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STANDARD ARTICLE

Luteinizing hormone concentrations in healthy horses and horses with trigeminal-mediated headshaking over an 8-hour period

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Background: Trigeminal-mediated headshaking results from a low threshold for firing of the trigeminal nerve. A seasonal component has been implicated in onset of clinical signs, which occur during the spring and summer months. Geldings are overrepresented in the affected population and hormonal differences as compared to a healthy control population of geldings might contribute to headshaking.

Objective/Hypothesis: To assess concentrations of luteinizing hormone (LH) over an 8-hour period in gelded healthy controls and horses affected with headshaking. Our hypothesis was that geldings with seasonal headshaking would have higher concentrations of LH over an 8-hour period compared to control horses during the summer when affected horses manifested headshaking.

Animals: Twelve geldings (6 controls and 6 affected).

Methods: Prospective controlled trial. Blood samples were drawn every 15 minutes over an 8-hour time period during summer from all horses to measure circulating LH concentrations by using a radioimmunoassay for equine LH. All affected horses were actively affected by headshaking at the time of sample collection.

Results: No statistically significant differences in LH concentrations were found throughout the study period in headshakers as compared to control horses. Time had no significant effect, but a slight decrease in LH concentrations was observed for all horses. The main limitation of the study was the low number of horses.

Conclusions and Clinical Importance: Horses affected with headshaking did not have significant differences in circulating LH during the late summer as compared to control horses.

KEYWORDS

equine, headshaking, luteinizing hormone, trigeminal

1 | INTRODUCTION

Trigeminal-mediated headshaking in horses is characterized by violent head tossing, snorting, rubbing the nose on limbs, and striking at the face.[1–3] These clinical signs are thought to be associated with neuropathic pain and can result in euthanasia in severe cases.[4] Recent studies using somatosensory nerve conduction on the trigeminal nerve in horses with headshaking have confirmed a low threshold for firing (2.5 mA) of the infraorbital nerve in affected horses compared to age-matched healthy control horses.[5,6] Reportedly, alleviation of clinical signs occurs during the winter months and exacerbation of signs occurs during the spring and summer months, highlighting a seasonal effect.[1–3,7,8] Furthermore, horses affected in a seasonal fashion have similar threshold for firing (≥10 mA with most ≥20 mA) to

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

control horses during the off-season.[5] The effects of season on the firing threshold of the trigeminal nerve remain unknown. This seasonally altered threshold in the absence of structural abnormalities of the trigeminal nerve complex supports a functional alteration.[5,6] Season, environmental factors, diet, and hormonal components all have been suspected to play a role in the pathophysiology of trigeminal-mediated headshaking.[1,3,5,6]

A possible cause for the seasonal effect seen in headshaking behavior is the reproductive hormonal changes associated with changes in photoperiod during the spring and summer months, which determines the breeding season.[9] In the spring and summer, the decrease in melatonin secretion allows for gonadotropin releasing hormone to be secreted, which then stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[10] Therefore, LH is increased in the summer months.[11] In mares, LH concentrations peak at ovulation and can remain increased for up to a week.[12] In contrast, LH can be persistently increased after castration in animals.[11,13,14] Castrated horses are more commonly affected (75% of cases) by trigeminal-mediated headshaking compared to intact horses and mares.[3,15–17] Therefore, our objective was to evaluate circulating concentrations of LH in geldings with headshaking as compared to control horses. We hypothesized that geldings with headshaking would have higher concentrations of LH over an 8-hour period compared to control horses during the summer, when affected horses manifested headshaking.

2 | MATERIALS AND METHODS

2.1 | Animals

Twelve geldings were included in the study. Inclusion criteria consisted of 6 horses that were diagnosed with trigeminal-mediated headshaking by exclusion of other identifiable causes of headshaking and 6 control horses that were closely matched for age and breed. At the time of the study, all horses had received all recommended vaccinations and deworming medications. Before entering the study, all affected horses had thorough physical and neurologic examinations performed by a board-certified large animal internist and neurologist followed by a detailed diagnostic evaluation. These horses did not display apparent nuchal or cervical pain based on observation, palpation, and manual manipulation of the neck by the examiner. Diagnostic evaluation included oral, ophthalmic and otoscopic examinations, CBC, serum biochemical profile, skull radiographs, and upper airway endoscopy. Other diagnostic testing such as computed tomography, magnetic resonance imaging, or somatosensory nerve conduction studies was not performed. However, other known causes of headshaking were deemed unlikely in these horses because they displayed headshaking behavior in a seasonal manner and appeared normal between seasons. Furthermore, seasonal allergies also were deemed unlikely because these horses were treated by their owners with corticosteroids and antihistamines before donation to our institution and showed no response to treatment. The 12 geldings enrolled included Quarter Horse breeds (N = 6) and Thoroughbreds (N = 6), ages 5-16 years. There were 4 Quarter Horses and 2 Thoroughbreds in each group (controls and affected). The horses were housed in 5 × 12 foot covered stalls adjacent to each another. All stalls had automatic watering devices and were bedded with shavings and cleaned daily. Horses were fed a hay diet twice daily. The study was approved by our institutional animal care and use protocol.

2.2 | Experimental design

The study was a prospective controlled trial. The horses were housed in their individual stalls for 24 hours before the start of the study for acclimatization. Intravenous catheters were inserted into the left jugular vein and sutured into place under aseptic technique the night before. Blood samples were taken from the catheter in each of the 12 horses every 15 minutes for 8 hours (T0, T15, T30, T45, T60, T75, T90, T105, T120 through T480) to assess circulating LH concentrations. The study was undertaken on all horses on 2 separate days from 7 AM to 5 PM during the end of July (July 24th) and beginning of August (August 5th). July 24th had an ambient temperature of 23.9°C-31°C (75°F-88°F), 30% humidity, and a daylight duration of 14h:22m:43s. August 5th had an ambient temperature of 26.1°C-32.2°C (79°F-90°F), 34% humidity, and a daylight duration of 14h:01m:00s. Because of difficulties in collecting blood samples every 15 minutes for 8 hours simultaneously in 12 horses, headshaking horses were sampled on July 24th and control horses were sampled on August 5th.

2.3 | Sample collection

Blood samples were collected in evacuated tubes containing heparin as anticoagulant. The heparinized blood samples were mixed and immediately placed on ice, centrifuged, and the plasma was harvested and transferred to cryovials and stored at −80°C. All blood samples were processed within 2 hours of collection.

2.4 | Luteinizing hormone quantification

Plasma samples were submitted for quantification of equine LH by radioimmunoassay at Louisiana State University AgCenter (School of Animal Sciences, LSU AgCenter, Baton Rouge, Louisiana).[18] This radioimmunoassay utilizes a highly purified, iodination grade, eLH standard, and measures LH concentration in 200 uL of plasma sample. Intra- and interassay coefficients of variation and sensitivity typically are 6, 9, and 0.2 ng/mL, respectively.

2.5 | Statistical analysis

All data was analyzed using Stata Statistical Software IC, Release 15.1, StataCorp LLC (College Station, Texas). Random effects analysis of variance was used to model the combined effects of breed, status, and time; all variables were categorical. Individual horse was a random effect. The final model contained a 3-way interaction among all variables. *P*-values <.05 were considered statistically significant.

3 | RESULTS

The overall mean and SD of LH concentrations for headshaking horses over 8 hours was 5.84 ± 2.84 ng/mL, whereas the mean and SD of

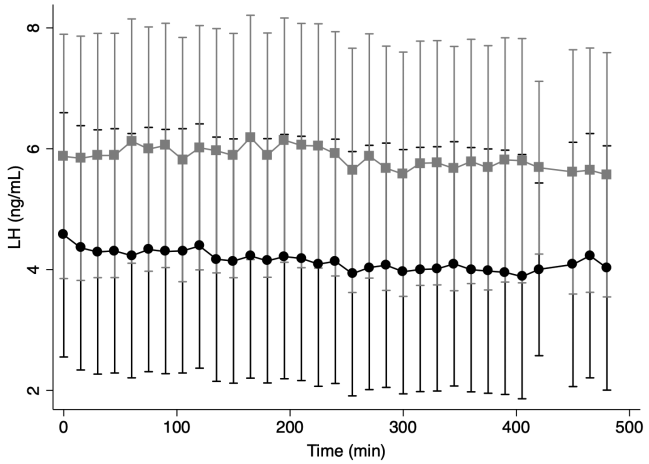

FIGURE 1 Mean circulating luteinizing hormone (LH) concentrations over time between control horses and horses affected with headshaking. Black circles indicate control horses and gray squares indicate headshaking horses

the mean of LH concentration in control horses over the same period was 4.14 ± 2.20 ng/mL (Figure 1). Similarly, over the 8-hour period, no significant difference was found between circulating LH concentrations in controls and affected horses. No effect of breed (Quarter Horse versus Thoroughbreds) or clinical status (headshaker versus control) on LH concentrations was found. An overall (both clinical statuses and breeds) slight decrease in LH concentration was observed over time. One headshaking horse consistently had a much higher LH concentration over the 8-hour study period, with a mean LH concentration of 11.30 ± 0.48 ng/mL. However, if the data from this horse is removed from the analysis, the mean and SD of the mean of the headshaking group was 4.75 ± 1.57 ng/mL.

4 | DISCUSSION

We studied circulating LH concentrations in 6 horses with trigeminal-mediated headshaking compared with 6 control horses over an 8-hour period, and showed that LH did not differ significantly between the 2 groups of horses. Although significant differences in LH concentrations were not found between the 2 groups of horses, we cannot exclude the possibility that LH affects headshaking behavior. Further studies are warranted to better clarify the effects of LH on headshaking.

No significant effect of time was found on LH concentrations over the 8-hour period, but a slight decrease in LH concentrations was observed for all horses. This finding was similar for control horses and headshaking horses. Other studies had similar findings to ours, with geldings having no significant hour-to-hour variations.[19,20] In contrast with other species such as cattle and sheep, LH concentration varies hour to hour.[13,21,22] The hypothesis for the lack of hour-to-hour variation in geldings is the long half-life of equine LH (5 hours), compared to that of sheep, in which the half-life is 43 minutes.[20]

Seasonality plays an important role in exacerbation of clinical signs of headshaking for many horses. The fact that geldings are over-represented raises questions about potential hormonal differences in geldings and stallions.[16] In stallions, photoperiod can drive seasonal changes in LH, FSH, and testosterone.[17,23,24] Plasma LH concentration in stallions is at its maximum in early spring and decreases until it reaches a minimum concentration in late fall.[17,23,24] In 1 study, stallions during summer had a mean LH concentration of 0.52 ± 0.13 ng/mL, which decreased to 0.25 ± 0.08 ng/mL in the winter.[11] Removal of testes leads to loss of testosterone negative feedback, resulting in an increase in LH in many species, including horses.[11,15,25,26] However, over a period of time after castration, geldings LH concentrations return to seasonal variation, although the average LH concentrations remain higher than that of stallions.[23] For instance, 1 study reports the average LH concentration for geldings in the summer as 4.97 ± 0.45 ng/mL and in the winter as 1.44 ± 0.25 ng/mL.[11] In another study, plasma LH concentration gradually decreased from early summer to winter, with a late summer mean of 7.1 ± 1.3 ng/mL.[27] No differences in LH concentrations were found throughout the 8-hour period in affected geldings compared to control geldings in our study. However, the role that LH might play in the pathophysiology of disease cannot be excluded entirely based on the small number of horses, and a larger number of horses might be needed to fully investigate our hypothesis. Furthermore, LH receptors are found in many tissues throughout the body including the trigeminal ganglion, as documented in rats in which LH receptor mRNA transcripts have been found.[28] Studies investigating the presence of LH receptors in the nervous system of horses have not been done. It remains to be determined if LH receptors are present or upregulated in equine nervous tissue, specifically in the trigeminal complex. If so, LH concentrations in horses with headshaking could have effects on neuropathic pain.

The main limitation of our study was the small number of animals. Another limitation was the fact that horses were not evaluated on the same day. However, both experiments were performed during the same season of the year and on days with similar environmental conditions (temperature, daylight duration, and humidity). Headshaking behavior can vary even within the same season, but all horses were confirmed to be headshakers at the time of the experiment.

In conclusion, no significant difference was found in circulating LH between our group of geldings affected with headshaking and control geldings during the summer. The role of other hormones or the presence or upregulation of receptors to different hormones between affected and healthy horses could play a role in the disease and would require further investigation with a larger population.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC of the University of California, Davis. No invasive procedures were done. No harm to any of these animals was done during this study.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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