Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Testing mammary gland secretions to predict foaling in jennies

Tiziana Nervo, Alessia Bertero, Patrizia Ponzio, Eleonora Julita, Mariagrazia Lucia Poletto & Gian Guido Donato

To cite this article: Tiziana Nervo, Alessia Bertero, Patrizia Ponzio, Eleonora Julita, Mariagrazia Lucia Poletto & Gian Guido Donato (2023) Testing mammary gland secretions to predict foaling in jennies, Italian Journal of Animal Science, 22:1, 35-44, DOI: 10.1080/1828051X.2022.2157764

To link to this article: https://doi.org/10.1080/1828051X.2022.2157764

Published online: 02 Jan 2023.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2023, VOL. 22, NO. 1, 35–44
https://doi.org/10.1080/1828051X.2022.2157764

PAPER

Testing mammary gland secretions to predict foaling in jennies

Tiziana Nervo[a], Alessia Bertero[a], Patrizia Ponzio[a], Eleonora Julita[b], Mariagrazia Lucia Poletto[a] and Gian Guido Donato[a]

[a]Department of Veterinary Sciences, University of Turin, Turin, Italy; [b]Practitioner, Novara, Italy

ABSTRACT

The surging demand for donkey milk has led to a stronger focus on donkey husbandry and reproductive management, particularly in the peripartum period. Techniques to predict parturition are needed to avoid losses due to dystocias, which are frequently related to unattended foaling. In mares, pH and electrolyte analyses of pre-colostrum are used to predict parturition with a good accuracy. The purpose of this study was to evaluate the pH of the mammary secretion using both a pH metre and test strips and the electrolytes ($Na+$, $K+$ and $Ca2+$) in the mammary gland secretions of 9 jennies during the 5 days preceding parturition, to understand if the trends of these cations were comparable to those of the mare and to identify possible markers/thresholds of impending parturition. $Ca2+$ concentrations increased in the days before delivery from day -2 ($p < 0.01$) and 78% of the jennies foaled within 48 h of reaching the threshold of 20 mmol/L. $Na+$ and $K+$ levels remained constant as parturition approached. The pH decreased in the days before foaling, starting from day -1 ($p < 0.05$). However, it was not possible to deduce certain indications on the date of delivery based on electrolyte concentrations, nor on pH and at the current state of knowledge foaling alert systems are the only methods for ensuring prompt assistance during parturition.

HIGHLIGHTS

- The ability to predict parturition for providing adequate assistance is essential in a donkey farm.
- pH and electrolytes of mammary gland secretions are used in mares to predict foaling, but no reference values are available for jennies.
- In our work, neither electrolyte nor pH evaluations proved to be reliable to predict with an adequate level of confidence the impeding parturition.

ARTICLE HISTORY
Received 19 September 2022
Revised 24 November 2022
Accepted 7 December 2022

KEYWORDS
Jenny; donkey; pregnancy; foaling; colostrum; electrolytes; pH

Introduction

Donkeys are still providing a fundamental contribution to the economies of some developing countries (McLean and Navas Gonzalez 2018; Davis 2019). In the Mediterranean area, with the advent of mechanisation, these animals had been gradually put asides, until recently when, for various reasons (milk production, onotherapy, breeding of mules for agricultural work in national parks, where use of farming machines is forbidden), researchers displayed renewed interest in this species (Carluccio, Panzani, et al. 2008).

Numerous studies have shown the similarities between horses and donkeys, nonetheless, the differences are many, thus knowledge cannot simply be transferred from one species to another (Veronesi et al. 2014).

Management of reproduction, pregnancy monitoring and methods for predicting parturition to provide assistance are essential in a dairy farm, where the good health of the dam as well as the foal are fundamental since the latter, by feeding, stimulates the udder to maintain an optimal milk production (Carluccio et al. 2015).

In the equine species, dystocias have an incidence that ranges from 1 to 10% (Lu et al. 2006). Parturition is an ‘explosive’ and very rapid event and the consequences of dystocia can be fatal; thus there is a great interest in assisting the animals to promptly intervene in case of any deviation from an eutocic delivery (Carluccio et al. 2015).

Historically, the prediction of parturition in the mare was based on the observation of physical changes associated with the end of the gestation

CONTACT Alessia Bertero alessia.bertero@unito.it

(Korosue 2013): mammary gland development with the presence of secretion or colostrum, waxing and relaxation of the sacro-ischial and sacro-iliac ligaments, of the tail and of the vulvar lips and duration of pregnancy that extends beyond 320 days. These signs are useful but not very accurate (Ley 2011), while the prediction of parturition based on the detection of electrolyte levels in pre-foaling mammary secretion has been successfully used in the mare for many years (Nagel et al. 2020). During the last 3 weeks of gestation, sodium and chlorine concentrations decrease while calcium, potassium, magnesium, protein and lactose concentrations increase (Peaker et al. 1979; Korosue 2013) until, between 9 and 1 day before birth (average of 3 days), a reversal of the sodium/potassium ratio can be observed (Ousey et al. 1984).

Multiparous mares show a more pronounced change in electrolyte concentration before parturition, while maidens often have a slower mammary development and electrolytes in the mammary secretion may not change until shortly before delivery (Macpherson and Paccamonti 2011).

Calcium concentrations in the pre-foaling mammary secretion are useful for predicting the end of gestation, particularly for determining the odds of foal survival following the induction of parturition (Leadon et al. 1984; Ley 2011).

The increase in calcium concentration in the pre-colostrum, together with the rise in potassium levels in relation to sodium, generally indicate foetal maturity in a normal gestation (Macpherson and Paccamonti 2011). Foals born after spontaneous or induced parturition before the increasing in calcium levels have inadequate adrenocortical function associated with prematurity (Canisso et al. 2013). Contrary to the other species, the equine foetus has a weak adrenocortical activity up to 24–48 h before birth, thus in case of preterm parturition there is a high risk of dysmaturity/prematurity (Macpherson and Paccamonti 2011). Precise measurement of electrolyte levels requires a biochemical analyser, but this is impractical since the change in electrolytes mainly occurs in the evening or at night (Macpherson and Paccamonti 2011). Therefore, numerous rapid tests have been developed to measure the calcium content of mares' milk in the field (Mancuso et al. 2004).

The concentration of electrolytes in the pre-foaling mammary secretion follows a pattern similar to that of pH, although not in all mares: animals that give birth with a high pH have low levels of calcium, magnesium and do not exhibit the inversion of sodium and potassium concentrations (Canisso et al. 2013). Conversely, mares that foal with a low pH have high levels of calcium and magnesium and show the classic sodium/potassium reversal (Canisso et al. 2013). However, at present, a standard reference pH value to predict foaling still need to be defined (Korosue 2013).

As for the donkey species, three studies have been published on the topic, with different results. One research reports only the calcium concentrations (Mancuso et al. 2004), while another also those of sodium and potassium (Carluccio, De Amicis, et al. 2008). A recently published article (Magalhaes et al. 2021) investigated pH and electrolyte concentrations of mammary secretions in jennies before parturition (from 350–355 days to delivery) but to date, no reference values are available, neither for electrolytes nor for pH.

The purpose of the study is to analyse the electrolyte trend in the five days preceding parturition in nine crossbred jennies to understand if it is comparable to that of the mare. The pH of the mammary secretion, which has been proposed in the literature as a potential parameter to predict birth in the mare, was also measured.

The ultimate goal is to provide the breeder with an economical and practical tool to help the prediction of parturition, in order to avoid unattended deliveries to ensure prompt intervention in case of need.

Materials and methods

Animals

The work was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin (Italy) (n. 311, 09/02/2021). All procedures were performed in compliance with the guidelines of the Italian Ministry of Health for the care and use of animals (D.L. 4 March 2014 n. 26 and D.L. 27 January 1992 n. 116) and with EU Directive 86/609/CEE.

Nine crossbred jennies of different ages (range 4–18 years) and weight (range 250–300 kg), housed in an organic dairy farm and kept in group stables and paddocks, were monitored daily during the peripartum period. One jenny was primiparous.

Sampling was performed daily, mainly during periods in which more deliveries were expected, namely from April to July, starting about one week before the expected date of parturition. Fewer jennies were sampled in the remaining months of the year. Pregnancy duration was calculated by counting 370 days from the last natural assisted mating (stable average). When the jennies were close to the delivery date and the mammary gland began to increase in volume, they were moved to the delivery room and monitored *via* a wireless camera.

During the study period, data regarding the duration of pregnancy, clinical observations related to impending parturition, season, time of delivery and weight of the foal were collected.

The collection of mammary secretions was performed on 23 animals, but only the samples from nine jennies have been analysed, namely those from which it was possible to obtain a sufficient quantity of material for at least 5 days before the birth.

Experimental protocol

Milking was done manually, in the late afternoon or early evening, always after 6 p.m.

After eliminating the first jets, the mammary secretions were collected from both teats in a 50 mL Falcon tube for a minimum total quantity of 3–4 mL. The samples were immediately subjected to a first macroscopic observation and pH measurement, using both test strips (range from 1 to 14, each unit corresponding to a different colour) (Merck Serono Spa, Rome, Italy), and a pH metre (Testo® 230, Testo Spa, Settimo Milanese, Milan, Italy). The test strips were dipped into the mammary secretion and the colour change was compared with the colour/pH scale reported on the package.

The pH metre was calibrated using solutions with known pH (4 and 7). The electrode was introduced into the sample and the value shown on the display was recorded. The electrode was thoroughly rinsed with distilled water and carefully dried between measurements.

After pH measurement, the samples were divided into several 1.5 mL Eppendorf tubes, labelled with the jenny's name and the collection date, and stored at -20° C for further analysis at the laboratory of the Veterinary University Hospital of the University of Turin.

The specimens, collected daily during the 5 days before delivery, were classified as -4, -3, -2, -1, 0. Parturition occurred within 24 h from the collection of the '0' sample.

In the laboratory, the samples were thawed at room temperature for 20 min, centrifuged and diluted 1:10 with bidistilled deionised water before analysis. The dilution was necessary to determine the calcium concentrations.

The calcium, sodium, and potassium concentrations were measured with a biochemical analyser (BT3500 VET, Biotecnica Instruments S.p.A., Rome, Italy).

Sodium and potassium concentrations were initially expressed in mmol/L, while calcium concentrations in mg/dL. The latter were then converted into mmol/L and ppm to be compared with the data provided by Carluccio et al. (Carluccio, De Amicis, et al. 2008) and Mancuso et al. (Mancuso et al. 2004), respectively.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Mac, Version 27.0 (Armonk, NY: IBM Corp.), while graphs were created with Prism for Mac, Version 9.2.0 (GraphPad Software Inc., La Jolla, CA, USA).

The normality of the distributions was assessed through the Shapiro-Wilk test.

The electrolyte concentrations following a normal distribution (Ca^{++}, K^{+}) were analysed with a two-way ANOVA model, considering 'time' as a fixed factor and 'jenny' as random factor. A Least Significant Difference (LSD) test was used to examine the difference between the various days in which sampling was performed. For the electrolytes (Na^{+} and Na^{+}/K^{+}) showing a non-parametric distribution, a Kruskal-Wallis test was performed.

Moreover, the presence of a possible correlation between the electrolyte concentrations and between the electrolyte concentrations and the day of sampling was evaluated with Spearman's test.

The pH (evaluated using a pH metre and test strips) differences in relation of the day of collection were analysed again, according to the data distribution, by means of two-way ANOVA model or Kruskal-Wallis test, while the presence of a possible correlation between pH values and day of sampling was assessed through Spearman's test.

In addition, a correlation analysis (Spearman's test) was performed on the two methods used for the pH measurement (strip and pH metre) and on electrolytes and pH values obtained with both methods.

Statistical significance was set at $p < 0.05$.

Results

During the study period, 23 jennies gave birth, but for only 9 of these it was possible to collect the mammary secretion for at least 5 days prior to delivery. Considering the 23 jennies that gave birth, the mean pregnancy length was 370.78 ± 15.22 (range: 340–398 days). The mean duration of pregnancy for jennies carrying a male foetus ($n = 10$) was 369.20 ± 15.70 (range 340–395) days, while in the case of the female foetus ($n = 13$) it was 372.00 ± 15.36 (range 346–398), but the difference was not statistically significant.

Fifteen of the 23 deliveries occurred in the late evening or during the night, particularly between 10 pm and midnight (5/23) and between 2 am and 4 am (7/23). Three out of 23 deliveries occurred between 2 pm and 4 pm, and one in the mid-morning.

The examined jennies ($n = 23$) had a gestation length of 367.00 ± 5.79 days when deliveries occurred between September and January ($n = 5/23$; range 361–273 days), of 367.91 ± 15.93 days ($n = 11/23$, range 340–395 days) when parturition occurred in June-August and of 378 ± 17.78 days ($n = 7/23$, range 346–398 days) from February to May, but these differences were not statistically significant.

The mean weight and standard deviation of foals at birth was 30.70 ± 5.17 kg ($n = 14$, range 24.50–41.10 kg). The weight of male foals was 29.78 ± 4.11 kg ($n = 6$, range 24.80–37.00), while it was 31.39 ± 6.02 kg ($n = 8$, range 24.50–41.10 kg) for the females, but the difference was not statistically significant.

The preparation for foaling and the related physical changes were highly variable. Mammary development began from 3 weeks to a few days before delivery. Initially, development and filling of the udder base was observed, followed by development and filling of the nipples. In some jennies, the nipples remained soft, in others they were hard on palpation until delivery, or from hard they gradually became softer until the day of parturition. The nipples began to fill with secretion 7 to 2 days before delivery.

The mammary secretion appeared initially watery and yellowish in colour. As parturition approached, it became thick, sticky and the colour tended more and more to white.

Waxing was observed just in one jenny, 6 days before delivery, in the form of tiny drops attached to the apex of the nipple.

In the primiparas, it was difficult to appreciate mammary development until a few days before delivery. It was not always possible to collect the mammary secretion or collect it in sufficient quantity to be analysed. As for the multiparous jennies, good mammary development was generally observed, and it has been almost always possible to collect a sufficient quantity of secretion for analysis in the correct days.

Relaxation of the sacroiliac and sacrotuberous ligaments proved to be an inconsistent sign for predicting foaling. Sometimes changes have been appreciated in the last 2–3 days, but the assessment is subjective, and it is not easy to use this information to predict the exact day of delivery.

Vulvar elongation is another sign that is difficult to detect and interpret.

Ventral oedema appeared from 1 week to 4 days before delivery, but it was not observed in all the jennies.

By the previously indicated criteria, the 9 jennies chosen for the electrolyte measurement gave birth spontaneously after 380.33 ± 13.91 days (range 361–398 days). Jennies carrying a male foetus ($n = 2$) had a pregnancy length of 381.00 ± 19.80 days (range 367–395 days), while for those carrying a female foetus ($n = 7$) it was of 380.14 ± 13.87 days (range 361–398 days).

The foals were all alive and viable, and the average birth weight ($n = 4$) was 32.43 ± 5.29 kg (range 24.80–37.00 kg).

Considering again the 9 jennies enrolled for the electrolyte evaluation, waxing was observed just in one jenny, 6 days before delivery, while ventral oedema occurred in 3 animals.

Electrolytes

Table 1 shows the concentrations of calcium, sodium and potassium in the mammary secretion of the 9 jennies included in the electrolyte evaluation, during the 5 days prior to parturition.

Considering the correlations among the different electrolytes (Table 2), a negative relationship between calcium and sodium (correlation coefficient = −0.389, $p < 0.05$) and between potassium and sodium (correlation coefficient = −0.363, $p < 0.05$) have been detected. As expected, the $Na+/K+$ ratio was positively correlated with sodium (correlation coefficient = 0.870, $p < 0.01$) and negatively correlated with potassium (correlation coefficient = −0.735, $p < 0.01$).

Calcium

Calcium in the mammary secretion increased in the days before delivery and, in particular, from day −2. This increase was most significant (reaching a p value

Table 1. Concentration of electrolytes during the 5 days prior to parturition in the 9 jennies examined.

Day of collection	Ca^{++} (mmol/L)	Na^{+} (mmol/L)	K^{+} (mmol/L)
−4	11.06 ± 4.11[a]	76.48 ± 73.01[a]	38.60 ± 10.13[a]
−3	13.20 ± 4.32[ab]	71.66 ± 54.92[a]	37.51 ± 7.36[a]
−2	15.49 ± 4.50[b]	67.11 ± 56.50[a]	35.68 ± 7.28[a]
−1	21.85 ± 4.42[c]	62.42 ± 47.99[a]	33.86 ± 6.35[a]
0 (day of delivery)	24.73 ± 4.74[d]	62.62 ± 42.10[a]	32.20 ± 5.04[a]

Within columns, means without a common superscript letter differ at $p < 0.05$.

Table 2. Correlation table between days, electrolyte concentrations and pH in the mammary secretion.

	Day	Ca^{++}	Na^{+}	K^{+}	Na^{+}/K^{+}	pH metre	pH test strips
Day	1	−0.769**	0.017	0.331*	−0.205	0.392*	0.855**
Ca^{++}	−0.769**	1	−0.389*	−0.208	−0.146	−0.615**	−0.808**
Na^{+}	0.017	−0.389*	1	−0.363*	0.870**	0.296	0.099
K^{+}	0.331*	−0.208	−0.363*	1	−0.735**	−0.109	0.209
Na^{+}/K^{+}	−0.205	−0.146	0.870**	−0.735**	1	0.219	−0.097
pH metre	0.392*	−0.615**	0.296	−0.109	0.219	1	0.537**
pH test strips	0.855**	−0.808**	0.099	0.209	−0.097	0.537**	1

*$p < 0.05$; **$p < 0.01$.

<0.01) between day −2 and day −1. Levels also increased between day −1 and day 0, but with a difference not as marked as between the two previous days ($p < 0.05$) (Figure 1).

Three of the 9 examined jennies had a calcium concentration equal to or greater than 10 mmol/L as early as day −4. On day −3 there were 6 donkeys with a concentration higher than 10 mmol/L, on day −2 there were 7, and on day −1 all the jennies had Ca levels greater than 10 mmol/L with 8 out of 9 animals showing Ca concentrations which had even reached 20 mmol/L (Figure 1).

It can be stated that 78% of the jennies showing calcium levels equal to or greater than 20 mmol/L foaled within 48 h.

Calcium levels in the mammary secretion were strongly inversely correlated with the day of collection (correlation coefficient: −0.769, $p < 0.01$; Table 2): as the days preceding parturition decreased, the calcium levels increased.

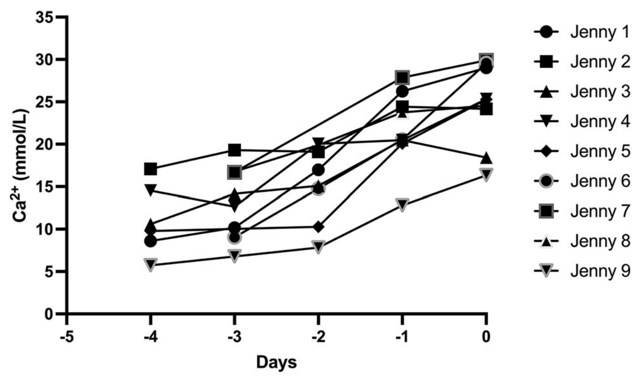

Figure 1. Calcium concentrations in the mammary secretions of the nine jennies during the 5 days prior to delivery.

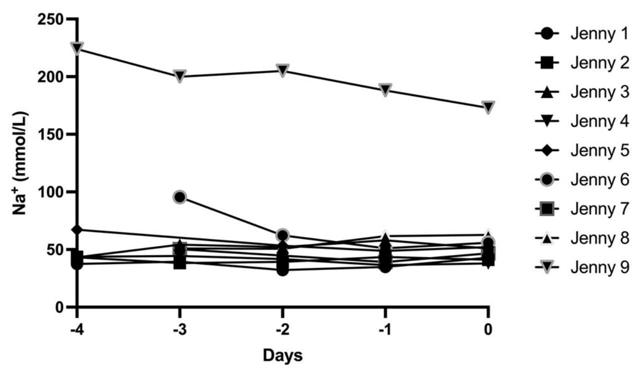

Figure 2. Sodium concentrations in the mammary secretions of the nine jennies during the 5 days prior to delivery.

Sodium and potassium

Sodium concentrations generally remained constant as parturition approached. No significant decreases were observed, although there was a slight downward trend, as can be seen in the chart (Figure 2). However, this tendency was weak and not statistically significant, thus sodium level cannot be used as a predictor of delivery.

The correlation between sodium concentrations and the day of sampling was not significant (Table 2).

Potassium, like sodium, showed almost constant levels in the last 5 days before delivery and, contrary to what is reported in the literature, a slight downward trend was observed (Figure 3).

A significant positive correlation has been found between potassium concentrations and the day of sampling (correlation coefficient = 0.331, $p < 0.05$)

In our work, sodium/potassium inversion did not occur, and the ratio remained roughly constant (around 2) never reaching values lower than 0.8, although a slight downward trend has been observed (Figure 4).

The correlation between the sodium/potassium ratio and the day of sampling was not significant (Table 2).

pH

The pH values measured in the mammary secretion in the 5 days prior to delivery are shown in Table 3.

The pH of mammary secretions, evaluated using a pH metre, decreased in the days before delivery. The pH difference was statistically significant between day −3 and −1, then between day −1 and 0 the pH remained constant (Table 3; Figure 5).

A similar trend has been observed measuring the pH with the test strips (Figure 6).

A positive correlation has been observed between the pH values measured using the pH metre and

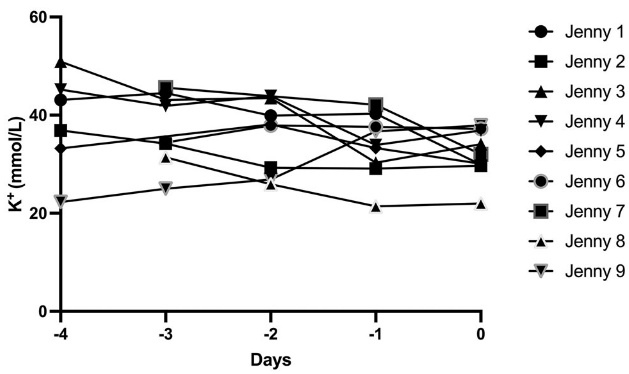

Figure 3. Potassium concentrations in the mammary secretions of the nine jennies during the 5 days prior to delivery.

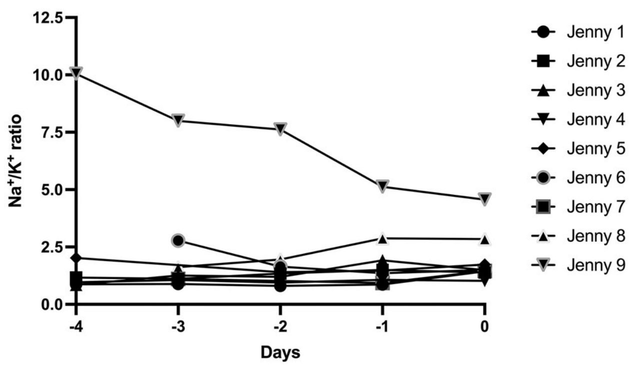

Figure 4. Sodium/potassium ratio in the mammary secretions of the nine jennies during the 5 days prior to delivery.

Table 3. Results of the pH measurement in the pre-foaling mammary secretion performed with a pH metre and with pH test strips in the 9 examined jennies.

	pH metre		pH test strips	
Day of collection	Mean ± SD	range	Mean ± SD	Range
−4	6.70 ± 0.14^{a}	6.55–6.90	6.86 ± 0.24^{a}	6.50–7.00
−3	6.65 ± 0.15^{a}	6.49–6.93	6.79 ± 0.27^{a}	6.50–7.00
−2	6.61 ± 0.12^{ab}	6.46–6.82	6.22 ± 0.26^{ab}	6.00–6.50
−1	6.54 ± 0.10^{b}	6.44–6.68	6.00 ± 0.00^{b}	6.00–6.00
0 (day of delivery)	6.54 ± 0.13^{b}	6.34–6.72	5.89 ± 0.33^{b}	5.00–6.00

Within columns, means without a common superscript letter differ at $p < 0.05$.

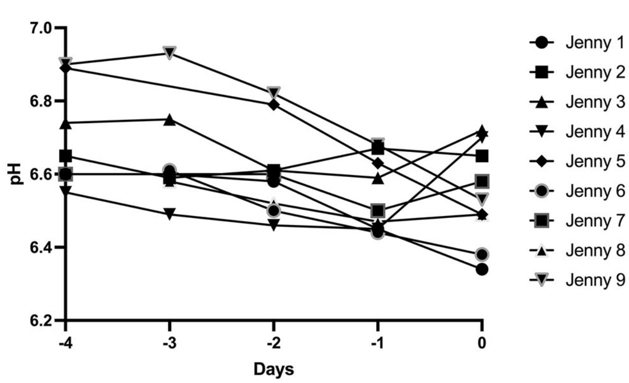

Figure 5. pH in the mammary secretions of the nine jennies measured using a pH metre during the 5 days prior to delivery.

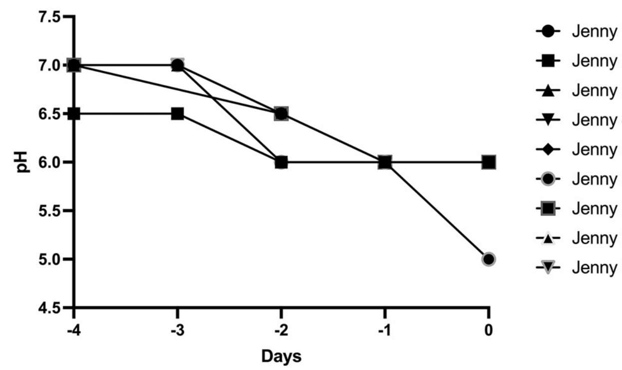

Figure 6. pH in the mammary secretions of the nine jennies measured using pH test strips during the 5 days prior to delivery.

those obtained with the test strips (correlation coefficient = 0.537, $p < 0.01$; Table 2).

A positive correlation has been also found between the pH values measured with a pH metre (correlation coefficient = 0.392, $p < 0.05$) as well as with test strips (correlation coefficient = 0.855, $p < 0.01$) and the day of sampling (Table 2).

Considering the relationship between pH and electrolytes, an association between pH values and calcium concentrations has been demonstrated using both pH metre (correlation coefficient = −0.615, $p < 0.01$) and test strips (correlation coefficient = −0.808, $p < 0.01$; Table 2): these two parameters were strongly negatively correlated.

No significant correlations have been found between the other electrolytes (sodium, potassium and Na+/K+ ratio) and pH, measured with both methods.

Discussion

The rearing of dairy donkeys requires a year-round breeding planning, which is possible since this species does not have a seasonal anoestrus. However, the number of matings and births during the spring - summer period is higher, and this may have affected the results obtained on pregnancy length in relation to the season.

The observed physical changes related to the preparation for foaling were mainly variable. The most detected signs were mammary development (development and filling of the udder base at first, then extension to the nipples was observed). Nipples began to show secretion 7 to 2 days before delivery. It was initially watery and yellowish in colour but, as parturition approached, it became thick and more whitish. The change in colour of the mammary secretion is in agreement with the work of Mancuso et al. (Mancuso et al. 2004) who reports that the only reliable indication for the prediction of parturition in the donkey is the 'black test', that becomes positive 24–36 h before parturition, when putting a drop of colostrum onto a black surface, the dark background is not visible due to the turbidity of the secretion.

According to Mancuso et al. (Mancuso et al. 2004), vulvar elongation becomes important 1–2 days before delivery and it is the only physical sign that undergoes a noticeable change as parturition approaches, but in our study, this was not the case since this modification was difficult to detect and interpret, being highly subjective due to the minimal vulvar modification observed. On the other hand, the development of ventral oedema was often detected from 7 to 4 days before delivery, but not in all the jennies.

Calcium

Ca2+ concentrations increased in the days before delivery (starting from day −2, $p < 0.01$) and 78% of the jennies foaled within 48 h of reaching the threshold of 20 mmol/L. This increase, common in several mammalian species, is governed by the complex mechanisms related to calcium homeostasis, involving the calcitropic hormones (calcitriol, calcitonin and parathyroid hormone), and is influenced by diet since rations rich in forage, as those fed to the jennies of our study, significantly affect the calcium content of colostrum (Polidori et al. 2022).

In the study by Carluccio et al. (Carluccio, De Amicis, et al. 2008), calcium levels showed a significant increase between day −10 and −6 and between day −6 and days −4 and −2, reaching the concentration of 10 mmol/L on the day of parturition. Similarly, Magalhaes et al. (Magalhaes et al. 2021) reported that Ca2+ concentrations increased from day −5 to the day of foaling (day 0, mean [Ca2+] = 10.6 ± 0.1 mmol/l).

In the mare, calcium concentrations can be already high several days before foaling. According to Peaker et al. (Peaker et al. 1979), the 10 mmol/L threshold can be reached 1 to 6 days before delivery. 98% of the mares with calcium concentrations below 400 ppm will not foal within 24 h, while 99% of the mares whose calcium concentration exceeds 400 ppm (or 10 mmol/L) will give birth within 72 h (Dascanio 2014).

As in the horse, also in the donkey this parameter may probably be more useful for predicting that foaling will not occur in the next 24 h if the concentrations are below a certain level (i.e. 10 mmol/L or 400 ppm) (Carluccio, De Amicis, et al. 2008).

In our work, only 2 out of 9 jennies gave birth within 72 h (or less) of reaching this threshold, since the calcium concentration at that time was already higher than this threshold in the other animals. Also in the study by Mancuso et al. (Mancuso et al. 2004) on 8 Ragusana donkeys, calcium concentrations have never been below 500 ppm in the 24 h prior to foaling, exceeding 900 ppm in 4 cases (corresponding to 22.5 mmol/L). Likewise, in our work the majority of the jennies had already exceeded the 500-ppm threshold (12.5 mmol/L) on day −3 and all the animals showed values above this threshold 48 h before delivery. On the day of parturition, 5 out of 9 jennies had reached and exceeded 1000 ppm (25 mmol/L) of calcium.

Values of 500–600 ppm were also recorded at 7–13 days prior to parturition, so it is not possible to deduce clear indications on the date of delivery based on calcium concentrations, as it has also been observed by Magalhaes et al. in jennies (Magalhaes et al. 2021).

The fact that calcium concentrations, expressed in mmol/L, differ considerably from those identified by Carluccio et al. (Carluccio, De Amicis, et al. 2008) and Magalhaes et al. (Magalhaes et al. 2021) may be due to the type of biochemical analyser used in those studies and to the applied methodology. A dilution with a factor of 1:10 was required for the analysis, while no dilution is reported by Carluccio et al. (Carluccio, De Amicis, et al. 2008). Similarly, the method Mancuso et al. (Mancuso et al. 2004) used to measure calcium is not clear, although their data are comparable to ours. Calcium is presumably an indicator of foetal maturity and readiness for birth also in the donkey, but, as in the mare, it is more useful for indicating when foaling is less likely to occur, rather than precisely indicating when it might occur.

Sodium and potassium

The standard deviation of sodium is large because one of the jennies showed values that were much higher than those of the other animals. We decided, however, to keep the data related to this subject because they showed the same trend. These high sodium levels depend on individual characteristics of the jenny and are not attributable to the analytic method used, since the same parameter evaluated in the other donkeys always fell in the same range. We also observed that this same animal reached a lower pre-foaling calcium concentration (day 0) in comparison with the other jennies (16.32 mmol/L vs 25.78 mmol/L of average).

In the mare, starting from 3 days before foaling, sodium levels drop from about 87 mmol/L to 37 mmol/L on the day prior to parturition (Christensen 2011). In the donkey, sodium concentrations decrease more gradually from day −10 to day −2 (Carluccio, De Amicis, et al. 2008). Rook et al. (Rook et al. 1997) indicated that the sharp decrease in sodium levels in the mare can be used as an indicator of imminent parturition, but in the donkey, since the decline is more gradual, this parameter does not seem to be a reliable predictive index (Carluccio, De Amicis, et al. 2008).

In the nine examined jennies, sodium concentrations remained high on the day of delivery, showing a mean value of 62.62 mmol/L (range 37.90–173.00 mmol/L) compared to the average of 19.0 ± 6.12 mmol/L reported by Carluccio et al. in their work (Carluccio, De Amicis, et al. 2008).

In the mare, potassium levels gradually increase up to 30 mmol/L 3 days before foaling, then rapidly rise to 47 mmol/L on the day prior to parturition (Christensen 2011). In the donkey, based on the available data, the trend appears different: there are two moments in which a significant increase in the potassium concentrations can be detected, namely between days −10 and −8 and between day −6 and day −4, without further variations until the time of delivery (Carluccio, De Amicis, et al. 2008).

Comparing the potassium levels in the last 5 days before parturition reported in our work with those of Carluccio et al. (Carluccio, De Amicis, et al. 2008), it can be seen that the values are quite similar. Approaching parturition, potassium concentrations remain constant and for this reason the statistical analysis carried out on the 9 jennies in the last 5 days before foaling did not show significant changes.

A positive correlation between potassium concentrations and day of sampling has been observed, this means that, in contrast to what is described in the literature, potassium levels undergo a slight decrease as the days preceding parturition decrease (Table 1).

In the mare, when evaluated together, sodium and potassium offer valuable information about the approach of foaling, and more precise than that provided by calcium levels alone. The inversion of the sodium/potassium ratio seemed to occur from 9 to 1 day before delivery, on average 3 days before (Ousey et al. 1984). However, more recent studies have shown that parturition, in the mare, takes place 24–36 h after the inversion of these two electrolyte concentrations (Bennett 1990; Rook et al. 1997). Therefore, that seems to be the best method to predict delivery. In the study by Carluccio et al. (Carluccio, De Amicis, et al. 2008) on 17 Martina Franca jennies, the Na^+/K^+ ratio reversed 48–24 h before foaling, appearing as the best indicator for impending parturition. Probably, a higher number of animals enrolled in our study would have led to greater significance. It should also be emphasised that the sodium and potassium trend is different from that reported in the literature; therefore, it is reasonable that also the ratio between the two electrolytes does not fully reflect the expected, based on the knowledge we have on the changes in electrolyte concentrations in the peripartum period in the donkey.

In the mare, it is reported that the electrolyte trend is not reliable in pathological pregnancies. In these cases, an early rise in calcium levels is generally observed. If this increase occurs before 310 days of pregnancy, it does not indicate foetal maturity but, rather, a placental abnormality (Ley 2011). In this case, the evaluation of other electrolytes could help in the diagnosis: if calcium levels are greater than 400 ppm, but sodium is still higher than potassium, there is a high probability of placentitis or twin pregnancy (Paccamonti 2001).

During the periodic clinical examinations of the jennies, we observed an abnormal ultrasound appearance of the placenta, compared to the mare. In horses, indeed, a thickened placenta with oedema is attributable to placentitis, but all the jennies in this study carried the pregnancy to term and gave birth to live and viable foals, without any therapeutic support and without evidence of placental lesions after the passage of the foetal membranes. It would be interesting to understand if this peculiar placental conformation could have somehow influenced the trend of the sodium and potassium concentrations in the mammary secretion. In fact, none of the examined jennies showed the inversion of the Na^+/K^+ ratio, as it has been hypothesised to occur in mares affected by placentitis.

pH

Few references can be found in the literature regarding the pH trend in the mare and jenny's mammary secretion during the days preceding foaling. It is, in fact, a recent discovery that pH decreases as parturition approaches and thus, that this information may be used to predict delivery, in the place of or in addition to the measurement of the electrolytes. A relationship between pH and calcium has been reported by Canisso et al. (Canisso et al. 2013), but the mechanism that leads to pH decrease in the days prior to parturition is still unknown. However, the pH drop is not regarded as an index of foetal maturity, as for calcium, but rather it is thought to be linked to other events that occur in the peripartum period (Canisso et al. 2013). According to this author (Canisso et al. 2013), the pH of mammary secretions decreases during the 4 days preceding delivery and, in particular, it significantly declines on the day of delivery, compared to the previous days. The pH, in their research, had been determined using two methods (semiquantitative evaluation with test strips and quantitative technique with pH metre), once a day (in the evening), and proved to be predictive for foaling, with 11 mares out of 14 that gave birth within 24 h of reaching the threshold of 7. Korosue et al. (Korosue et al. 2013) measured instead the pH twice a day, in 27 mares, using a semiquantitative method, and reported a positive predictive value (PPV) for parturition within 72 h of 97.9% and a negative predictive value (NPV) within 24 h of 99.4% applying a standard value set at 6.4. In the jenny, Magalhaes et al. (Magalhaes et al. 2021) obtained PPV and NPV values of 40% and 97%, respectively, with a 90% sensitivity and 70% specificity for foaling within 24 h. In this work, the majority of the animals (65%) foaled with a mean pH of 6.4 ± 0.02.

All the 9 jennies enrolled in our study showed a pH $\leq$ 7 starting from day −4 and the values obtained using both the pH metre and the test strips varied significantly between day −3 and −1.

Considering the values obtained using the pH metre, only in 2 donkeys the birth took place within 24 h of reaching the threshold value of 6.4. The mean pH value on day −1 as well as on day 0 was 6.54 (Table 3). Thus, achieving the threshold of 6.4 could not be regarded as a benchmark for predicting parturition in the subjects of this study. In general, at day −1, a pH lower or equal to 6.7 was recorded, in accordance with the work of Korosue et al. (Korosue et al. 2013) in which the pH of the mammary secretion, measured through test strips, showed a similar trend.

As for the pH values obtained using the test strips, the mean was 6.79 on day −3 and 6.22 on day −2. In this case, the birth took place within 72 h of achieving the pH threshold of 6.5 in 6 out of 9 donkeys, while 2 jennies presented this value already at day −4 and one at day −3.

In the research by Canisso et al. (Canisso et al. 2013) pH test strip with a range of 6.2 to 7.6 were used and the colour change was expected every 0.2 units. On the other hand, the strips used in our study had a very wide range, from 0 to 14, and each unit corresponded to a different colour. Such a large scale, which does not allow a clear distinction of the numerous shades of colour included between 7 and 6, certainly influenced the analysis results, which therefore diverged from those obtained with the pH metre.

In fact, the correlation between the two pH measurement methods proved to be rather weak, with a coefficient equal to 0.537 ($p < 0.01$). On the contrary, in the study by Canisso et al. (Canisso et al. 2013) the two methods were highly correlated ($r = 0.93$).

In any case, although the two methods are not highly correlated and it was not possible to determine an optimal cut-off value for predicting parturition, it can be observed that, considering the test strips (a method more suitable for the field conditions) most of the animals gave birth within 72 h of reaching the pH threshold of 6.5.

Conclusions

At the present time, a perfect test able to predict with certainty the moment of parturition does not exist, neither in the horse nor in the donkey, although the simultaneous use of multiple methods (clinical evaluation and laboratory analyses or field tests on the mammary secretion) can increase the ability to identify a jenny close to parturition. Moreover, on a dairy farm, together with the economic aspects, management implications must be considered. Often these farms have limited personnel, which besides the other work, is required to monitor the donkeys at night in case a delivery occurs, with a high percentage of failure in the possibility of actually seeing it and providing assistance if needed. The use of field tests is currently unable to guarantee the presence of staff at the appropriate time, even if parameters such as pH, measured with test strips, would be a quick, practical, and economical method. However, at the current state of research, it is too early to say whether the drop in pH in the donkey and the mare may indicate impending parturition: further studies on a more significant number of animals are needed to assess if this or other parameters can help predict delivery. Indeed, at present state of knowledge, the only way of ensuring prompt assistance at parturition is based on foaling alert systems. These systems are quite costly, but the expense would be amply justified by the enhancements in reproductive efficiency and postpartum health of foal and dam, with obvious repercussion on the farm economy.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

The project is unanimously approved by the Commission of Ethics and Animal Welfare of the Department of Veterinary Science, University of Turin.

ORCID

Alessia Bertero http://orcid.org/0000-0001-8181-5866

Data availability statement

The data that support the findings of this study are available from the corresponding author, [A.B.], upon reasonable request.

References

Bennett DG. 1990. Confronto tra parto indotto artificialmente e parto spontaneo nella cavalla. Ippologia. 3:11–18.

Canisso IF, Ball BA, Troedsson MH, Silva ES, Davolli GM. 2013. Decreasing pH of mammary gland secretions is associated with parturition and is correlated with electrolyte concentrations in prefoaling mares. Vet Rec. 173(9):218.

Carluccio A, De Amicis I, Panzani S, Tosi U, Faustini M, Veronesi MC. 2008. Electrolytes changes in mammary secretions before foaling in jennies. Reprod Domest Anim. 43(2):162–165.

Carluccio A, Gloria A, Veronesi MC, De Amicis I, Noto F, Contri A. 2015. Factors affecting pregnancy length and phases of parturition in Martina Franca jennies. Theriogenology. 84(4):650–655.

Carluccio A, Panzani S, Tosi U, Riccaboni P, Contri A, Veronesi MC. 2008. Morphological features of the placenta at term in the Martina Franca donkey. Theriogenology. 69(8):918–924.

Christensen BW. 2011. Parturition. In: mcKinnon AOSE, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. Hoboken, New Jersey: Wiley-Blackwell; p. 2268–2276.

Dascanio JJ. 2014. Assessment of mammary gland secretions to predict foaling. In: Dascanio JJ, McCue PM, editors. Equine reproductive procedures. 1st ed. New Jersey: John Wiley & Sons, Inc.; p. 249–252.

Davis E. 2019. Donkey and mule welfare. Vet Clin North Am Equine Pract. 35(3):481–491.

Korosue K. 2013. Testing mammary gland secretions to help predict when a mare will foal. Vet Rec. 173(9):216–217.

Korosue K, Murase H, Sato F, Ishimaru M, Kotoyori Y, Tsujimura K, Nambo Y. 2013. Comparison of pH and refractometry index with calcium concentrations in preparturient mammary gland secretions of mares. javma. 242(2):242–248.

Leadon DP, Jeffcott LB, Rossdale PD. 1984. Mammary secretions in normal spontaneous and induced premature parturition in the mare. Equine Vet J. 16(4):256–259.

Ley WB. 2011. Pre-foaling mammary secretions. In: McKinnon AOSEL, Vaala WE, Varner DD, editor. Equine reproduction. 2nd ed. UK: Blackwell Publishing Ltd.; p. 2733–2737.

Lu KG, Barr BS, Embertson R, Schaer BD. 2006. Dystocia—A true equine emergency. Clin Techniq Equine Prac. 5(2):145–153.

Macpherson ML, Paccamonti DL, et al. 2011. Induction of parturition. In: mcKinnon AO, Squires EL, Vaala WE, editors. Equine reproduction. 2nd ed. UK: Blackwell Publishing Ltd; p. 2262–2267.

Magalhaes HB, Canuto LEF, Canisso IF. 2021. Electrolytes and pH of Mammary Gland Secretions Assessments to Detect Impending Parturition and Associations With Placental and Neonate Features in Donkeys. J Equine Vet Sci. 102:103636.

Mancuso R, Torrisi C, Catone G. 2004. Experiences in the management of the reproductive activity of dairy jennieass. Ippologia. 15:5–9.

McLean AK, Navas Gonzalez FJ. 2018. Can scientists influence donkey welfare? Historical perspective and a contemporary view. J Equine Vet Sci. 65:25–32.

Nagel C, Aurich J, Aurich C. 2020. Prediction of the onset of parturition in horses and cattle. Theriogenology. 150:308–312.

Ousey JC, Dudan F, Rossdale PD. 1984. Preliminary studies of mammary secretions in the mare to assess foetal readiness for birth. Equine Vet J. 16(4):259–263.

Paccamonti D. 2001. Milk electrolytes and induction of parturition. Pferdeheilkunde. 17:616–618.

Peaker M, Rossdale PD, Forsyth IA, Falk M. 1979. Changes in mammary development and composition of secretion during late pregnancy in the mare. J Reprod Fertil Suppl. 27:555–561.

Polidori P, Rapaccetti R, Klimanova Y, Zhang J-J, Santini G, Vincenzetti S. 2022. Nutritional parameters in colostrum of different mammalian species. Beverages. 8(3):54.

Rook JS, Braselton WE, Nachreiner RF, Lloyd JW, Shea ME, Shelle JE, Hitzler PR. 1997. Multi-element assay of mammary secretions and sera from periparturient mares by inductively coupled argon plasma emission spectroscopy. Am J Vet Res. 58(4):376–378.

Veronesi MC, Dall'Ara P, Gloria A, Servida F, Sala E, Robbe D. 2014. IgG, IgA, and lysozyme in Martina Franca donkey jennies and their foals. Theriogenology. 81(6):825–831.