Evaluation of squeeze-induced somnolence in neonatal foals

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Objective—To test the hypothesis that application of a rope restraint device would result in behavioral, electroencephalographic, and humoral changes consistent with sleep and analgesia in neonatal foals.

Animals—8 healthy neonatal foals.

Procedures—Following acclimatization to experimental conditions, each foal underwent a series of assessments before and during or at the end of a period of restraint via application of a restraint device (soft linen rope). Assessments included measurements of heart and respiratory rates, rectal temperature, and circulating β-endorphin and steroid hormone concentrations and evaluations of mentation and body position (behavior), electroencephalographic patterns, and pain tolerance.

Results—All foals were lively with apparently normal behavior prior to restraint. During application of the restraint device, foals assumed lateral recumbency with relaxed, somnolent behavior. Heart and respiratory rates and rectal temperature uniformly decreased as a result of the procedure. Electroencephalographic recordings (completed for 3 foals only) revealed patterns consistent with slow wave sleep. Plasma ACTH, dehydroepiandrosterone sulfate, and androstenedione concentrations significantly increased during restraint, compared with prerestraint values. The foals’ tolerance to noxious stimuli significantly increased during restraint; however, this was independent of the concentration of circulating β-endorphin.

Conclusions and Clinical Relevance—In neonatal foals, the evaluated form of restraint resulted in a decrease in heart and respiratory rates and rectal temperature. Squeeze-induced somnolence may resemble the effects of compression of the fetus in the birth canal and lead to inhibition of voluntary activity. Use of this technique to safely restrain neonatal foals during minor procedures warrants further evaluation. (Am J Vet Res 2012;73:1881–1889)
not been extensively investigated, to our knowledge. In rabbits, gentle but firm pinching of the skin with padded clips will lead initially to arousal, followed by decreased muscle tone, altered mentation, and the synchronization of EEG activity.10 In cats, rubbing and gentle pinching of a paw will decrease tonic activity in the dorsal column nuclei and somatosensory cortex. In an experiment involving nonhuman primates, baby monkeys introduced to fear stimuli preferred to cling and compress themselves against a soft cloth mother surrogate that provided contact comfort and deep touch (psychological bonding) over a wire surrogate that provided milk.8 Sheep become relaxed when entering a squeeze chute to receive drugs during pharmacological studies. The reactions of cattle to restraint via a strong pressure initially causes them to relax, but will lead to struggling and discomfort as they habituate.9 Infant swaddling, which has been a commonly applied procedure in several cultures during different eras, relies on the compression and restraint created by cloth material wrapped around a child’s body.10 Dr. Temple Grandin, a renowned animal welfare scientist, constructed a squeeze machine based on conventional cattle chutes for autistic individuals with hypersensitivity.11 Physical restriction has been hypothesized to reduce the sympathetic stimulation to the ascending reticular activating system and decrease the level of arousal in human infants.12 Restraint, in the form of physical restriction, of nonhuman animals may also influence the ascending reticular activating system, thereby having a profound effect on consciousness and physical tone. The purpose of the study reported here was to test the hypothesis that application of a restraint device would result in detectable alterations in behavioral, EEG, and humoral variables consistent with sleep and analgesia in neonatal foals.

**Materials and Methods**

**Animals**—Following approval by the University of California-Davis Institutional Animal Care and Use Committee, neonatal foals born at the Center for Equine Health at the University of California-Davis were evaluated for use in the study. The inclusion criteria were as follows: foals had to be born at >330 days of gestation with a normal, uncomplicated delivery and had to be healthy without apparent neurologic deficits (determined on the basis of neurologic and physical examination findings).

**Restraint device and technique**—A soft linen rope (6.1 m [20 feet] in length and 1.27 cm [0.5 inch] in diameter) was used to construct the restraint device. A modified rope squeeze technique, adopted from an earlier publication,13 was applied. The technique has been used in cattle and adult horses for restraint; however, to the authors’ knowledge, the use of this technique in foals had not been previously described. First, a bowline knot was used to secure the rope around the neck and under the shoulder of each foal to prevent tightening of that segment (which could result in pressure on the trachea or jugular veins; Figure 1). Two half-hitch knots were used to loop the rope around the thorax and abdomen 5 to 25 cm (2 to 10 inches) from each other and perpendicular to the vertebral column. The half-hitch knots were positioned directly on the dorsal thoracolumbar area. A designated person (JEM) stood behind the foal and pulled on the rope, which resulted in a generalized squeezing of the foal, as a second person (BT) held the foal and assisted as it lay down. Tension was maintained on the rope (by the person holding the rope) until the experiment was completed.

**Study design overview**—Animals served as their own controls in this study. Prior to application of the restraint device, the 2 individuals performing the restraint procedure spent a period of 15 minutes with the foal to allow acclimatization to experimental conditions. After the period of acclimatization, pre-restraint (baseline) heart rate, respiratory rate, and rectal temperature measurements (determined 3 times every 5 minutes except for rectal temperature, which was assessed once); blood samples; and pain threshold measurements were obtained. An attempt to place the EEG electrodes was made following completion of the aforementioned procedures. The restraint device was then fitted to the foal, and pressure was applied without additional acclimation time. For foals that did not tolerate the placement of electrodes when standing, the EEG electrodes were placed with the foals in lateral recumbency. During restraint, heart rate, respiratory rate, and rectal temperature measurements were obtained at 5-minute intervals (3 times) except for rectal temperature, which was assessed once at 15 minutes following the onset of restraint. After 15 minutes of applied pressure (ie, during the EEG evaluation), blood samples were again collected. Analgesia and pain threshold testing during restraint was performed after the EEG electrodes were removed, and post-restraint testing was performed 5 minutes after removal of the restraint device.

Assessment of physiologic variables and behavior—For each foal, heart and respiratory rates were determined via auscultation with a stethoscope* and recorded before device application and during restraint.

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*Figure 1—Photograph of a foal fitted with a restraint device constructed by use of a soft linen rope. After the rope is positioned in this manner on a standing foal, a squeeze effect is generated by a person who stands behind the foal and pulls on the rope, while another person holds the foal and assists it to lie down. Tension is maintained on the rope for the required period of restraint and SIS.
Three heart rate and respiratory rate measurements, obtained at 5-minute intervals, were recorded during both the baseline and restraint periods; means were calculated for each period. The multiple heart and respiratory rate measurements were performed to obtain more consistent data. Rectal temperature was measured with a digital thermometer once prior to device application and 15 minutes into restraint. Behavior was assessed on the basis of categorical visible changes in mentation and body position (ie, bright and active or sleepy and recumbent).

EEG—Electroencephalographic recordings were performed with a 32-channel telemetry unit, an acquisition system, and a notebook computer that had acquisition, review, and file utility software installed. Stainless steel needle electrodes were placed SC via a modified (reduced number of electrodes) protocol used in foals at the University of California-Davis as described in detail elsewhere. Three central electrodes (left [C3], midline [Cz], and right [C4]), 2 auricular electrodes (left [A1] and right [A2]), and a ground electrode (midline [Z]) were applied (Figure 2). Electrode impedance was determined acceptable at ≤10 kΩ. A transverse bipolar montage was used to review and analyze all EEGs. The settings for EEG recordings were as follows: sensitivity, 70 μV/cm; time constant, 0.1; and high-frequency cutoff, 35 Hz. No sedatives were administered to any of the foals used in this study to avoid alterations of consciousness. Electrode placement was attempted with each foal standing following the completion of other prerestraint measurements but prior to the application of the restraint device. When electrode placement was not possible because of a lack of patient cooperation, the electrodes were placed immediately after the foal attained lateral recumbency with the restraint device on and pressure maintained. In those foals, the EEG recording started within <20 seconds from the onset of restraint and recumbency. The EEG evaluation was performed continuously for a minimum of 15 minutes. The recordings were visually examined to determine state of consciousness by use of previously described criteria for humans and horses.

Analgesia and pain threshold testing—Pain threshold and analgesic effects associated with application of pressure via the restraint device were tested with a square pulse stimulator. For stimulation, two 27-gauge 5/8-inch needle electrodes were positioned so that one was placed 50 mm lateral to the base of the tail and the second was placed 50 mm distal to the first over the semitendinosus muscle. The stimulation rate was 50 pulses/s, and pulse duration was 10 milliseconds each. Voltage range was 0.1 to 20 V with a ramp rate of 0.2 V/s. For test purposes, positive conscious pain responses were defined as purposeful avoidance movements of tail, limbs, trunk, and head and neck or attempts to kick or turn the head toward the site of electrical stimulation at the time the stimulation was applied; at each assessment, response was recorded as a dichotomous variable (yes or no).

For each foal, the experimental session was started at a stimulus intensity of 0.1 V, with the anodal electrode positioned distal to the cathodal electrode; if no response was elicited, the voltage was gradually increased in increments of 0.2 V/s until a response was observed. At each assessment, response was evaluated by 3 independent observers (NN, BT, and JEM) who were unaware of the voltage being applied. The threshold stimulation was repeated at least 3 times at 2-minute intervals at each of 3 assessments: before restraint, during restraint, and 5 minutes following removal of the restraint device. Measurements obtained at each 3 assessments were averaged.

Blood sample collection, handling, and analysis—For each foal, venipuncture was performed on the
left or the right jugular vein before (following 15 minutes of experimental condition acclimatization [baseline]) and 15 minutes after continuous application of pressure via the restraint device. At each time point, a venous blood sample (2-mL) was collected into a 3-mL syringe containing heparin (1,000 U/mL), which was then closed with a plastic stopper and placed on ice immediately. Venous blood gas analysis was performed with a commercially available blood gas machine, and values of blood pH, Pco2, and Pvo2 were corrected to the foal’s rectal temperature.

Immediately after the first blood sample collection, a second blood sample (20-mL) was collected with a 20-gauge 1-inch needle into a 25-mL plastic syringe, aliquoted to tubes containing no anticoagulant, glass and plastic tubes containing EDTA, and tubes containing heparin; tubes were then placed on ice until the experiments were finished. Samples were centrifuged for 10 minutes at 1,000 X g and stored at –80°C in a freezer until processing. Measurements of plasma ACTH and serum cortisol concentrations were performed with a solid-phase, 2-site sequential chemiluminescent immunoassay as described elsewhere. The technique uses monoclonal and polyclonal antibodies for the capture and detection, respectively, of ACTH and cortisol. Plasma ACTH concentrations were also classified as physiologic (≤9.9 pmol/L) or supraphysiologic (>9.9 pmol/L) for purposes of analysis.

Serum samples were used to determine the concentrations of circulating steroid hormones (nandrolone sulfate; boldenone sulfate; 17β estradiol sulfate; testosterone sulfate; 1,4-androstadien-3,17-one; testosterone glucuronide; 19-norandrostenedione; boldenone; androstenedione; nandrolone; estrone; testosterone; epitestosterone; progesterone; 6α,7α,11α-trihydroxy androstenedione; nandrolone glucuronide; 17β estradiol; 17α estradiol; 17β hydroxy progesterone; 19-norepiandrosterone; DHEA; DHEAS; 17β hydroxy pregnenolone; 5α dihydroandrosterone; 5α estradiol; 17α dihydroprogesterone; 19-norandrostenedione; 5α dihydrotestosterone; 5α dihydroprogesterone; pregnenolone; allopregnalone; pregnanediol; and estrone sulfate) via liquid chromatography–mass spectrometry with online sample extraction by turbulent flow chromatography and detection by standard reference material on a triple quadrupole mass spectrometer. This method has been described in detail elsewhere.

For measurement of plasma β-endorphin concentration, blood samples were placed in tubes containing heparin; tubes also contained bacitracin (2.5 U/mL) and aprotinin (1,000 U/mL) in 0.1M PBS solution (pH 7.0 with 0.01% thimerosal [10,000 kU/mL]). Measurements were performed with β-endorphin radio-immunoassay tests that have been previously validated for use in horses.

**Study design and statistical analysis**—For each individual foal, paired comparisons were made between prerestraint baseline values and values obtained during restraint (heart rate, respiratory rate, rectal temperature, pain threshold, blood parameters) or values obtained after restraint (pain threshold). Statistical analyses were performed with a commercially available program. Distribution of the data was tested with the Kolmogorov-Smirnov normality test. Wilcoxon signed rank tests were used to compare values before and after restraint. A Fisher exact test was used to test the difference for dichotomous categorical variables (male vs female and physiologic vs supraphysiologic concentration of ACTH). Spearman rank correlation analysis was performed to reveal any association between percentile change (ratio of restraint and baseline values) of plasma β-endorphin concentration and percentile change (ratio of restraint and baseline values) of pain threshold level, between plasma ACTH concentration and androstenedione concentration, and between plasma ACTH concentration and DHEAS concentration. Behavior and EEG waveforms were not evaluated statistically. Values of P < 0.05 were considered significant in all analyses.

**Results**

Eight foals were used in the study. There were 4 males and 4 females. The median age of the foals was 2.5 days (range, 1 to 4 days). All 8 foals were Quarter Horses.

**Behavior and physiologic variables**—All 8 foals were lively and active, and their behavior was consid-
erected normal prior to restraint. All foals tolerated the rope well. Somnolence and decreased voluntary activity became apparent in all foals within seconds after the restraint device and pressure were applied. Three foals exhibited what appeared to the authors to be dream-like behavior (eg, unconscious whinnying, rolling, and kicking out with their limbs) for a period of a few seconds at 10 to 12 minutes after initiation of restraint. After pressure was released and the restraint device was removed, all foals stood up immediately, stretched their limbs, and approached their dams. Compared with prerestraint baseline values, foals had significantly ($P = 0.014$ for each variable) lower heart rate, respiratory rate, and rectal temperature during restraint (Table 1).

**EEG findings**—Electroencephalographic recordings were attempted in all 8 foals; however, application of electrodes and recording was only possible in 4 foals because of lack of compliance. The EEG recording from 1 foal was excluded from further analysis because of excessive movement artifacts. Statistical analysis was not performed on the EEG data. One of the foals (foal 1) underwent 2 EEG evaluations; the first EEG recording was obtained during application of pressure via the rope restraint device, and the second EEG recording was obtained during manual restraint. Following the first EEG evaluation, the rope device was removed from the foal, and it was allowed to recover for 4 minutes. The second EEG evaluation subsequently performed under manual restraint was not part of the study, but it was conducted because at the time the foal stood up after being released from the first evaluation, the needle electrodes remained in place and EEG recording continued. The foal was manually restrained (no rope) and manipulated into lateral recumbency. The EEG electrodes were maintained from the first evaluation through completion of the second evaluation. Foals 2 and 3 each underwent 1 EEG evaluation during application of pressure via the rope restraint device. For 2 foals, an EEG recording was obtained during the transition from a standing position to recumbency (Figure 3). All 3 foals displayed sleeping behavior alternating with periods of wakefulness, which manifested on the EEG as sleep spindles, vertex sharp waves, slow waves, and K-complexes (all of which are consistent with slow-wave sleep) and as high-frequency waves (consistent with wakefulness). No REM

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**Figure 3** part D and E appear on the next page

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AJVR, Vol 73, No. 12, December 2012

1885
sleep was evident at the beginning of the recording for any of the 3 foals; for 1 foal, REM sleep (not shown because of movement artifacts during recording) was detected several minutes after the recording started.

Analgesia and pain threshold testing—Prior to placement of the restraint device, the median pain threshold was 3.11 V (range, 1.93 to 5.5 V) at rest; during the restraint, it increased to 3.85 V (range, 2.83 to 12.6 V). After the experiment, baseline threshold values were retaken, and they decreased to 2.35 V (range, 1.2 to 3.9 V). Results indicated that all foals had an increased pain threshold during restraint, compared with mean prerestraint baseline and postrestraint pain threshold levels ($P = 0.028$). The median baseline and postrestraint values were not significantly ($P = 0.295$) different. There was no significant linear correlation between the percentile increase in pain threshold levels and the percentile increase in serum $\beta$-endorphin concentration during restraint ($P = 0.345$).

Venous blood gas analysis—Due to availability of equipment, venous gas analyses were performed in only 7 foals. During restraint, median venous blood pH did not differ significantly ($P = 0.830$) from prerestraint baseline values (Table 1). Median values of $P_{\text{CO}_2}$ and $P_{\text{O}_2}$ determined during restraint did not differ significantly ($P = 0.060$ and $P = 0.400$, respectively) from prerestraint baseline values.

Plasma ACTH concentration—Median plasma concentration of ACTH in the 8 foals was significantly ($P = 0.014$) increased during restraint, compared with the prerestraint baseline value (Table 1). All 8 foals had physiologic concentrations of ACTH ($\leq 9.9$ pmol/L) prior to restraint; during restraint, 5 of the 8 foals had supraphysiologic concentrations ($P = 0.030$).

Serum cortisol concentration—Serum cortisol concentration increased during restraint in most foals, compared with findings before restraint (Table 1). However, the median concentration during restraint was not significantly ($P = 0.080$) different from the prerestraint baseline value.

Plasma $\beta$-endorphin concentration—Because of availability of the assay, measurements were performed in only 6 foals. Median serum $\beta$-endorphin concentration was significantly ($P = 0.036$) increased during restraint, compared with the prerestraint baseline value (Table 1).

Serum concentrations of steroid hormones—Serum concentrations of 34 steroid hormone metabolites were measured, of which 27 were not detectable either before or during restraint. Seven metabolites had measurable serum concentrations before and during restraint (Table 2). Steroid hormone metabolites for which the median concentration was 0 (in the mea-
Cataplexy is also associated with a strong stimulus) is considered a pathological finding in all species.27 Cataplexy during restraint as proposed by Adams and Mayhew.1 Cataplexy (loss of muscle tone, which is often associated with a strong stimulus) is considered a pathological finding in all species.27 Cataplexy is also accompanied by narcolepsy and manifests in sudden onset of REM sleep (rapid eye movements as well as high-frequency waves detected via EEG).28 The appearance of sleep spindles, K-complexes, vertex sharp waves, and slow waves is not consistent with REM sleep.23 Furthermore, this phenomenon is a well-recognized and physiologic event in young foals, and we propose to name it SIS on the basis of its phenotypic characteristics.

It must be noted that low-frequency waves and sleep spindles have been detected in awake neonatal foals.24 However, those foals were also under restraint at the time of EEG recording. Thus, the impact of restraint on EEG characteristics remains unclear because neither those investigators24 nor our research group obtained sufficient EEG data without restraint in neonatal foals. Unfortunately, the EEG data obtained in our study was limited by the low number of foals used in the investigation and the inability to obtain EEG recordings for all foals as restraint was applied and recumbency was induced. Furthermore, to our knowledge, EEG patterns in awake, unsedated, unrestrained neonatal foals have not been described to date. Therefore, our findings are not sufficient to draw conclusions regarding the relationship between SIS and brain wave activity.

Although foals in the present study had a significant increase in pain thresholds during restraint, it was unlikely that there was sufficient analgesia to allow surgical procedures causing moderate or severe pain to be performed. An investigation16 in which responses to noxious electrical stimuli were assessed in horses receiving opioids via epidural administration revealed threshold responses > 40 V, in contrast to threshold values < 13 V in foals in the present study. It is unknown whether the analgesia induced by use of the rope restraint device would be sufficient to allow the foals to undergo minor procedures. From data obtained from 6 of the 8 study foals, it was apparent that median 

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<th>P value†</th>
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<td>17-OH pregnenolone (ng/mL)</td>
<td>32 (5.5–92.7)</td>
<td>31.40 (5.95–94.8)</td>
<td>0.620</td>
</tr>
<tr>
<td>Androstenedione (pg/mL)</td>
<td>76.5 (0–598.2)</td>
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<td>Cortisol (µg/dL)</td>
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<td>DHEAS (pg/mL)</td>
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<td>Epitestosterone (pg/mL)</td>
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*Serum concentrations of 34 steroid hormone metabolites were measured at each time point; however, only 8 metabolites had measurable concentrations prior to and during the restraint period. Steroid hormones for which the median concentration was 0 (in the measured units) before or after restraint were tested statistically only when the other value was not 0.

See Table 1 for remainder of key.

Discussion

In the present study, application of pressure via the described restraint device induced neonatal foals to lie down and remain in lateral recumbency. Physical restraint appears to decrease a foal’s voluntary motor activity; it also triggers somnolence, as observed in all study foals. In 3 of the 8 foals, the physical restraint applied resulted in periods of wakefulness and drowsiness and late-onset slow-wave sleep (delta waves and K-complexes) recorded during EEG evaluation. The latter findings are in agreement with the previous descriptions of slow-wave sleep in humans and horses.25–27 In human infants, slow-wave activity (detected via EEG),26 deceleration in heart rate, and diminished brain metabolism25 are evident when increased pressure is applied by maternal pushing in the second stage of labor (similar to the pressure exerted on the foals of the present study during restraint). However, the methods and design of our study preclude making further comparisons regarding these apparent similarities.

The foals in the present study did not develop cataplexy during restraint as proposed by Adams and Mayhew.1 Cataplexy (loss of muscle tone, which is often associated with a strong stimulus) is considered a pathological finding in all species.27 Cataplexy is also accompanied by narcolepsy and manifests in sudden onset of REM sleep (rapid eye movements as well as high-frequency waves detected via EEG).28 The appearance of sleep spindles, K-complexes, vertex sharp waves, and slow waves is not consistent with REM sleep.23 Furthermore, this phenomenon is a well-recognized and physiologic event in young foals, and we propose to name it SIS on the basis of its phenotypic characteristics.

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are also potent psychotropes and anxiolytics,25 it is unlikely that the detected concentrations of β-endorphin alone could have caused this profound change in consciousness and physical tone when one considers the reported concentrations for normal adult horses.26 It is important to note that reference values for serum β-endorphin concentration have not been established for neonatal foals.

Although activation of the HPA axis and production of cortisol may only take minutes during periods of stress in adult horses,27 significant increases in serum cortisol concentration were not evident in the foals of the present study; however, results of independent studies2,12,13 have indicated that, in neonatal foals, an increase in circulating cortisol concentration in response to cosyntropin (tetracosactrin) administration may not be detected before 30 minutes has elapsed. Additionally, the small sample size in the present study may also preclude making further conclusions regarding the lack of a significant increase in serum cortisol concentration. However, serum concentrations of 2 steroid hormones increased during restraint: 1 of those was DHEAS, a neurosteroid and an agonist of sigma-1 receptors, which has potent neuromodulatory effects.28 The potential role of DHEAS in the development of SIS remains to be determined.

Limitations of the present study include a lack of standardization regarding pressure exerted via the restraint device on the foals. It is possible that differing degrees of compression contributed to the variability of the results. The hugging machine devised by Dr. Temple Grandin delivers pressure of 30 to 80 psi, depending on the age of the user, and squeezes almost the entire lateral body surface.12 Therefore, it is possible that a more profound response from the foals could have been obtained with increasing the pressure or surface area.

Electroencephalographic evaluations were not performed in all foals in the present study because of lack of compliance; however, the characteristics of the 4 EEG recordings obtained from 3 foals were consistent. It should be noted that EEG evaluation was attempted prior to placement of the restraint device but was unsuccessful in most cases; in general, unrestrained and unsedated foals did not tolerate placement of the electrodes, and movement artifact resulted in uninterpretable recordings. In another study,24 disc electrodes were used, and multiple tracings from foals that were restrained in lateral recumbency or a standing position were obtained. The degree of restraint in that study24 was not well described. Given the expense and difficulties associated with placement and maintenance of surface electrodes (eg, achieving appropriate contact; presence of sweat or thick dense hair; and lack of cooperation from unsedated foals), they were not used in our study.

Application of pressure via the rope restraint device used in the present study appeared to activate the HPA axis, decrease heart and respiratory rates, and cause dormancy in neonatal foals. This SIS may be a reflection of complex autonomic responses that persist from the early perinatal period, where both the activation of HPA axis and the quiescence could be essential to the transition from fetus to newborn (by preventing malpositioning during birth). It is possible that this squeeze technique could be used as a method of restraint for performing minor procedures in young foals. Adverse effects attributable to restraint and SIS were not detected in the present study; thus, use of the restraint device could be a safe alternative to mild sedation. Although the EEG data were not definitive, in part because of the small number of foals evaluated, the findings did not support narcolepsy or cataplexy as the mechanism for the observed behavioral response and collapse of the foals upon restraint. Further studies with a larger group of foals are warranted to elucidate additional physiologic and receptor pathways that are involved in SIS.

References


