensive and costly<sup>8</sup>.

Clinical signs of the mare's approaching readiness for foaling have long been noted to include an appropriate gestation length of greater than 320 days, udder enlargement with the presence of colostrum, relaxation of the perineal region, and cervical relaxation<sup>5,6,7,14</sup>. None of these signs are extremely useful as a means of predicting foaling time to within a 4-10 day timespan<sup>14</sup>. The mare, like most species with comparatively long gestational lengths, has a wide 95% confidence interval for full term gestation<sup>14,16</sup>. The concept of readiness for birth relates this physiological phenomenon of variability in mean gestational time to *in utero* maturation of the fetus which individually controls its own gestational length<sup>16</sup>. Recent studies of mammary development and pre-foaling mammary secretion electrolyte changes have demonstrated an increased ability to narrow the prediction of foaling time to within a 24-48 hour timespan<sup>2,3,9,12,13</sup>. The electrolyte changes (especially with regard to calcium level) that occur within the pre-foaling mammary secretions have also been shown to relate to fetal maturity and viability of the equine neonate<sup>9,10</sup>.

This study was designed to develop a method of convenient and reliable daytime foaling management for the mare using pre-foaling mammary secretion changes as a decision-making tool to indicate the mare's readiness for birth and the fetal foal's *in utero* gestational maturity. Three different tests available commercially in the U.S. were evaluated; 2 that are used in the water quality industry: 1) Sofchek™ Test Strips<sup>a</sup> for determination of total water hardness, 2) Titrets™ Calcium Hardness Test Kit<sup>b</sup> for determination of water calcium carbonate content; and 1 that is marketed as a predictor of mare foaling time: 3) Predict-A-Foal™ Test<sup>c</sup>.

## MATERIALS AND METHODS

Fifty-six mares of Thoroughbred, American Saddlebred and Arabian breeding, during the 1987 and 1988 foaling seasons, were used in this field trial. Mares were under similar management and maintained on pasture through mid-gestation and supplemented with roughage and concentrate during their last 90 days of pregnancy. Routine preventive measures for infectious and parasitic diseases were employed, including pre-foaling booster immunizations with tetanus toxoid, eastern and western encephalomyelitis, influenza and EHV-1 vaccines at 10 months of gestation.

Beginning 10 days in advance of their expected foaling date (calculated as 340 days from their last known breeding date), all mares were sampled for pre-foaling mammary

secretion. One to 5 ml of secretion were obtained per mare, collected in a clean manner by stripping a small amount from each teat into a clean plastic test tube. Samples were then diluted in a 1:6 ratio with double distilled water as previously recommended<sup>2,3</sup>. Tests for secretion water hardness were performed on the farm by the first of 3 methods:

### 1. Sofchek™ Test Strips<sup>a</sup>

Raw and diluted samples were then identified as to mare, sampling date and time and transported back to the laboratory where they were frozen (-70°C) in storage until subsequent testing by the following methods could be performed:

### 2. Predict-A-Foal™ Mare Foaling Predictor Kit<sup>b</sup>

### 3. Titrets™ Calcium Hardness Test Kit<sup>c</sup>

### 4. Samples were retested with Sofchek™ Test Strips

Sampling schedule was once daily until the secretion total hardness as indicated by the Sofchek™ test equalled or exceeded 120 ppm; subsequent to this, mares were sampled on a twice daily basis (early a.m. and late p.m.). Mares were then assigned to 1 of 2 groups:

#### 1) spontaneously foaling group,

#### 2) induced foaling group.

A decision to induce parturition was based upon pre-foaling mammary secretion water hardness reaching, or exceeding, 250 ppm for the first time as per the Sofchek™ test along with appropriate gestational days in excess of 330, adequate mammary development, and perineal relaxation. Foaling date, time of day, ease of foaling, foaling difficulties and complications in the postpartum period were recorded for all mares.

All foals born were evaluated clinically for neonatal viability as soon after birth as feasible<sup>11</sup>; routine neonatal care for all foals included umbilical area treatment with 2% iodine twice daily for 3 days, enema administration if indicated, observation for suckling and general physical health, and plasma IgG determination at 18-24 hours of age by either CITE™ Foal IgG Test<sup>d</sup> or single radial immunodiffusion technique.

Statistical analyses were performed by comparison of mean intervals from first obtaining a decision point of readiness for birth (i.e.  $\geq 250$  ppm water hardness for Sofchek™ test;  $\geq 4$  color bar changes for Predict-A-Foal™ test;  $\geq 250$  ppm calcium carbonate content for Titrets™ test) to time of foaling. All 3 methods of pre-foaling mammary secretion were evaluated in both spontaneously foaling and induced-to-foal mare groups using a matched-pair t test<sup>4</sup>. Within-test comparisons for differences between mean intervals for spontaneously foaling and induced foaling groups were made using 1 factor ANOVA tests (e.g. Scheffe F-test & Fisher's Least Significant Difference test)<sup>4,15</sup>. The probability of each test to indicate a mare's readiness to foal within a given 24 hour time period was calculated and comparisons between tests were performed using Chi-square contingency analysis.

<sup>d</sup>CITE™ Foal IgG Test, AgriTech Systems, Inc. Portland, ME 04101

Table 1. Mean mammary secretion test values at time of foaling

Test	Mare Groups	
	Spontaneous	Induced
Sofchek™ (water hardness ppm)	329	293
Predict-A-Foal™ (color bar changes)	4.2	4.1
Titrets™ (calcium carbonate ppm)	298	281

## RESULTS

There were no differences ( $p > 0.05$ ) in the mean pre-foaling mammary secretion test values between mare groups at the time of foaling (time=0) for any of the 3 testing methods employed (Table 1). There was a significant difference ( $p < 0.05$ ) between the Sofchek™ and Titrets™ tests in the mean interval from first reaching the decision point of readiness for birth to the time of foaling in both spontaneously foaling and induced to foal mare groups. No differences ( $p > 0.05$ ) were determined between the Sofchek™ and Predict-A-Foal™ tests in the spontaneously foaling group, however there was a significant difference ( $p < 0.05$ ) between these 2 tests in the induced to foal mare group (Figs 1 & 2).

Within test comparisons were performed to determine differences between the induced and spontaneously foaling mare groups. Mean intervals to foaling after first reaching the decision point for readiness for birth ( $> 250$  ppm water hardness) as determined by the Sofchek™ test were significantly different ( $p < 0.05$ ). The induced group foaled a mean of 22 hours earlier than the spontaneously foaling group (Fig 3). No difference ( $p > 0.05$ ) was determined between induced and spontaneously foaling group mean intervals to foaling after reaching a 4 color bar change for the first time using the Predict-A-Foal™ test (Fig 4); nor was there a significant difference ( $p > 0.05$ ) between group mean intervals to foaling after reaching 250 ppm calcium carbonate content of pre-foaling mammary secretions as tested by the Titrets™ method (Fig 5).

The patterns of pre-foaling mammary secretion changes were recorded for all mares in each group from 10 days prior (-10d), to the time of foaling, or time zero (0). The mean values and 95% confidence intervals are presented for each of the 3 testing methods used in Figures 6 to 8. Twelve hours prior to foaling, in the spontaneously foaling group, 95% of all mares tested were within a range of 180-280 ppm, 3-4 color bar changes and 175-320 ppm for the Sofchek™, Predict-A-Foal™ and Titrets™ testing methods, respectively. The confidence interval lower limits for 95% of all mares tested in the induced-to-foal group at foaling time (time = 0) were 250 ppm, 3.8 color bar changes and 230 ppm for the Sofchek™, Predict-A-Foal™ and Titrets™ methods, respec-

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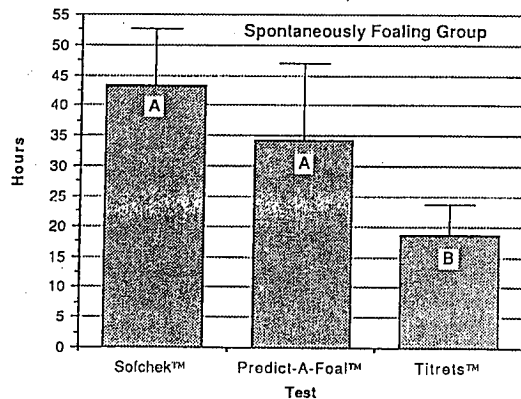


Figure 1. Mean interval to foaling after reaching 250 ppm water hardness (Sofchek™), 4 color bar changes (Predict-A-Foal™), or 250 ppm calcium carbonate content (Titrets™); columns with different letters are significantly different ( $p < 0.05$ ).

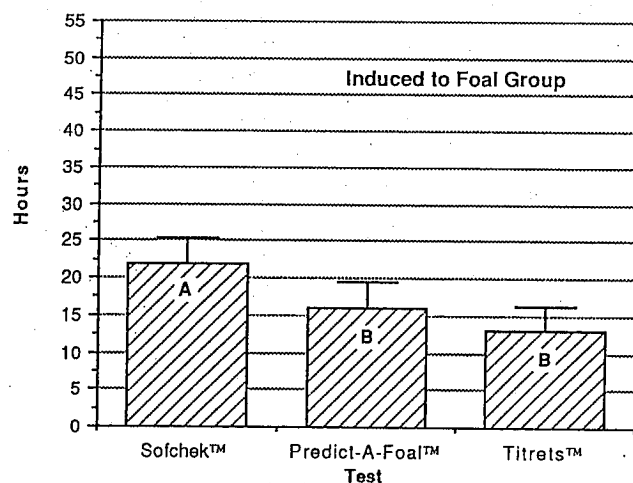


Figure 2. Mean intervals to foaling after reaching 250 ppm water hardness (Sofchek™), 4 color bar changes (Predict-A-Foal™), or 250 ppm calcium carbonate content (Titrets™); columns with different letters are significantly different ( $p < 0.05$ ).

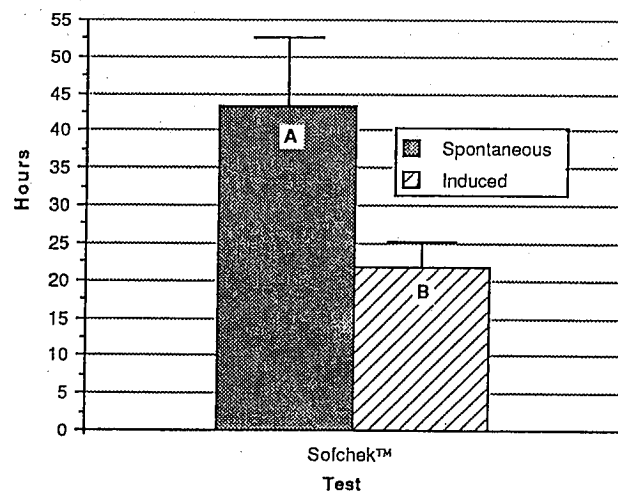


Figure 3. Mean intervals from foaling after reaching 250 ppm; columns with different letters are significantly different ( $p < 0.05$ ).

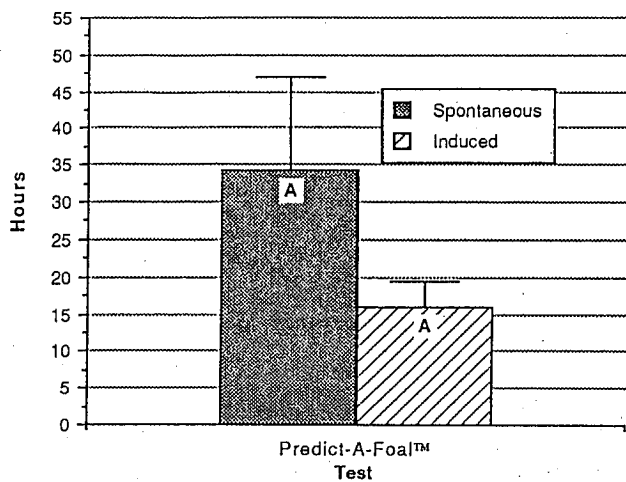


Figure 4. Mean intervals from foaling after reaching 4 color bar changes; columns with different letters are significantly different ( $p < 0.05$ ).

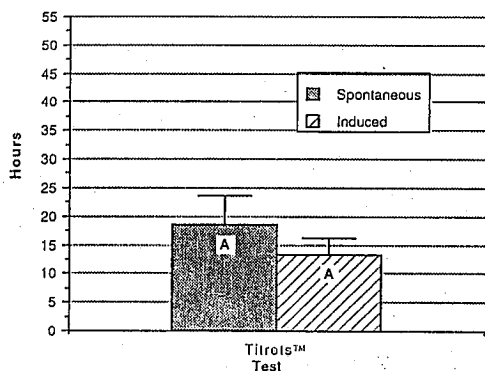


Figure 5. Mean intervals from foaling after reaching 250 ppm calcium carbonate content; columns with different letters are significantly different ( $p < 0.05$ ).

tively. These limits were all well within the ranges for mares in the spontaneously foaling group at 12 hours prior to foaling for each of the 3 tests evaluated.

The number of mares that foaled within 24 hours of reaching the decision points used in this study to indicate readiness for birth in the spontaneously foaling and induced-to-foal groups are presented in Table 2 for all 3 testing methods used. By the Sofchek™ test, there was a 72% probability (no. mares that foaled / total no. mares at risk of foaling) that the mare would foal spontaneously within 24 hours of reaching, or exceeding, 250 ppm water hardness of the pre-foaling mammary secretion on the first occasion. There was also a 22% chance that the mare would not foal within 24 hours and a 6% chance that she would foal prior to reaching that change in pre-foaling mammary secretion water hardness. The Predict-A-Foal™ test indicated a 53% probability that the mare would spontaneously foal within 24 hours after first reaching a 4 color bar change, a 20% chance that she would not foal within 24 hours, and a 27% chance that she would foal without reaching this point. The Titrets™ test indicated a 59% probability of the mare spontaneously foaling within 24 hours after reaching

Volume 9, Number 2, 1989

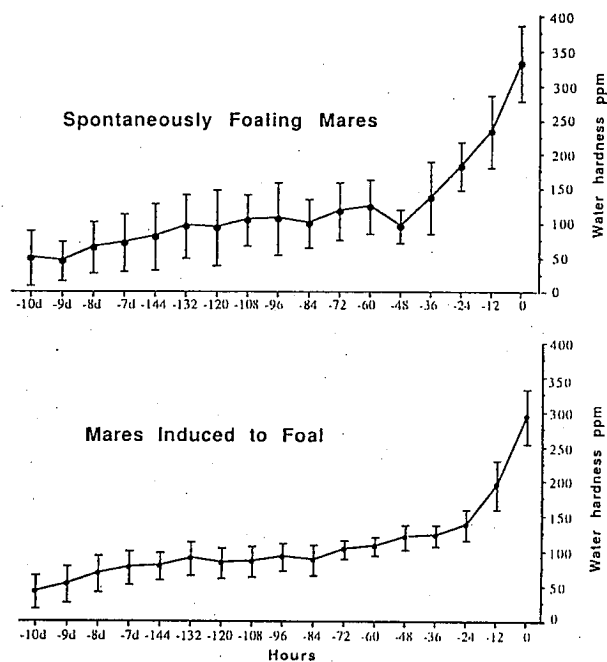


Figure 6. Mean and 95% confidence intervals for Sofchek™ water hardness test strip values on pre-foaling mammary secretions from 10 days prior to foaling (-10d) to time of foaling (0).

250 ppm calcium carbonate content in pre-foaling mammary secretion on the initial occasion; there was a 23% chance that she would not foal within 24 hours and 18% chance that she would foal without reaching this decision point. There were no differences ( $p > 0.05$ ) between testing methods for the number of mares that foaled within 24 hours of reaching the decision point of readiness for birth in the spontaneously foaling group in this study. There were significant differences ( $p < 0.05$ ) between the Sofchek™ and both of the other two tests in the number of mares that foaled within 24 hours of reaching the decision point of readiness for birth in the induced-to-foal mare group.

The interpretation of the Sofchek™ test by the methods employed in this trial was fairly subjective; color changes were not consistent with those represented by the manufacturer on the test's label. This introduced some error in the decision point as to when a specific test was truly equal to or greater than 250 ppm water hardness. The Predict-A-Foal™ test was much easier to read and interpret than the Sofchek™ test but had greater inherent variability in the readings it gave from sampling period to sampling period. This can be appreciated from the pattern of parturition

Table 2. Testing method versus foaling mare groups

	Sofchek™		Predict-A-Foal™		Titrets™	
	Spontaneous	Induced	Spontaneous	Induced	Spontaneous	Induced
No. that foaled within 24h of reaching decision point <sup>a</sup>	13	26 <sup>b</sup>	8	16 <sup>c</sup>	10	18 <sup>c</sup>
No. that foaled more than 24h after reaching decision point <sup>a</sup>	4	1 <sup>b</sup>	3	7 <sup>c</sup>	4	3 <sup>b</sup>
No. that foaled without reaching decision point <sup>a</sup>	1	2 <sup>b</sup>	4	2 <sup>b</sup>	3	8 <sup>c</sup>

<sup>a</sup>Decision point of readiness for birth was >250ppm water hardness of pre-foaling mammary secretion for Sofchek™, >4 color bar changes for Predict-A-Foal™, and > 250 ppm calcium carbonate for Titrets™ tests  
<sup>b,c</sup>Values with different superscripts within rows are significantly different (p<0.05)

values in Figure 7, and the large 95% confidence limits associated with this test at each time period pre-foaling. The Titrets test was technically more difficult to perform than the other 2, but once a few trial samples were performed and the operator became familiar with its use, it was readily adaptable for on-the-farm application. The Titrets™ was the most consistent of the 3 methods evaluated in its ease of interpretation and repeatability. The variance in mean values and 95% confidence limits as seen in Figures 6-8 at each sampling period pre-foaling were also influenced by factors indigenous to the mare.

## DISCUSSION

Two of the 3 pre-foaling mammary secretion testing methods evaluated in this trial gave similar responses with regard to the interval from first indicating a readiness for birth to actual foaling time in the spontaneously foaling

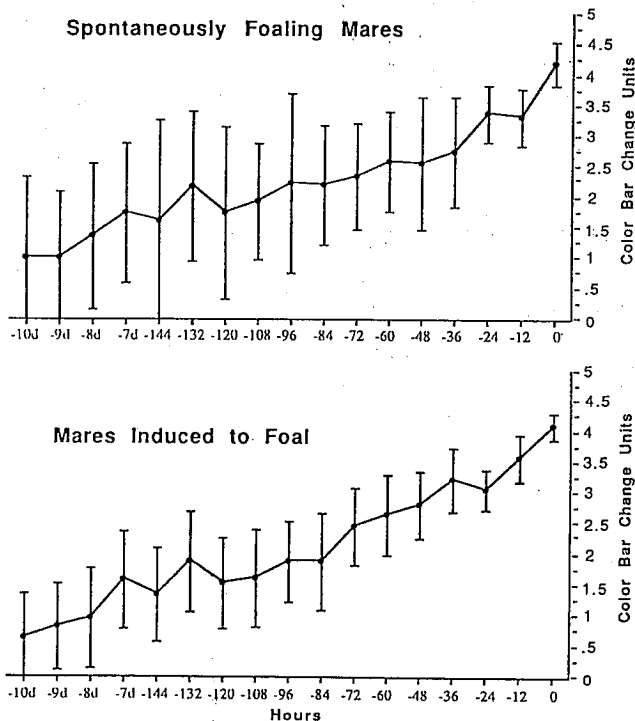


Figure 7. Mean and 95% confidence intervals for Predict-A-Foal™ test strip values on pre-foaling mammary secretions from 10 days prior to foaling (-10d) to time of foaling (0).

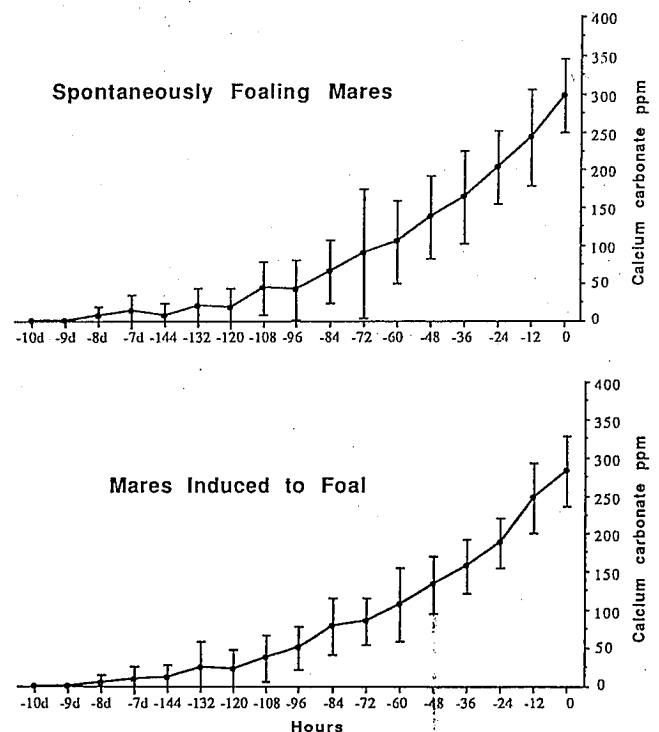


Figure 8. Mean and 95% confidence intervals for Titrets™ water hardness test values on pre-foaling mammary secretions from 10 days prior to foaling (-10d) to time of foaling (0).

group of mares. These were the Sofchek™ and Predict-A-Foal™ tests which were both designed for use as water hardness test strips and should have similar sensitivities for the electrolyte concentrations of magnesium and calcium in solution. (The Predict-A-Foal test™ employs the use of a water hardness strip marketed in Europe<sup>e</sup>). There was a difference in the interval from first reaching the decision point of readiness for birth between both the Sofchek™ and Predict-A-Foal™ tests and the Titrets™ test; the latter only measures calcium content in solution as a means of indicating water hardness. It has been shown previously that pre-foaling mammary secretion total calcium levels rise in association with approaching readiness for birth in the mare, and that magnesium levels also contribute to the prepartum mammary secretion electrolyte changes<sup>13</sup>. It is not surprising that the Sofchek™ and Titrets™ methods would differ therefore, since the water hardness changes of the mammary secretions seen earlier in the prepartum period are due primarily to magnesium elevation followed thereafter by a sharp rise in calcium. The Sofchek™ and Predict-A-Foal™ tests would indicate a slightly earlier readiness for birth due to their magnesium sensitivities than the Titrets™ test which is only designed to detect changing levels of calcium.

In the group of mares that were induced to foal, the mean intervals to foaling after reaching the decision point of readiness for birth were different between the Sofchek™ test and both of the other two tests. Due to the biological nature of the animal involved, it cannot be expected that every mare would follow a consistent pattern. However, the tendency to follow the patterns described make the use and interpretation of these testing methods most useful on the farm as a means of predicting readiness for birth. Variation in intervals to foaling following the decision point of readiness for birth were in the range of 12 to 54 hours for all tests. Using as a safeguard the mare's known gestational length of greater than 330 days, the threat of inducing a previsible or premature foal to be born is much reduced, if not eliminated, by judicious use of these testing methods.

The probabilities of parturition occurring within the spontaneously foaling group of mares as indicated by each test were quite different. The Sofchek™ test indicated that 94% of all mares evaluated reached or exceeded 250ppm prior to foaling, but 6% did not. With the Predict-A-Foal™ test, 73% of all mares reached or exceeded 4 color bar changes prior to foaling. This is not as high as the number of mares reported previously, where 80% of mares having a 4 color bar change were likely to foal within a 12 hour period<sup>2</sup>. In that report 2% of the mares studied delivered foals prior to obtaining even 1 color bar change, 3 of which delivered premature foals<sup>2</sup>; the report does not mention how many actually foaled with a mammary secretion of less than 4 color bar changes, but did state a 40% probability of the

mare foaling within 12 hours of obtaining a value of 3 color changes. The inability of the Predict-A-Foal™ test to discriminate or detect readiness for birth within a more confined interval could lead to early induction of foaling prior to the foal's readiness, as this test found many mares with a color value of 3 as early as 7 days prior to spontaneously foaling. The Titrets™ method indicated that 82% of all mares evaluated reached or exceeded 250ppm prior to foaling, but 18% did not. The calcium carbonate level ( $\geq 250$  ppm) selected as the decision point of readiness for birth in this trial may have been too high. Recalculation of probabilities using a  $\geq 200$  ppm value indicated an 88% chance of spontaneously foaling within 24 hours, 94% of all mares that foaled equalled or exceeded this value, and only 6% foaled spontaneously without reaching it. One previous report found that the mean total calcium levels of mammary secretion in full term mares that foaled spontaneously was  $10.6 \pm 2.7$  mmol/litre (which equates to a mean of  $400 \pm 108$  ppm calcium) on the day of parturition<sup>9</sup>; the authors stated that lower values than this did not necessarily mean that parturition would not take place, however they did not discuss probabilities. Their technique for calcium determination (an O-cresolphthalein complexone method) was different from that used in the Titrets™ method (a zinc EGTA-zincon complex method), thus further comparison between results are beyond the scope of this paper. The Titrets™ values obtained, however were similar, although lower, than those previously reported<sup>9</sup>.

## CONCLUSION

All 3 methods evaluated for pre-foaling mammary secretion hardness testing were easily performed and adaptable for on-the-farm use. They each demonstrated the ability to predict readiness of approaching parturition; however, within a time span of 24 hours or less, under the design of this trial, it was felt that the Titrets™ test was most reliable and repeatable. Further work needs to be performed on larger populations of late term pregnant mares to better quantify the predictability of the hardness changes observed. Routine application of these testing methods as a means of better managing the foaling mare and safeguarding her parturition through controlled observation should be encouraged based upon the findings of this trial.

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<sup>e</sup>Merckoquant Water Hardness Test Strip, E. Merck, Post Fach 4119, 6100 Darmstadt, West Germany