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BRIX refractometric analysis of mammary secretions is a poor predictor of impending parturition in mares

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Abstract

Equine dystocia can rapidly progress to life-threatening neonatal hypoxia and/or maternal trauma. Since the prognosis can often be improved by timely intervention, it is advisable to attend all foalings; tests that reliably predict imminent parturition are therefore invaluable. Sugar (BRIX) refractometry is accepted as a mare-side test for evaluating colostrum quality, and some studs use BRIX refractometry to indicate approaching parturition. This study compared the accuracy of BRIX refractometry with those of laboratory analysis of [Ca^{2+}], [Na^{+}] and [K^{+}] and the Predict-a-foal™ test for indicating when a mare is or is not about to foal, using mammary secretions collected daily from day 325 of gestation from 30 Dutch Warmblood mares. The mean BRIX score rose progressively as foaling approached in multiparous mares: 5/22 (22%) foaled within 1 night, 8 (36%) within 36h and 11 (50%) within 60 h of achieving a BRIX score of 22%; however, 5 mares (22%) failed to achieve >20% BRIX pre-foaling. Primiparous mares reached a (near) peak BRIX score further in advance of foaling with little subsequent rise, rendering the test a poor predictor of foaling in maiden mares. Moreover, a Predict-a-foal score of 4 (14/17: 82%) and a [Ca^{2+}] ≥ 7.5 mmol l^{-1} (20/26: 77%) were better indicators of foaling within 60 h; while below threshold values were reliable indicators that foaling would not occur within 24 h (>95%). Not all mares followed the general rules; one multiparous mare showed negligible pre-foaling changes in milk electrolytes or BRIX, whereas one maiden mare showed repeated inversions of [Na^{+}] and [K^{+}] between days 10 and 1 pre-partum; in this maiden and a multiparous mare with an early Na-K inversion, [Ca^{2+}] first exceeded 7.5 mmol l^{-1} 9-10 days before foaling.

Keywords: Foaling prediction, mammary secretions, sugar refractometry, milk electrolytes, reproduction

Die Analyse von Eutersekret mittels BRIX Refraktometer ist bedingt geeignet zur Vorhersage des Geburtstermins bei multiparen aber nicht bei primiparen Stuten

Dystokien bei der Stute können schnell zur Ursache einer lebensbedrohlichen Hypoxie des Fohlens und/oder eines Traumas der Stute werden. Je früher professionelle Geburtshilfe geleistet wird, umso besser ist die Prognose für Stute und Fohlen. Daraus ergibt sich die Empfehlung der Überwachung jeder Geburt. Hierbei sind zuverlässige Tests zur Vorhersage des Geburtszeitpunktes von unschätzbarem Wert. Das Zucker-(BRIX)-Refraktometer wird genutzt, um die Qualität von Kolostrum zu bewerten und kann direkt vor Ort verwendet werden. Außerdem wurde es in einzelnen Studien zur Erkennung bevorstehender Geburten eingesetzt. Diese Studie vergleicht die Genauigkeit des BRIX-Refraktometers mit der herkömmlicher Laboranalysen von [Ca^{2+}], [Na^{+}], [K^{+}] und dem Predict-a-foal™-Test. Alle Verfahren sollen Indikatoren liefern, die eine Aussage darüber zulassen, ob sich eine Stute um den Zeitraum der Geburt befindet. Es wird jeweils das Eutersekret von insgesamt 30 holländischen Warmblutstuten untersucht, welches ab dem 325. Tag der Trächtigkeit in täglichem Abstand gewonnen wird. Der mittlere BRIX Score multiparer Stuten stieg mit herannahender Geburt progressiv an. 11/22 (50%) der Stuten fohlten 60 Stunden nach Erreichen eines BRIX Scores von 22%; fünf Stuten (22%) überschritten vor der Geburt einen BRIX Score von 20% jedoch nicht. Bei primiparen Stuten werden annähernd Höchstwerte des BRIX Score schon früher vor der Geburt erreicht, welche dann in Geburtsnähe weiter ansteigen. Aus diesem Grund kann anhand dieses Tests nur eine vage Voraussage bezüglich des Abfohltermins bei Maidenstuten gemacht werden. Vielmehr ist ein Predict-a-foal Score von 4 (14/17:82%), als auch ein [Ca^{2+}]-Gehalt ≥ 7.5 mmol l^{-1} (20/26: 77%) besser geeignet, um ein Prognose bezüglich einer Abfohlung innerhalb der nächsten 60 Stunden zu geben. Werden Werte unterhalb dieser Schwellenwerte gemessen, ist dies ein verlässlicher Indikator für ein Ausbleiben des Abfohlens innerhalb der nächsten 24 Stunden (>95%). Nicht alle Stuten lassen sich in dieses Raster einordnen: Eine multipare Stute zeigte vor der Geburt nur unwesentliche Veränderungen in der Zusammensetzung der Elektrolyte der Milch oder im BRIX Score. Eine Maidenstute zeigte zwischen dem 10. und 1. Tag pre partum wiederholt eine Umkehrung von [Na^{+}] und [K^{+}]. Sowohl bei dieser Maidenstute, als auch bei einer anderen multiparen Stute, mit einer frühen Umkehrung von [Na^{+}] und [K^{+}], überstieg der gemessene [Ca^{2+}] Wert die 7.5 mmol l^{-1} - Grenze bereits am 9. bis 10. Tag vor der Abfohlung.

Schlüsselwörter: Vorhersage des Geburtzeitpunktes, Eutersekret, Zucker-Refraktometrie, Elektrolyte der Milch, Reproduktion

Introduction

Parturition in the mare is normally a rapid event involving considerable force and, while the majority of foalings (>90%: *Frazer* et al. 1999) progress without significant complications, any delay or deviation from the norm can have serious repercussions for both neonate and dam. Indeed, beyond 30 minutes after the onset of second stage labour, every additional 10 minute delay to delivery results in a 10% increase in the likelihood of the foal being born dead (*Lynch Norton* et al. 2007), where foal death results primarily from hypoxia due to reduced placental perfusion and/or detachment (*Hong* et al. 1993). Prolonged unproductive straining to expel the foal can also lead to severe trauma to the mare's reproductive tract or other caudal abdominal and pelvic structures. In many cases, however, the damage can be limited or avoided by timely performance of a relatively straightforward intervention (e.g. extending a flexed neonatal fore-limb, or clearing the foal's muzzle of amniotic membrane). As a result, it is recommended that all foalings are attended, and that competent (veterinary) assistance is sought in the case of complicated abnormalities of posture, position or presentation, or if parturition is not completed within 20-30 minutes

of chorio-allantoic rupture or the onset of abdominal contractions (*Frazer* et al. 1999).

While most breeders accept the need to supervise foaling, it is difficult to reliably predict when a mare probably is, or definitely is not, going to foal; ensuring that no births are missed may therefore entail many additional nights 'sitting up'. For example, while mean gestation length in the mare is generally quoted as 335-345 days, it varies between breeds and is affected by both fetal gender (longer for colt foals: *Davies-Morel* et al. 2002, *Valera* et al. 2006) and the time of year of anticipated foaling (longer for early foals: *Davies-Morel* et al. 2002, *Valera* et al. 2006); moreover, gestation lengths ranging from 320 to 370 days are accepted as normal (*Davies-Morel* et al. 2002), and viable foals have been reported after gestations of just under 300 and over 400 days (*Davies-Morel* et al. 2002, *Valera* et al. 2006). Whereas the length of previous gestations can be a useful indicator of expected foaling date for an individual mare (*Valera* et al. 2006), this data is not available for primiparous (maiden) mares and can be misleading in aged mares or in the case of underlying pathology. Similarly, most of the physical changes associated with impending parturition are insufficiently sensitive or consistent for reliable prediction of impending foaling (see *Christensen* 2011). These physical changes include udder enlargement, which may begin as early as 4-6 weeks before foaling in some mares, but may not be evident until very shortly before, or even after, foaling in primiparous mares. Likewise, softening of the sacro-sciatic ligaments (indicating pelvic relaxation) and elongation, swelling, and relaxation of the vulva are often too subtle or too variable in time of onset in relation to foaling to be helpful.

The most useful physical indicator of impending parturition is the appearance of colostrum in the udder and, in particular, a change in the consistency of the mammary secretion from serum-like to colostrum-like as parturition approaches (*Peaker* et al. 1979). Indeed, the most reliable current method for predicting parturition in the mare is analysis of mammary secretion electrolyte concentrations, although even this is far from perfect (Brown Douglas et al., 2002). Changes in the sodium, potassium and, in particular, calcium concentrations ($[Na^+]$, $[K^+]$ and $[Ca^{2+}]$) in pre-partum mammary secretions are, in the absence of pathology (*Rossdale* et al. 1991, *Paccamonti* 2001), fairly good indicators of 'fetal readiness for birth' and whether a mare is or isn't likely to foal within the following 24-72 h (*Peaker* et al. 1979, *Leadon* et al. 1984, *Ousey* et al. 1984, *Ley* et al. 1993). The classic pre-partum changes in mammary secretion electrolyte concentrations include a fall in $[Na^+]$ and a rise in $[K^+]$ such that $[Na^+]$ and $[K^+]$ 'cross' (invert) between 9 and 1 days prior to foaling (median 3 days: *Ousey* et al. 1984). Even more suggestive of imminent parturition is a steep rise in $[Ca^{2+}]$, or a single measurement in excess of 10 mmol l-1 or 400 p.p.m. (*Peaker* et al. 1979, *Leadon* et al. 1984, *Ousey* et al. 1984, *Ley* et al. 1993, *Paccamonti* 2001). While mammary secretion electrolyte concentrations can be measured with standard clinical biochemical analyzers, it is common to use mare-side test kits developed or modified to detect the increase in $[Ca^{2+}]$. Among these, commercial 'water hardness' test kits have been reported to be useful, primarily for indicating when a mare does not need to be observed overnight for possible foaling (*Ousey* et al. 1989, *Ley* et al. 1993). There is some discussion as to whether there should be a preference for kits that measure only $[Ca^{2+}]$ (*Ousey* et al. 1989) or whether kits that also measure $[Mg^{2+}]$ are just as reliable (*Ley* et al. 1993). Because $[Mg^{2+}]$ rises gradually during the last 7-10 days of pregnancy, Ca-Mg combined test kits have been reported to yield more 'false positive' diagnoses of imminent foaling (*Ousey* et al. 1989).

On many large stud-farms it has become routine practice to assess mare colostrum quality soon after foaling using a BRIX refractometer; this is possible because the sugar concentration correlates closely with the IgG concentration (*Cash* 1999). The preferential sequestration of IgG into mammary secretions is of course a critical feature of late gestation (*Pearson* et al. 1984) and, based on the assumption that it will be accompanied by a parallel increase in sugar concentration, some stud farms have started to use mammary secretion sugar concentrations (as determined using a BRIX-refractometer) to indicate when a mare is close to foaling. *Brown Douglas* et al (2002) reported a fairly steep rise in the lactose concentrations of mammary secretions (from 1.5 to >3%) in the last 48-60 h before foaling, however it is not clear how this corresponds to the BRIX score or, indeed, how useful BRIX refractometry is as an indicator of approaching parturition. The aim of this study was to investigate whether BRIX refractometry was a useful indicator of imminent parturition and, if so, what aspect of increasing sugar concentration was most useful e.g. the pattern of rise or an absolute cut-off value. The usefulness of BRIX analysis would be compared to a commercially available Ca-Mg based test kit and laboratory analysis of $[Na^+]$, $[K^+]$ and $[Ca^{2+}]$.

Materials and methods

Animals

The study was performed using 30 pregnant Dutch Warmblood mares resident at either the "Waiboerhoeve" experimental farm in Lelystad or the "Tolakker" teaching farm at Utrecht University, both in the Netherlands. The mares were aged between 4 and 18 years and weighed between 616 and 707 Kg (mean ± sem: 661.3±14.6 Kg) at the start of the study. Seven of the mares were primiparous (aged 4-6 years) and the remaining 23 were multiparous and had previously produced between 1 and 10 foals (mean±sem: 3.9±2.1). The mares were inseminated between 6th May and 2nd September with chilled transported semen from commercial stallions and examined at least every second day during oestrus to determine the day of ovulation. During the experimental period, the mares were maintained in groups at pasture during the day and stabled individually overnight, when they were fed additional concentrates and hay. All of the mares foaled spontaneously between 3rd April and 11th August.

Collection of mammary secretions

Attempts to collect mammary secretions daily were initiated from day 325 of gestation, or earlier if mares already displayed clear physical signs of impending parturition (e.g. 'waxing up'). For practical reasons, samples were collected in the early afternoon, between 12:00 and 16:00. An attempt was made to collect approximately 2.5 ml of mammary secretion into a sterile plastic vial, so that there would be sufficient secretion for the various tests that were to be compared, namely i) BRIX refractometric analysis of sugar concentration,

ii) laboratory analysis of [Na^+], [K^+] and [Ca^{2+}], and iii) the Predict-a-Foal™ test (monitors combined Ca and Mg ion concentrations). In a number of cases however, insufficient mammary secretion was recovered to perform all 3 of the planned tests. In the case of limited secretion volume, the tests were executed in the following order of preference (approximate amount of secretion required in parentheses): sugar-refractometry (a drop), laboratory analysis of [Na^+], [K^+] and [Ca^{2+}] (1.5ml), Predict-a-Foal™ test (0.6ml). Sugar refractometry and the Predict-a-Foal™ tests were performed on-site within 1 h of sample collection. The 1.5ml of secretion destined for laboratory analysis was transported at 4°C and stored overnight at the same temperature before being analyzed the following morning for [Na^+], [K^+] and [Ca^{2+}] at Utrecht University's clinical pathology laboratory.

BRIX sugar-refractometer
BRIX sugar refractometers are designed for measuring the sugar concentration of a solution. Moreover, BRIX refractometry has been validated as a quick and reliable method for evaluating mare colostrum quality post-partum (Cash 1999). Before use, the refractometer was 'zeroed' using distilled water. Measurement of the sugar concentration in mammary secretions was then performed by applying a drop of well-shaken sample onto the prism and closing the cover. The sugar percentage was indicated by the position of the light-dark border on the refractometer scale.

Laboratory analysis of electrolyte concentrations
Laboratory analysis of [Na^+], [K^+] and [Ca^{2+}] was performed using a Beckman-Coulter CX7 Delta Chemistry analyzer (Bekkman-Coulter Nederland B.V., Woerden, the Netherlands) in urinalysis mode; if the secretions were particularly thick and viscous they were diluted 1:3 prior to analysis.

Predict-a-Foal™ test kit
The Predict-a-foal™ test is a semi-quantitative test for determining combined calcium and magnesium ion concentrations in mammary secretions. The test strips contain five reaction squares with decreasing sensitivity to calcium and magnesium. The test was performed in accordance with the manufacturer's instructions. Briefly, the provided pre-marked test-tube was filled to the line with 'test fluid', to which 0.6ml of mammary secretion was added. The tube was then capped and inverted gently 2-3 times to mix the solutions. A test strip was dipped into the mix such that all 5 squares were covered; after removing the strip from the tube, the excess fluid was shaken off. After one minute, the number of squares that had changed from green to mauve was recorded.

Analysis
The time of foaling with respect to mammary secretion analysis was classified as <12h (before 4 a.m. on the morning after sample collection), <36 h (before 4 a.m. the following day) , and <60 h (before 4a.m. two days after sampling). The endpoints considered to be of interest were; 1) the percentage of mares that actually foaled within 36 or 60 h of the first time that a given test indicated imminent foaling (i.e. the 'positive predictive value'); 2) the percentage of mares that foaled without indication of imminent foaling (from which was derived the 'negative predictive value'; *Brown Douglas* et al. 2002); 3) the number of days between the first indication that foaling was imminent and actual foaling.

Results

All 30 mares spontaneously delivered a normal live foal after gestations ranging from 324 to 356 days in length (mean ± sem: 339.3 ± 8.3 days). In eight cases, monitoring of changes in mammary secretion electrolyte concentrations was restricted to a period of only 2-3 days, either because the mare foaled shortly after the onset of sample collection (2 mares) or because no more than a drop of mammary secretion could be expressed until the last 1-2 days before foaling (6 mares). However, because refractometry requires only a drop of secretion, sugar concentrations could be measured serially in most cases. In fact, serial BRIX values were recorded for 29 mares, all except one of which achieved a BRIX score of at least 14 % before foaling; one mare achieved a maximum BRIX score of only 8%, and also failed to show any of the normal pre-foaling changes in mammary secretion electrolyte concentrations. For this mare, none of the tests examined successfully indicated approaching parturition.

There was a difference in the general pattern of change in mammary secretion sugar concentrations between multiparous and primiparous mares. In the former, the mean BRIX score rose progressively as foaling approached (Figure 1) to reach mean values consistent with 'adequate' colostrum quality (>20%: *Cash* 1999); indeed, in the multiparous mares a 'cut off value' of 22% would have successfully predicted foaling within 60 h in 11/22 mares (i.e. a positive predictive value of 50%: Table 1). However, it would also have resulted in 5/22 of the foalings being missed (22%; i.e. a negative predictive value of 78%: Table 1); in these mares, the peak BRIX scores were 8, 14, 15, 15 and 18 %, i.e. indicative of poor to borderline colostrum quality (Table 2). The remaining 6 mares foaled 3, 3, 4, 5, 8 and 12 days after first achieving a BRIX score of >22%. On the other hand, two mares achieved a BRIX score of 21%, respectively, 17 and 16 days before foaling; they eventually foaled 1 and 3 days after the BRIX score reached 22% following a period in which the sugar concentration fluctuated between 9 and 20%. In primiparous mares, by contrast, BRIX scores were almost always relatively

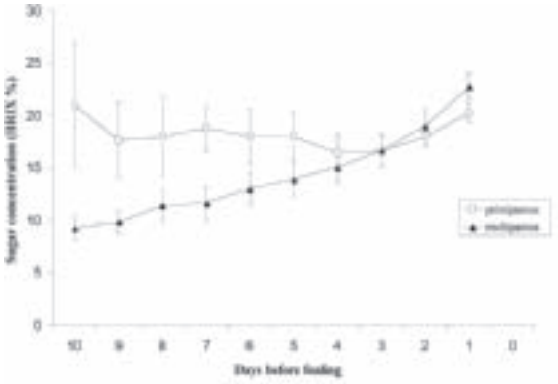

Fig. 1 Brix refractometric percentage sugar scores in mammary secretions collected from Warmblood mares in the days immediately preceding foaling. In multiparous mares (n=22), BRIX percentage rose progressively to reach a peak of 22.7±0.86% (mean ± s.e.m) on the day prior to foaling. In primiparous mares (n=7), mammary secretion BRIX scores were already high when first measured (4-12 days before foaling) and increased little as foaling approached. A score of 22% was a reasonable predictor of foaling within 60 h in multpiparous mares (50%), but was associated with a relatively high risk of failing to identify approaching parturition (22%).

Table 1 Predictive value of tests for mammary secretion constitution in terms of the likelihood of foaling within the following 24-60 h, based on values recorded for 23 multiparous and 7 primiparous Dutch Warmblood mares. Positive prediction implies that foaling followed within the given number of hours (36 or 60h) of the given result being recorded for the first time. Negative prediction refers to the absence of foaling in the 24 h following a value below the documented threshold values. The maximum delay from first recording of a threshold level to actual foaling is also given.

		Positive predictive value		Negative predictive value	Maximum wait (days)
Test	Threshold value	36 h	60 h	24 h	
BRIX	22% (multiparous)	36%	50%	78%	12 days
Na-K inversion		61%	71%	96%	10 days
$[Ca^{2+}]$	7.5 mmol l^{-1}	62%	77%	96%	10 days
	10 mmol l^{-1}	55%	82%	81%	9 days
Predict-a-foal	4 squares	65%	82%	89%	10 days
	5 squares	69%	92%	68%	3 days

high on the first occasion that they could be measured (14-32%), which could be a number of days before foaling (4-12 days), and rose little or not at all as foaling approached (Figure 1; Table 1). In primiparous mares, therefore, the BRIX score was of little predictive value with regards to indicating whether the mare was or was not about to foal.

Laboratory analysis of $[Na^+]$ and $[K^+]$ was possible for 28 mares; as previously mentioned, one of these mares failed to show either a Na-K inversion or indeed any significant change in concentration of the 3 electrolytes measured. In another 4 mares, the $[Na^+]$ and $[K^+]$ were already inverted when the first sample was collected, and their profiles could not therefore be used to determine when $[Na^+]$ and $[K^+]$ first crossed; all 4 of these mares did, however, foal soon after the first measurement (1, 2, 2, and 3 days later). For the 23 mares for which the time of Na-K inversion was recorded, it ranged from 0.5-10 days pre-foaling (mean±sem, 2.7±2.8 days: mean and mode, 1 day). In total, 17/23 of these mares foaled within 60 h of first detection of Na-K inversion (positive predictive value of 71%: Table 1), and fourteen (61%) had foaled within 36 h; only one mare failed to show a Na-K inversion before foaling (i.e. a negative predictive value for the absence of the Na-K inversion of 96%). On the other hand, one maiden mare first showed a Na-K inversion 10 days before foaling; thereafter the $[Na^+]$ and $[K^+]$ crossed back and forth a number of times. For a further 3 multiparous mares, the $[Na^+]$ and $[K^+]$ also crossed back after initial inversion, although all 3 still foaled within 3 days of first recorded inversion of the electrolyte concentrations.

Laboratory analysis of mammary secretion $[Ca^{2+}]$ yielded usable results for 27 mares. The one mare that failed to show a Na-K inversion also failed to show a rise in $[Ca^{2+}]$; two mares (both maidens) already had a mammary secretion concentration of >10 mmol l^{-1} when the first sample was recovered; both foaled within 36 h. For the other mares, the mean $[Ca^{2+}]$ showed a progressive rise in the last 4 days prior to foaling; mean $[Ca^{2+}]$ 3.5, 2.5, 1.5 and 0.5 days prior to foaling were, respectively, 5.4±0.8 (n=18), 6.6±1 (n=17), 8.5±0.9 (n=24) and 14.3±0.9 mmol l^{-1} (n=26). However, while 22 of 27 mares attained the generally quoted peak pre-foaling mammary secretion calcium concentration of 10 mmol l^{-1}, five (19%) did not, i.e. the negative predictive value of a $[Ca^{2+}]$ < 10 mmol l^{-1} for indicating the absence of foaling within 24 h was only 81% (Table 1); these foalings would have been unsupervised if the 10 mmol l^{-1} cut-off had been used as the only criterion for whether or not to monitor the mare overnight.

Table 2 Sugar concentrations (BRIX %) in the mammary secretions of 30 Warmblood mares in the 16 days preceding foaling

Days pre partum	Mare																												
	Ir	No	175	196	391	393	438	451	564	573	608	609	611	626	662	675	723	785	788	792	872	975	873*	874*	876*	950*	965*	Su*	So*
16			6	10		2		5	7	18	15		6		16			7	21	14	7							
15			5	11		3		5		18	15		7		17			5	12	11	10							
14			8	16		3		7		14	14		6		17			5	12	9	8	6						
13	8.5		7	15	6	4		6		15	12		6		17	21		4	11	8	12							
12			8	14	8	3		4	9	15	10		6		17	23		8	15	8	6			17	32			
11			9	12	6	5		4	12	14	10		6	3	19	20	5	3	14	10	12			17	30			
10			8	12	8	5	3	4	10	17	9		7	4	18	18	5	3	13	9	13	10		15	27			
9			8	10	8	4	3	6	12	17	10		6	4	15	17	6		13	12	13	13	14	14	25			
8	22		10	13		4	2	4	15	17	10		6	4	12	26	5		13	12	13	17	12	17	25			
7			11	12	8	4	2	5	17	20	14		5	4	18	31	6	7	17	14	13	13	16	16	25		18		
6	25		11	16		8	2	4	17	17	16	10	6	4	19	26	5	7	17	18	18	13		17	25		14		
5			18	19	8	6	4	6	18	20	24	12	5	5	19	30	9	7	18	21	14	13		14	23		18		
4			18	27	8	7	5	6	18	21	20	13	6	5	21	31	16	7	19	21	12	16	14	15	22		12	21	
3	25		19	21	7	10	5	6	16	22	23	16	10	6	27	30	16	12	16	21	17	15	15	18	20	20	12		
2	23		19	25	10	10	5	8	22	23	25	24	14	8	29	29	16	14	13	22	18	16	17	17	18	22	16		
1	25	33	27	27	23	14	8	22	25	22	26	31	25	15	30	26	23	16	22	22	22	13	20	19	18	24	18		
0		30							24	21			25					18	24	20		15			20			22	14

* Indicates maiden (primiparous) mares

When mammary secretion [Ca^{2+}] did reach 10 mmol l^{-1}, the mare generally foaled within 60 h (18/22: 82%), with 12 (55%) foaling within 36 h. The four mares (18%) that did not foal within 60 h had intervals to parturition of 3, 3, 6 and 9 days. If the [Ca^{2+}] cut-off value considered as indicating imminent foaling was reduced to 7.5 mmol l^{-1}, only the mare that showed no changes in milk electrolytes would have foaled unsupervised (i.e. negative predictive value of 96%). Moreover, reducing the cut-off to 7.5 mmol l^{-1} did not dramatically reduce the positive predictive value for foaling within 60 h since 20/26 (positive 77%) did so, including 16 that (62%) foaled within 36 h (Table 1). Of the six mares that had not foaled within 60 h, the 'waiting periods' were 3, 3, 4, 4, 9 and 10 days.

The Predict-a-foal™ test was performed for 19 mares, thirteen of which (68%) reached the maximum score of 5 prior to foaling. Most mares that attained a score of 5 subsequently foaled within 36 h (9/13: 69% positive predictive value) and only one had not foaled within 60 h (92%); this mare eventually foaled 3 days after the first recording of a score of 5. However, if 'sitting-up' had been delayed until mares reached a score of 5, then six (32%) foalings would have been unattended (i.e. negative predictive value of only 68%). If a score of 4 was taken as the point at which 'sitting up' was initiated, 17 of the 19 foalings (89%) would have been attended, of which eleven (65%) would have required less than 2 nights of observation (<36 h), and 14 were within 60 h (82%: Table 1). The remaining 'sitting-up' periods were 3, 6 and 10 days. One of the mares that would have been missed was the mare that showed no change in [Na^{+}], [K^{+}] and [Ca^{2+}]; it achieved a maximum predict-a-foal test score of only 1 square.

Collection of more than a few drops of mammary secretion was a challenge in primiparous mares; in fact, collection of more than two pre-partum mammary secretion samples was only successful in two of the seven maiden mares included in the study. One of the two primiparous mares from which serial samples could be collected displayed Na-K inversion, a [Ca^{2+}] of 7.5 mmol l^{-1} and a predict-a-foal score of 4 as early as 10 days pre-partum. Three of the maiden mares from which only two analysable samples were recovered showed Na-K inversion the first time secretion was analysed (2-4 days pre-partum).

Discussion

Mammary secretion sugar concentration turned out to be a moderate predictor of foaling in multiparous mares, but to be of little value for indicating imminence of foaling in primiparous mares. Since large studs will often have a BRIX refractometer available, however, it does represent a simple and low-cost manner of predicting likely foaling within 60 h in multiparous mares (50% positive predictive value). Nevertheless, even in multiparous mares there were individual differences in mammary secretion sugar concentration profiles in the days leading up to foaling, and BRIX refractometry was not as sensitive or reliable for indicating whether a mare should or should not be kept under 24 h observation as milk electrolyte based tests; in particular, there was a relatively high risk of failing to indicate imminent parturition (22%; i.e. the negative predictive value was only 78%). In addition, the selected cut-off value of 22% requires verification since it is based on a relatively small number of mares (22) and, moreover, because dropping the cut-off value by a single percentage point (to 21%) would have resulted in intensively monitoring 2 mares for a much longer period before they foaled; i.e. 16-17 days instead of 1-3. Not surprisingly, the cut-off value of 22% is consistent with the sugar concentration generally recommended for adequate colostrum quality (20%: *Cash* 1999); as such, the test does have the added value of indicating in advance that mares are accumulating colostrum of adequate quality. The foals of mares that do not reach the 22% cut-off value would be expected to be at higher risk of failure of passive transfer of immunity, since inadequate colostral IgG concentration is associated with an increased risk of poor IgG uptake by the foal (*Leblanc* et al. 1986). On the other hand, if a BRIX score of 22% was used as the only indicator of whether to observe a mare for foaling or not, the likelihood that parturition would be unattended might make it difficult to verify whether or not pre-suckle colostrum quality had reached 'adequate' and whether the resulting foals should therefore receive donor colostrums; in short, BRIX refractometry should not be considered a suitable stand-alone test for determining whether or not to sit-up with a mare. Not surprisingly, the rise in BRIX scores recorded in the pre-foaling period was very similar in profile to that for lactose concentrations described previously by *Brown Douglas* et al. (2002).

Primiparous mares showed a very different pattern of increase in sugar concentrations, and presumably IgG, into the mammary secretions in the pre-foaling period. This may relate to their general tendency to accumulate relatively little secretion until very close to, or even after, foaling. Presumably, the secretion that does accumulate has a higher percentage of IgG and associated sugars, and a lower concentration of other milk constituents. In any case, it was clear that sugar concentrations were of little practical use for indicating whether primiparous mares should be observed for foaling or not, at least for the 7 maiden mares included in the current study. This was unfortunate in that the biggest challenge when attempting to use mammary secretions to indicate likely foaling in primiparous mares was inability to collect sufficient secretion to perform an electrolyte based test; refractometry had the potential advantage of requiring only a single drop of secretion to perform the test.

BRIX refractometry was, however, a less accurate predictor of impending foaling than electrolyte concentrations (Table 2), both in terms of its ability to detect foaling within 60 h (positive predictive value) and in its suitability for confirming that foaling was not going to take place in the coming 24 h (negative predictive value). With respect to the electrolyte concentrations, while laboratory analysis and the Predict-a-foal test were not performed on exactly the same subsets of mares (because the amount of secretion was often limiting) there was little obvious difference between [Ca^{2+}] and the combined [Mg^{2+}] and [Ca^{2+}] test (i.e. the Predict-a-foal test) in their ability to predict whether foaling was or wasn't about to occur. On the other hand, in our hands, lower levels of Ca or Ca-Mg than those generally recommended in the literature (7.5 instead of 10 mmol l^{-1} Ca^{2+}) or in the Predict-a-foal users instructions (4 instead of 5 squares) needed to be used to indicate that sitting up was necessary; otherwise too many foalings would have been missed. Nevertheless, Na-K inversion, [Ca^{2+}] ≥ 7.5 mmol l^{-1} and a Predict-a-foal score of 4 were all equally useful, if not fail-safe, positive indicators of foaling within 60 h (71% of 23 mares, 77% of 26 mares, and

81% of 19 mares, respectively; Table 1). In all cases, however, at least one mare would have been observed for 10 nights before foaling using the chosen criteria; this highlights why electrolyte levels are not accepted as absolute indicators of the time of foaling but considered more useful as indicators that parturition is not imminent and, subsequently, that the fetus is preparing itself for birth (*Ousey* et al. 1984 and 1989, *Brown Douglas* et al. 2002). In practice, it is more important not to miss foaling than to be able to predict exactly when it is going to happen and, in this respect, the negative predictive value of all of the latter 3 tests (with the modified cut-off values) was very good (96%, 96% and 89%, respectively: Table 1). Using the cut-off values generally quoted in the literature of 10 mmol l^{-1} [Ca^{2+}] or 5 strips on the Predict-a-foal test, could not be recommended on the basis of this study because of their reduced ability to rule out foaling within the coming 24 h (only 81% and 68% accurate, respectively), even though their ability to predict foaling within 60 h (81% and 92%, respectively) were better. That one mare failed to show any of the normal changes in Na^+, K^+ and Ca^{2+} concentrations associated with foaling, but still produced a normal foal after a normal parturition, underlines the conclusion that mammary secretion electrolyte tests are not perfect indicators of imminent foaling, or indeed of fetal readiness for birth. In this respect, we concur with *Brown Douglas* et al. (2002) that some parameter other than the various aspects of colostrum constitution may be required before truly accurate prediction of impending parturition is possible in the mare. On the other hand, both in this study and on the basis of our clinical experience, the percentage of mares that fail to show either Na-K inversion or a rising [Ca^{2+}] is low, and milk electrolyte analysis remains the best available indicator of fetal maturity; it is also a useful tool for monitoring fetal well-being or stress in mares showing signs of threatened abortion (*Rossdale* et al. 1991, *Paccamonti* 2001).

The reason for the lower threshold values for Ca or Ca-Mg ion concentrations generated on the basis of the current study may relate to the timing of sampling; it is generally recommended to perform sampling as late in the day as possible because [Ca^{2+}] can rise fairly abruptly in the last 12 h before foaling in some mares (*Peaker* et al. 1979, *Ousey* et al. 1984, *Cash* et al. 1985, *Paccamonti* 2003). In practice, this would generally require testing to be performed on farm, and therefore involve the use of a calcium or calcium-magnesium based 'water-hardness' type test kits. Even then, it may be prudent to observe the lower thresholds for calcium or calcium-magnesium, since most owners/breeders would consider the potential inconvenience of sitting up for an additional 1-2 days to outweigh the risks of failing to be present at parturition. Similarly, while it would be difficult to recommend the use of BRIX refractometry to indicate imminent foaling for owners of one or two mares who want to know when to observe their mare overnight, in a large stud-farm situation where round-the-clock foaling assistance is provided, it may be a useful tool for identifying the mares that need to be watched more carefully.

In conclusion, BRIX refractometry is a moderately accurate (positive predictive value of 50%) method for predicting foaling within 60 h in multiparous mares, but is fallible as a method for determining when a mare does not need to be observed overnight (negative predictive value of 78%) and is of little predictive value in maiden mares. It is, however, not as sensitive as electrolyte based tests (e.g. positive predictive value of 77% and negative predictive value of 96% for [Ca^{2+}] > 7.5 mmol l^{-1}), and may be best suited to a large stud situation in which it can be used to identify mares for closer observation and/or act as an early warning sign for mares that need to be checked carefully for the adequacy of colostrum quality between foaling and first suckle.

References

Brown Douglas C. G., Perkins N. R., Staffor K. J. and *Hedderley D. I.* (2002) Prediction of foaling using mammary secretion constituents. New Zeal. vet. J. 50, 99-103

Cash R. S. G. (1999) Colostral quality determined by refractometry. Equine Vet. Educ. 11, 36-38

Cash R. S. G., Ousey J. C. and *Rossdale P. D.* (1985) Rapid strip test method to assist management of foaling mares. Equine Vet. J. 17, 61-62

Chistensen B. W. (2011) Partutition. In Equine Reproduction Second Edition Ed: A. O. McKinnon, E. L. Squires, W. E. Vaala, D. D. Varner. Wiley-Blackwell, Chichester, UK. Pp 2268-2276.

Davies Morel M. C., Newcombe J. R. and *Holland S. J.* (2002) Factors affecting gestation length in the Thoroughbred mare. Anim. Reprod. Sci. 74, 175-185

Frazer G. S., Perkins N. R. and *Embertson R. M.* (1999) Normal parturition and evaluation of the mare in dystocia. Equine vet. Educ. 11, 41-46

Hong C. B., Donahue J. M., Giles R. C. Jr., Petrites-Murphy M. B., Poonacha K. B., Roberts A. W., Smith B. J., Tramontin R. R., Tuttle P. A. and *Swerczek T. W.* (1993) Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. J. Vet. Diagn. Invest. 5, 560-566

Leadon D. P., Jeffcott L. B. and *Rossdale P. D.* (1984) Mammary secretions in normal spontaneous and induced premature parturition in the mare. Equine Vet. J. 16, 256-259

LeBlanc M. M., McLaurin B. I. and *Boswell R.* (1986) Relationships among serum immunoglobulin concentration in foals, colostral specific gravity, and colostral immunoglobulin concentration. J. Am. Vet. Med. Assoc. 189, 57–60

Ley W. B., Bowen J. M., Purswell B. J., Irby M. and *Greive-Crandell K.* (1993) The sensitivity, specificity and predictive value of measuring calcium carbonate in mares' pre-partum mammary secretions. Theriogenology 40, 189-198

Lynch Norton J., Nolen-Walston R. D., Underwood C., Slack J., Boston R. and *Dallap B. L.* (2007) Retrospective study of dystocia in mares at a referral hospital. Equine Vet. J. 39, 37-41

Ousey J. C., Delclaux M. and *Rossdale P. D.* (1989) Evaluation of three strip tests for measuring electrolytes in mares' pre-partum mammary secretions and for predicting parturition. Equine Vet. J. 21,196-200

Ousey J. C., Dudan F. and *Rossdale P. D.* (1984) Preliminary studies of mammary secretions in the mare to assess foetal readiness for birth. Equine Vet. J. 16, 259-263

Paccamonti D. L. (2001) Milk electrolytes and induction of parturition. Pferdeheilkunde 17, 616-618

Peaker M., Rossdale P. D. and *Forsythe I. A.* (1979) Changes in mammary development and the composition of secretion during late pregnancy in the mare. J. Reprod. Fert. Suppl. 27, 555-561

Pearson R. C., Hallowell A. L., Bayly W. M., Torbeck R. L. and *Perryman L. E.* (1984) Times of appearance and disappearance of colostral IgG in the mare. Am. J. Vet. Res. 45,186-190

Rossdale P. D., Ousey J. C., Cottril C. M., Chavatte P., Allen W. R. nd *McGladdery A. J.* (1991) Effects of placental pathology on maternal plasma progestagen and mammary secretion calcium concentrations and on neonatal adrenocortical function in the horse. J. Reprod. Fertil. Suppl. 44, 579-590

Valera M., Blesa F., Dos Santos R. and *Molina A.* (2006) Genetic study of gestation length in andalusian and arabian mares. Anim. Reprod. Sci. 95, 75-96

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