Received: 22 March 2022 | Accepted: 23 November 2022

DOI: 10.1111/jvim.16604

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

The hypothalamic-pituitary-adrenal axis response to ovine corticotropin-releasing-hormone stimulation tests in healthy and hospitalized foals

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Abstract

Background: The hypothalamic-pituitary-adrenocortical axis (HPAA) response to sepsis can be impaired in critical illness. Corticotropin-releasing hormone (CRH) stimulation test might assess HPAA function in foals.

Objective: To evaluate plasma cortisol, ACTH, arginine vasopressin (AVP), and endogenous CRH (eCRH) response to different doses of ovine CRH (oCRH).

Animals: Healthy (n = 14) and hospitalized (n = 15) foals <7 days of age.

Methods: In this prospective randomized study, oCRH (0.1, 0.3, and 1 μg/kg) was administered intravenously and blood samples were collected before, 15, 30, 60, and 90 minutes after administration of oCRH to determine plasma hormone concentrations. The hormonal response was evaluated as the difference (Delta; μg/dL or pg/mL) or percent change between baseline hormone concentration and each time point after oCRH stimulation.

Results: Cortisol concentrations increased from baseline at 15 minutes with 0.1 and 0.3 μg/kg and at 30 and 60 minutes from baseline with 1 μg/kg oCRH ($P < .05$) in healthy and hospitalized foals. ACTH concentrations increased from baseline at 15 minutes with 0.1 μg/kg and at 30 minutes with 1 μg/kg oCRH ($P < .05$) in hospitalized foals. Delta cortisol 0 – 30, ACTH 0 – 30, and eCRH 0 – 30 was higher for the 1 μg/kg compared with 0.1 μg/kg oCRH in healthy foals ($P < .05$). Delta ACTH 0 – 15 and eCRH 0 – 30 was higher for the 1 μg/kg compared with the lower doses of oCRH in hospitalized foals ($P < .05$).

Conclusions and Clinical Importance: Cortisol, ACTH, and eCRH concentrations increased in response to administration of all doses of oCRH. One microgram per kilogram of oCRH appears to be optimal for the assessment of HPAA in healthy and hospitalized foals.

KEYWORDS
adrenocorticotropic hormone, arginine vasopressin, cortisol, endocrine, sepsis

Abbreviations: ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; eCRH, endogenous corticotropin-releasing hormone; oCRH, ovine corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; RAI, relative adrenal insufficiency.

1 | INTRODUCTION

Sepsis is a leading cause of morbidity and mortality in foals.[1-5] The hypothalamic–pituitary-adrenocortical (HPA) axis is activated in response to sepsis-associated stress leading to a cascade of hormone signaling.[1,2,6,7] Corticotropin-releasing hormone (CRH), released from the hypothalamus, stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pars distalis, and ACTH stimulates secretion of cortisol from the adrenal cortex. Cortisol is directly involved in whole-body metabolism for the provision of nutrients, maintenance of blood pressure, and regulation of immune function.[8-11] Arginine vasopressin (AVP) is another important hormone within the HPA axis.[6,8] AVP also regulates the secretion of ACTH, and in horses, has a central role in the control of short-term fluctuations and secretion, in stressful conditions, (eg, hypoglycemia) of ACTH.[8,12,43]

The HPA axis response can be impaired during critical illness, such as sepsis, and in severe cases can lead to relative adrenal insufficiency (RAI) (also termed critical illness-related corticosteroid insufficiency [CIRCI] or ACTH:cortisol imbalance [ACI]).[5,7,11,13,14] RAI is defined as an inadequate cortisol response for the severity of disease and is associated with decreased survival in humans and equine neonates.[5-7,15-17] Recognition, diagnosis, and timely treatment with corticosteroids are crucial to prevent death in septic foals and people.[14,16,17] The definition and optimal diagnostic test for RAI in critically ill human patients remains controversial despite a large body of literature.[18] In humans and foals, the ACTH stimulation test has been used to evaluate adrenal function and cortisol response.[5,7,16,20] However, this test has limited ability to differentiate pituitary dysfunction from adrenal dysfunction.[5,16] Additionally, the ACTH stimulation test has remained controversial because of its poor diagnostic ability in critically ill people.[19] Therefore, further research is needed to determine a more accurate test for the diagnosis of HPA axis dysfunction.

CRH stimulation tests have been used to evaluate the pituitary and adrenal response in healthy people, horses, and other species using a variety of doses.[8,12,21-24] Information regarding the use of CRH to evaluate the response of the HPA axis in foals is lacking. Understanding the HPA axis response to exogenous CRH in foals and establishing a useful dose is the first step in determining the usefulness of this dynamic test.

The objective of this study was to evaluate the cortisol, ACTH, AVP, and endogenous CRH (eCRH) responses to three different doses of ovine CRH (oCRH) in healthy and hospitalized foals. We hypothesize that cortisol and ACTH will increase and eCRH and AVP will decrease in response to oCRH in a dose-dependent manner.

2 | MATERIALS AND METHODS

2.1 | Animals/inclusion criteria

This prospective clinical study was performed at Iowa State University Veterinary Teaching Hospital during one foaling season between January and July 2020. Fifteen hospitalized foals and 14 healthy foals were included. The hospitalized group consisted of client-owed neonatal foals <7 days old of both sexes and different breeds. Healthy foals <72 hours old of either sex and different breeds maintained at the Iowa State Horse Barn served as controls. Hospitalized foals were presented for the following conditions: sepsis, diarrhea, neonatal maladjustment syndrome, failure transfer of passive immunity, meconium impaction, aspiration pneumonia, and prematurity. Foals were classified as healthy based on physical examination, normal complete blood count (CBC; Advia 212Qi Hematology Analyzer, Seimens Healthineers, Malvern, Pennsylvania), normal serum biochemistry profile (Vitros 4600 Chemistry System, Ortho Clinical Diagnostics), serum immunoglobulin G (DVM Rapid Test II-Multi-Test Analyzer, MAI Animal Health, Elmwood, Wisconsin; IgG) concentrations (>800 mg/dL), normal parturition, and a sepsis score of <4.[25] Foals with a history of receiving glucocorticoids or plasma transfusions before admission were excluded from the study. Survival was defined as survival to discharge. Foals that died or were euthanized because of grave prognosis were defined as nonsurvivors. Foals euthanized because of financial constraints were excluded from the study.

2.2 | Data collection

Clinical history obtained upon presentation included expected foaling date, duration of pregnancy, parity of the mare, maternal illness, observed or assisted parturition, dystocia, passing and appearance of fetal membranes, and administered medications. Clinical data collected from each hospitalized foal included signalment, physical examination findings, CBC, biochemistry profile, plasma fibrinogen concentration, IgG concentrations, and blood culture results. Endocrine measurements included eCRH, AVP, ACTH, and cortisol concentration. The sepsis score for each foal was calculated based on history, physical examination, and laboratory findings. For consistency, the sepsis score was calculated by a single author (KMJ).

This study was approved by the Institutional Animal Care and Use Committee of Iowa State University, the Clinical Research Advisory Committee of the College of Veterinary Medicine and adheres to the principles for the humane treatment of animals in veterinary clinical investigations as stated by the American College of Veterinary Internal Medicine. Owner consent was obtained before inclusion in the study.

2.3 | Study design

A repeated measures design was used to compare plasma eCRH, AVP, ACTH, and serum cortisol response to three different doses of oCRH in healthy and hospitalized foals. The oCRH dose was randomly assigned by alternating each dose from a list. Nine foals received 0.1 μg/kg of oCRH (4 healthy; 5 hospitalized), 10 foals received 0.3 μg/kg of oCRH (5 healthy; 5 hospitalized), and 10 foals received 1 μg/kg of oCRH (5 healthy; 5 hospitalized).

An intravenous catheter was placed in the left or right jugular vein and a blood sample was collected on admission and immediately following collection, foals received a bolus dose of oCRH intravenously. Additional

blood samples were collected through the IV catheter at 15, 30, 60, and 90 minutes after oCRH. During CRH stimulation and blood collection, no treatments were administered to the hospitalized foals. CRH stimulation tests in healthy foals were performed on the farm after a routine newborn foal examination. Blood was placed in plain serum and chilled aprotinin-EDTA tubes. Aprotinin (500 kU/mL of blood) was added to preserve hormone integrity. Serum and plasma were stored at −80°C until analyzed. Blood samples for CBC, serum biochemistry, and IgG concentrations were processed within 1 hour. Blood culture was performed routinely, and microorganisms were identified using standard identification methods.

2.4 | Determination of hormone concentrations

Serum cortisol concentrations were determined using immunoassays previously validated for equine samples according to the manufacturer's instructions (Cortisol Coated Tube Radioimmunoassay Kit, MP Biomedicals, LLC, Solon, Ohio).[26,27] ACTH concentrations were measured with an immunochemiluminometric assay previously validated for horses (ACTH Immulite Kit, Siemens Medical Solutions USA, Tarrytwon, New York).[26] A validated assay is not commercially available for quantification of equine CRH in blood, but a radioimmunoassay (RIA) has been validated to measure human CRH (hCRH). According to amino acid sequence analysis, human and equine CRH are 100% homologous, therefore we used a validated human CRH RIA (Corticotropin Releasing Factor, RIA Kit Assay Protocol, Phoenix Pharmaceuticals, Inc, Burlingame, California) with a working range between 10 and 1280 pg/mL and 1.8% cross-reactivity with ovine CRH estimated by the manufacturer. The CRH intra-assay and inter-assay coefficients of variation (CVs) were <10%. Plasma AVP concentrations were measured using a multispecies enzyme immunoassay (Arg-Vasopressin Enzyme Immunoassay Kit, Arbor Assays, Ann Arbor, Michigan) with a detection limit of 2.11 pg/mL and a working range between 4.09 and 1000 pg/mL. Plasma samples were extracted with the provided extraction solution following the manufacturer's instructions before running the assay. The AVP intra-assay and inter-assay CVs were 8.9% and 8.6%, respectively.

2.5 | Data analysis

Data were tested for normality with the Shapiro-Wilk test and were noted to be normally distributed. Data are presented as mean ± SD. A 2-way (time and dose) repeated measures ANOVA was used to compare cortisol, ACTH, AVP, and eCRH concentrations at baseline and 15, 30, 60, and 90 minutes after each dose of oCRH (0.1, 0.3, and 1 μg/kg). Post hoc comparisons for significant findings were conducted with a Tukey test. Delta cortisol, ACTH, and eCRH concentration was defined as the difference (μg/dL or pg/mL) or percent change (%) between baseline concentrations at 15 (Delta 0-15), 30 (Delta 0-30), and 60 (Delta 0-60) minutes after oCRH stimulation. Differences in delta cortisol, delta ACTH, and delta eCRH were analyzed with 2-way repeated measures ANOVA with a Tukey post hoc test for multiple comparisons.

Peak eCRH, ACTH, and cortisol concentrations were defined as the single highest hormone concentration achieved in each individual foal. Time of peak cortisol, ACTH, and eCRH concentration was the time point at which peak hormone concentration was achieved in each foal for each dose of oCRH. One way ANOVA analysis was used to compare the time of peak eCRH, ACTH, and cortisol concentration between three doses of oCRH. Relationships between categorical variables were analyzed using contingency tables and Fisher's exact test. Statistical analysis was performed using statistical software (SPSS, IBM SPSS Statistics, Armonk, New York and GraphPad Prism, GraphPad Software, San Diego, California).

3 | RESULTS

3.1 | Study sample

A total of 29 foals were included in the study. Fifty-two percent (15/29) were hospitalized and 48% (14/29) were healthy. Thirty-four percent (10/29) were fillies and 66% (19/29) were colts. The mean age for hospitalized foals was 47 hours (±25 hours) and for healthy foals was 41 hours (±15.5 hours). There was no difference in age between the two groups of foals ($P = .88$). Foals were full term based on gestation length (330-345 days) and lack of clinical signs of prematurity. One foal in the study was premature (320 days; born 2 weeks earlier than expected) and did not survive. Breeds represented included Thoroughbred (n = 18), Quarter Horse (n = 4), Paint Horse (n = 3), Standardbred (n = 1), Warmblood (n = 1), Tennessee Walker (n = 1), and Percheron (n = 1). Of the hospitalized foals, 38% (5/13) had positive blood cultures and 62% (8/13) had negative blood cultures. Two of the hospitalized foals did not have blood cultures reported. Of the healthy foals, 29% (4/14) had positive blood cultures and 79% (11/14) had negative blood cultures. There was no difference in the proportion of positive blood culture results between healthy and hospitalized foals ($P = .29$). The mean sepsis score was 1 (±2) and 6 (±5) for healthy and hospitalized foals, respectively ($P = .001$). Survival rate was 100% in the healthy foals and 99% in the hospitalized foals. One of the hospitalized foals was euthanized because of grave prognosis. All foals completed the oCRH stimulation test, and no adverse effects attributed to the test were identified.

3.2 | Cortisol, ACTH, AVP, and eCRH concentrations on admission in healthy and hospitalized foals

Cortisol concentration on admission was 0.7 ± 0.8 μg/dL in healthy and 1 ± 1.4 μg/dL in hospitalized foals ($P = .38$). Baseline ACTH and eCRH concentrations were 29.2 ± 10.4 and 12.76 ± 7 pg/mL in healthy foals, and 39.2 ± 40.6 and 11.4 ± 5.6 pg/mL in hospitalized foals, respectively ($P = .57$ and $P = .23$). AVP concentration on presentation was 14.6 ± 7.7 pg/mL in healthy and 14.3 ± 10.7 pg/mL in hospitalized foals ($P = .72$).

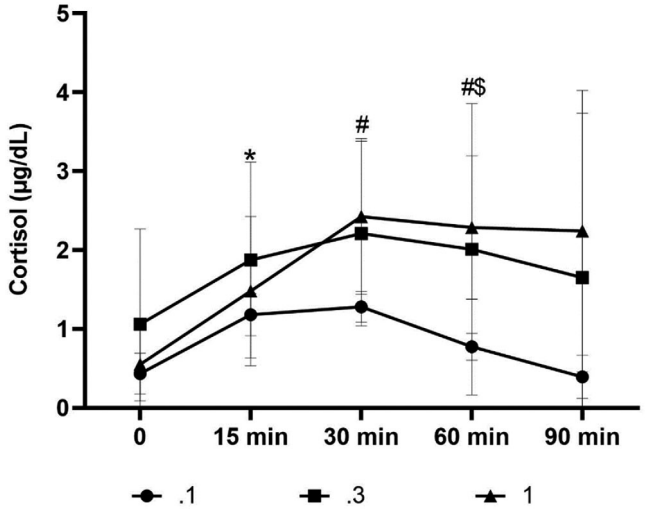

FIGURE 1 Serum cortisol concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 µg/kg of oCRH in healthy foals (n = 14; mean and SD). *P < .05; compared with baseline for 0.1 and 0.3 µg/kg dose of oCRH.#P < .01; compared with baseline for 1 µg dose of oCRH. $$P$ < .05; 0.1 compared with 1 µg/kg dose of oCRH within 60 minutes time point

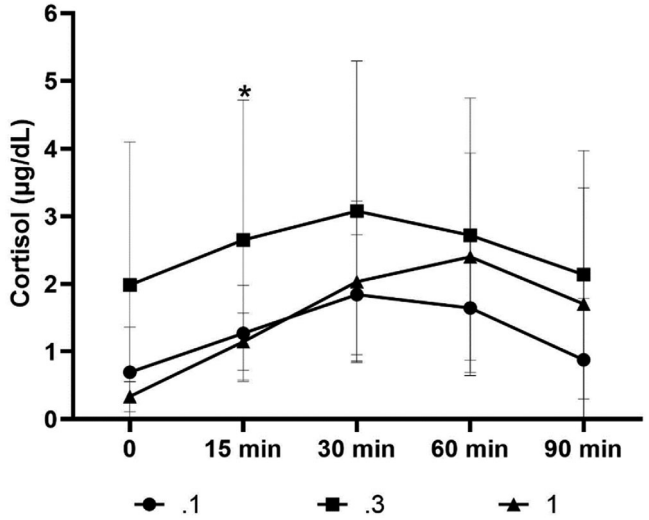

FIGURE 2 Serum cortisol concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 µg/kg of oCRH in hospitalized foals (n = 15; mean and SD). *P < .05; compared with baseline for 0.1 µg/kg dose of oCRH

3.3 | Cortisol concentrations at 0, 15, 30, 60, and 90 minutes after oCRH stimulation in hospitalized and healthy foals

Cortisol concentrations before and after oCRH stimulation in two groups of foals are presented in Figures 1 and 2. There was a significant effect of time on cortisol concentration after administration of 0.1, 0.3, and 1 µg/kg of oCRH in healthy and hospitalized foals (P = .0003). In healthy foals, serum cortisol concentration increased 15 minutes after administration of 0.1 and 0.3 µg/kg of oCRH compared with baseline (P = .04, P = .01). For the 1 µg/kg dose of oCRH, serum cortisol concentration was higher 30 and 60 minutes after stimulation compared with baseline values (P = .007, P = .01). Sixty-minute cortisol concentrations were higher in foals that received 1 µg/kg of oCRH compared with those that received the 0.1 µg/kg oCRH dose. In hospitalized foals, serum cortisol concentration increased 15 minutes after oCRH stimulation with 0.1 µg/kg dose compared with baseline (P = .01). There were no differences noted in cortisol response to the 0.3 and 1 µg/kg doses of oCRH dose in hospitalized foals (P > .05).

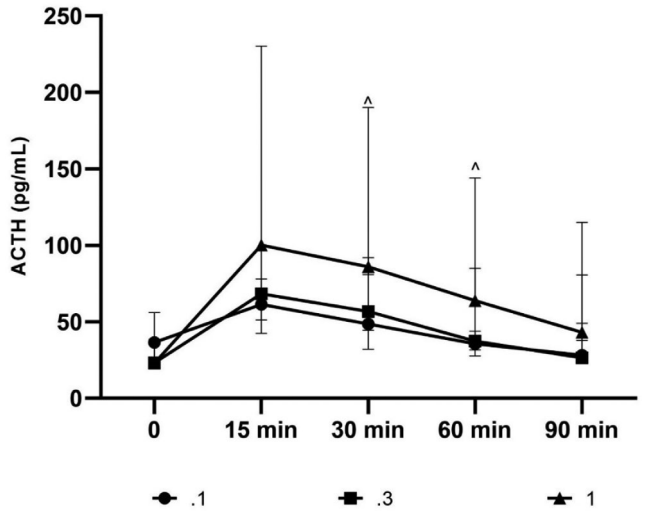

FIGURE 3 Plasma ACTH concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 µg/kg of oCRH in healthy foals (n = 14; mean and SD). ^P < .05; compared with 90 minutes for 1 µg/kg dose of oCRH

3.4 | ACTH concentrations at 0, 15, 30, 60, and 90 minutes after oCRH stimulation in hospitalized and healthy foals

ACTH concentrations before and after oCRH stimulation in two groups of foals are presented in Figures 3 and 4. There was a significant effect of time on ACTH concentration after administration of 0.1, 0.3, and 1 µg/kg of oCRH in healthy and hospitalized foals (P = .001). Compared with 90 minutes, plasma ACTH concentration was higher at 30 and 60 minutes after administration of 1 µg/kg of oCRH in healthy foals (P = .01, P = .0004). There were no differences noted in ACTH response to 0.1 and 0.3 µg/kg dose of oCRH in healthy foals (P > .05). In hospitalized foals, ACTH concentrations increased 15 and 30 minutes from baseline after administration of 0.1 and 1 µg/kg of oCRH, respectively (P = .009, P = .03).

3.5 | Endogenous CRH concentrations at 0, 15, 30, 60, and 90 minutes after oCRH stimulation in hospitalized and healthy foals

Endogenous CRH concentrations before and after oCRH stimulation in two groups of foals are presented in Figures 5 and 6. There was a

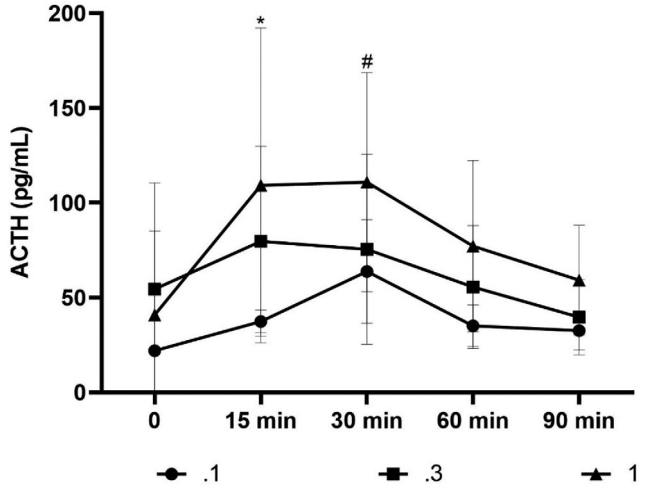

FIGURE 4 Plasma ACTH concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals (n = 15; mean and SD). *P < .05; compared with baseline for 0.1 μg/kg dose of oCRH. #P < .05; compared with baseline for 1 μg/kg dose of oCRH

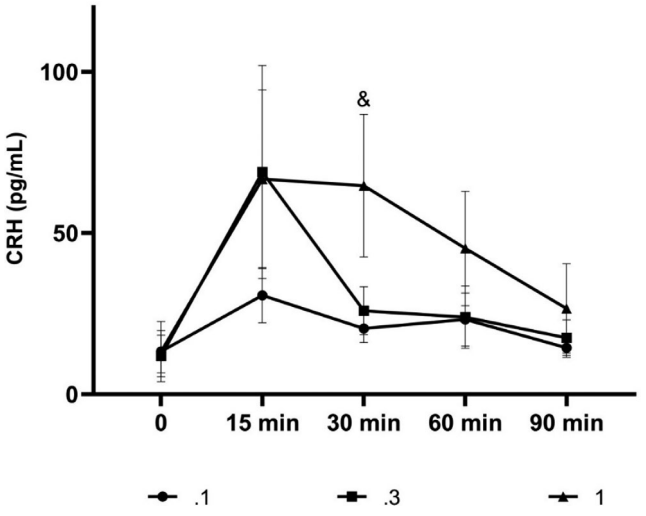

FIGURE 5 Plasma endogenous CRH concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals (n = 14; mean and SD). &P < .05; 1 compared with 0.1 and 0.3 μg/kg of oCRH within the same time point

significant effect of time and dose on eCRH response after administration of 0.1, 0.3, and 1 μg/kg of oCRH in healthy and hospitalized foals (P = .001, P = .005). Thirty-minute eCRH concentration was higher in healthy foals that received 1 μg/kg of oCRH compared with those that received 0.1 and 0.3 μg/kg oCRH dose (P = .02, P = .03). In hospitalized foals, eCRH concentrations increased 15 and 30 minutes from baseline after administration of 1 μg/kg of oCRH (P = .006, P = .02). There was also an increase in eCRH concentrations 15 minutes after administration of 0.1 μg/kg of oCRH (P = .001). eCRH concentrations

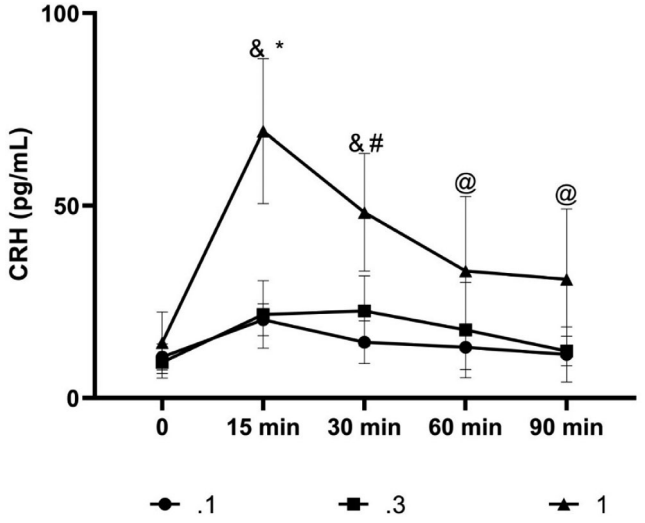

FIGURE 6 Plasma endogenous CRH concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals (n = 15; mean and SD). *P < .01; compared with baseline for 0.1 and 1 μg/kg dose of oCRH. #P < .05; compared with baseline for 1 μg/kg dose of oCRH. @P < .05; compared with 15 minutes for 1 μg/kg dose of oCRH. &P < .05; compared with 0.1 and 0.3 μg/kg dose of oCRH within the same time point

were higher in response to 1 μg/kg of oCRH compared with 0.1 and 0.3 μg/kg at 15 minutes (P = .008, P = .006) and 30 minutes (P = .01, P = .03) time points in hospitalized foals.

3.6 | AVP concentrations at 0, 15, 30, 60 and 90 minutes after oCRH stimulation in hospitalized and healthy foals

AVP concentration before and after oCRH stimulation in two groups of foals are presented in Figures 7 and 8. There was no effect of time or dose on AVP concentration after administration of all three doses of oCRH in healthy and hospitalized foals (P = .8, P = .35). Consequently, delta AVP as well as peak AVP and time of peak AVP were not calculated.

3.7 | Delta blood cortisol, ACTH, and eCRH concentrations after administration of 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals

Delta cortisol, ACTH, and eCRH concentration 15, 30, and 60 minutes after oCRH stimulation is presented in Table 1. For the 1 μg/kg dose of oCRH, delta cortisol 0 to 30 was higher compared with delta cortisol 0 to 15 in healthy foals (P = .006). Delta cortisol 0 to 30 and 0 to 60 for the 1 μg/kg of oCRH was increased compared with 0.1 μg/kg dose of oCRH (P = .03, P = .006). There were no differences detected

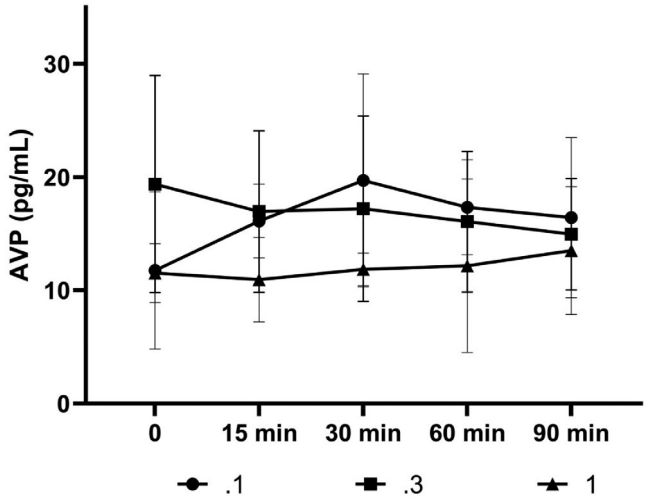

FIGURE 7 Plasma AVP concentrations before (time 0) and 15, 30, 60, and 90 minuts after intravenous administration of 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals (n = 14; mean and SD)

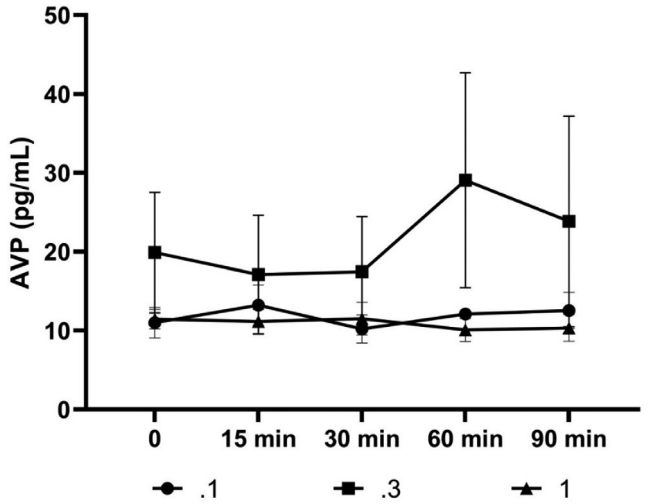

FIGURE 8 Plasma AVP concentrations before (time 0) and 15, 30, 60, and 90 minutes after intravenous administration of 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals (n = 15; mean and SD)

in percent change in cortisol concentration at 15, 30, and 60 minutes after administration of three doses of oCRH. There was also an overall effect of time and dose of oCRH on delta ACTH ($P = .0007$, $P = .01$). For the 0.1 μg/kg dose of oCRH, delta ACTH 0 to 15 and 0 to 30 were higher than delta ACTH 0 to 60 in healthy foals ($P = .04$, $P = .03$). Delta ACTH 0 to 30 and 0 to 60 were higher in 1 μg/kg than 0.1 μg/kg dose of oCRH ($P = .04$, $P = .03$). Percent change in ACTH concentration was higher 15 and 30 minutes after 1 μg/kg of oCRH administration compared with 60 minutes ($P = .04$, $P = .008$). In addition, ACTH response (%) 30 minutes after administration of 1 μg/kg of oCRH was higher compared with 0.1 μg/kg of oCRH ($P = .04$). Percent change in ACTH concentration 15 and 30 minutes after oCRH stimulation with 0.1 μg/kg dose was increased compared with 60 minutes ($P = .03$, $P = .04$). Overall, there were no differences in cortisol and ACTH response to 0.3 μg/kg dose of oCRH compared with 0.1 and 1 μg/kg ($P > .05$). Delta eCRH 0 to 30 was higher in 1 μg/kg compared with 0.1 and 0.3 μg/kg dose of oCRH ($P = .04$). There were no differences detected in percent change in eCRH concentration at 15, 30, and 60 minutes after administration of three doses of oCRH.

3.8 | Delta blood cortisol, ACTH, and eCRH concentrations after administration of 0.1, 0.3, and 1 μg/kg of CRH in hospitalized foals

Change in cortisol, ACTH, and eCRH concentration 15, 30, and 60 minutes after CRH stimulation is presented in Table 1. For the 0.3 μg/kg dose of oCRH, delta cortisol 0 to 30 was higher compared with 0 to 15 in hospitalized foals ($P = .02$). ACTH response (pg/mL) 15 minutes after oCRH stimulation with 1 μg/kg dose was higher compared with 0.1 μg/kg dose at the same time point ($P = .04$). There were no differences in cortisol and ACTH percent change at all three time points after 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals ($P > .05$). Delta eCRH (pg/mL) and percent change 15 ($P = .006$, $P = .04$) and 30 minutes ($P = .01$, $P = .04$) after 1 μg/kg oCRH stimulation was higher compared with 0.1 and 0.3 μg/kg dose of oCRH at the same time point. For the 1 μg/kg dose of oCRH, Delta eCRH 0 to 15 and percent change were higher compared with Delta eCRH 0 to 60 and percent change in hospitalized foals ($P = .008$, $P = .03$). Delta cortisol, ACTH, and eCRH were compared between healthy and hospitalized foals and no statistical differences were found.

3.9 | Peak cortisol, ACTH, and eCRH concentrations, and time to peak cortisol, ACTH, and eCRH concentrations in healthy and hospitalized foals

Peak cortisol, ACTH, and eCRH concentrations and time to peak cortisol, ACTH, and eCRH concentrations in healthy foals are presented in Table 2. Compared with 0.1 and 0.3 μg/kg of oCRH, peak ACTH concentration was higher for the 1 μg/kg dose of oCRH and occurred at 18 ± 6.7 minutes in healthy foals ($P = .04$). Peak eCRH concentration was higher for the 1 μg/kg dose of oCRH compared with 0.1 and 0.3 μg/kg doses of CRH in hospitalized foals ($P = .0001$). The times of peak cortisol, ACTH, and eCRH concentrations, and peak cortisol concentrations were not different between three doses of oCRH in healthy foals. There were no differences detected in the times of peak cortisol, ACTH, and eCRH, and peak cortisol and ACTH concentrations in hospitalized foals (Table 2).

4 | DISCUSSION

The main results of our study demonstrate that cortisol, ACTH, and eCRH concentrations increased in response to all three doses of oCRH in healthy and hospitalized neonatal foals. Dose-dependent

TABLE 1 Delta cortisol, ACTH, and eCRH in response to three doses of oCRH (0.1, 0.3 and 1 μg/kg) in healthy and hospitalized foals (mean and SD)

Cortisol response to 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals				**Cortisol response to 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals**		
oCRH dose	*Delta 0-15 (μg/dL)*	*Delta 0-30 (μg/dL)*	*Delta 0-60 (μg/dL)*	*Delta 0-15 (μg/dL)*	*Delta 0-30 (μg/dL)*	*Delta 0-60 (μg/dL)*
0.1 μg/kg	0.74 ± 0.27	0.84 ± 0.4	0.34 ± 0.33	0.57 ± 0.2	1.15 ± 0.73	0.95 ± 0.72
0.3 μg/kg	0.82 ± 0.28	1.15 ± 1.03	0.95 ± 1.1	0.67 ± 0.56	**1.09 ± 0.55***	0.73 ± 0.5
1 μg/kg	0.92 ± 0.54	**1.87 ± 0.5*#**	**1.73 ± 0.5#**	0.79 ± 0.51	1.68 ± 1.3	2.05 ± 1.6
oCRH dose	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*
0.1 μg/kg	301.6 ± 297	427 ± 555	227 ± 371	182 ± 180	393 ± 457	365 ± 563
0.3 μg/kg	211 ± 265	344 ± 516	290 ± 595	81 ± 88	137 ± 160	82.8 ± 73.5
1 μg/kg	183 ± 105	565 ± 454.5	519 ± 362	433.5 ± 575.5	931 ± 1250	1237 ± 1864
ACTH response to 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals				**ACTH response to 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals**		
oCRH dose	*Delta 0-15 (pg/mL)*	*Delta 0-30 (pg/mL)*	*Delta 0-60 (pg/mL)*	*Delta 0-15 (pg/mL)*	*Delta 0-30 (pg/mL)*	*Delta 0-60 (pg/mL)*
0.1 μg/kg	23.3 ± 14.6	10.7 ± 9.9	**−2.2 ± 12.5*^**	15.5 ± 4.8	41.84 ± 29.5	13.2 ± 11.4
0.3 μg/kg	35 ± 20.8	36.7 ± 31.2	20.7 ± 26	25.2 ± 42.6	21 ± 35.5	1.18 ± 35.4
1 μg/kg	110 ± 66.8	**97.4 ± 51.4#**	**65.7 ± 38.2^#**	**68.2 ± 43#**	70 ± 32.5	36.2 ± 27.3
oCRH dose	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*
0.1 μg/kg	77.8 ± 58.6	40.5 ± 45.8	**3.7 ± 44.7*^**	71 ± 21.5	205 ± 163	62.3 ± 50.78
0.3 μg/kg	153 ± 97	164 ± 135	94.5 ± 113.6	83.4 ± 114	70 ± 105.6	35.4 ± 77.43
1 μg/kg	427 ± 246	**388 ± 209.8#**	**268.7 ± 182.8*^**	236.8 ± 174.5	332 ± 366.6	215.2 ± 313
Endogenous CRH response to 0.1, 0.3, and 1 μg/kg of oCRH in healthy foals				**Endogenous CRH response to 0.1, 0.3, and 1 μg/kg of oCRH in hospitalized foals**		
oCRH dose	*Delta 0-15 (pg/mL)*	*Delta 0-30 (pg/mL)*	*Delta 0-60 (pg/mL)*	*Delta 0-15 (pg/ml)*	*Delta 0-30 (pg/mL)*	*Delta 0-60 (pg/mL)*
0.1 μg/kg	17.43 ± 10.1	7.13 ± 3.7	9.94 ± 9.25	9.7 ± 1.7	3.85 ± 7.36	2.5 ± 8.2
0.3 μg/kg	57 ± 30.8	14 ± 9.25	12 ± 14.6	12.45 ± 11.42	13.4 ± 9	8.4 ± 14
1 μg/kg	53.45 ± 32.7	**51.4 ± 18.04#**	32 ± 18	**55 ± 15.71$**	**34 ± 13.5#**	**18.64 ± 19.5***
oCRH dose	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*	*Delta 0-15 (%)*	*Delta 0-30 (%)*	*Delta 0-60 (%)*
0.1 μg/kg	178.3 ± 143.5	78.3 ± 70.5	117.1 ± 167.2	96.6 ± 26.4	51.8 ± 82.4	43 ± 92
0.3 μg/kg	527 ± 313.2	162.7 ± 106.4	182.2 ± 221	174.4 ± 148	166 ± 120	117 ± 152.5
1 μg/kg	608 ± 493	580 ± 421	330 ± 195	473 ± 255	**281.2 ± 140.8#**	**183 ± 234.1***

*$P < .05$; compared with Delta 0 to 15 within the same dose of oCRH. #$P < .05$; compared with 0.1 μg/kg of CRH within the same Delta.
^$P < .05$; compared with Delta 0 to 30 within the same dose of oCRH.
$$P < .01$; compared with 0.1 or 0.3 μg/kg of CRH within the same Delta.

effects were demonstrated by both the magnitude and prolonged rise in the hormone concentrations (cortisol, ACTH, and eCRH) above baseline with the higher dose of oCRH. These findings agreed with the HPA axis response to CRH stimulation in adult horses and other species.[21-23,28]

Healthy and hospitalized foals in this study had lower and less sustained mean peak cortisol concentrations above baseline compared with healthy adult horses stimulated with 1 μg/kg oCRH.[21] Differences in age and maturity of the adrenal gland between neonatal foals and horses might influence the HPA axis response to oCRH. In neonatal foals, the adrenal gland does not synthesize cortisol until 5 days before parturition and continues to mature within the first week of life.[29-31] During the maturation process, the adrenal gland could have limited glucocorticoid synthetic capacity when stimulated with exogenous CRH in healthy and noncritically ill foals versus adult horses. There are differences in cortisol homeostasis (production rate and clearance) between adult horses and neonatal foals, which could influence the mean peak cortisol concentrations.[32]

Foals in this study had similar mean peak cortisol concentrations compared with calves and pigs and higher mean peak cortisol concentrations compared with sheep.[23,28,33] Although cortisol increased in response to exogenous CRH in all these species, precise comparisons of adrenal responses between species is complex because of many factors. Some of these factors include dose and biologic form of CRH, age of the animal, and cortisol measurement method. There are also species differences in HPA axis perinatal development and function that should be considered; these can affect the pituitary and adrenal response.[30,34]

TABLE 2 Cortisol, ACTH, and eCRH peak and time to peak after oCRH stimulation (0.1, 0.3, and 1 μg/kg) in healthy foals and hospitalized foals (mean SD)

Cortisol in healthy foals			Cortisol in hospitalized foals	
oCRH dose	**Time to peak (min)**	**Peak cortisol (μg/dL)**	**Time to peak (min)**	**Peak cortisol (μg/dL)**
0.1 μg/kg	22.5 ± 8.7	1.3 ± 0.1	39 ± 20.2	1.9 ± 0.8
0.3 μg/kg	42 ± 32.5	2.65 ± 1.7	36 ± 13.4	3.1 ± 2.2
1 μg/kg	54 ± 25.1	2.8 ± 1.5	48 ± 16.4	2.4 ± 1.5
ACTH in healthy foals			**ACTH in hospitalized foals**	
oCRH dose	**Time to peak (min)**	**Peak ACTH (pg/mL)**	**Time to peak (min)**	**Peak ACTH (pg/mL)**
0.1 μg/kg	15 ± 0	61.15 ± 8.2	27 ± 6.7	64.8 ± 25.8
0.3 μg/kg	21 ± 8.2	67.5 ± 22.2	27 ± 35.8	85.1 ± 56.5
1 μg/kg	18 ± 6.7	**139 ± 67.4***	24 ± 8.2	121.7 ± 76.2
CRH in healthy foals			**CRH in hospitalized foals**	
oCRH dose	**Time to peak (min)**	**Peak CRH (pg/mL)**	**Time to peak (min)**	**Peak CRH (pg/mL)**
0.1 μg/kg	37.5 ± 26	33.1 ± 5.8	27 ± 19.6	22.1 ± 2.4
0.3 μg/kg	24 ± 20	70.5 ± 30	27 ± 19.6	25.4 ± 9.2
1 μg/kg	30 ± 18.4	71.2 ± 26.4	18 ± 6.7	**70.7 ± 16.6****

*$P < .05$; Compared with peak ACTH for 0.1 and 0.3 dose of oCRH.**$P < .01$; Compared with peak CRH for 0.1 and 0.3 dose of oCRH.

The mean basal cortisol and ACTH concentrations in healthy and hospitalized foals in this study were comparable to reported values in healthy foals.[9,10,35] The cortisol values obtained from the hospitalized foals would suggest that these foals had an intact HPA axis and were not critically ill. It is also possible that a higher dose than the 1 μg/kg is required to discriminate dysfunction of the HPA axis. The low severity of disease in our hospitalized foals likely explains why there was no difference in mean baseline or delta cortisol, ACTH, and eCRH concentrations between foal groups. In septic foals, ACTH concentrations are reported to be high and cortisol concentrations are high or low; these values are associated with non-survival.[6,15] The majority of our hospitalized foals had low sepsis scores and high survival rates, which supports lack of critical illness or RAI. There was a subset of healthy foals with positive blood cultures. Transient bacteremia in healthy foals occurs and might be one explanation for this finding.[36] Our study reported the percentage of foals with a positive blood culture (29%), which is higher when compared with the previous study (13%).[36] However, that study reported the percentage of positive blood culture samples not positive foals. The percentage of positive blood culture samples would be comparable, if the current study reported the percent based on total blood culture samples. Another possibility is contamination during the blood collection on farm. Although blood was collected using sterile technique, contamination might have occurred from using the IV catheter after placement or break in sterility of the sample syringe.

This study demonstrated that oCRH appears to have a stimulatory effect on the pituitary gland in neonatal foals, which is also found in other species.[12,22,23,28,33,37,38] Time to reach peak ACTH concentrations is similar among different species, and although it is dose-dependent it appears to be between 10 and 20 minutes.[21-24,28,39] In healthy and hospitalized foals, mean peak ACTH concentrations were lower compared with calves; however, bovine CRH (bCRH) was used in calves.[23] Additionally, mean peak ACTH concentrations in our foals stimulated with the 1 μg/kg oCRH were higher than sheep and humans stimulated with the same dose of oCRH.[22,33,38] These findings might suggest the pituitary gland of foals produces and secretes more ACTH when stimulated by oCRH compared with sheep and humans.

CRH and AVP work synergistically to regulate ACTH secretion in humans and animals.[6,8,9,22,23] To further understand the HPA axis response to oCRH in foals, we measured AVP concentrations and found that AVP concentrations did not increase in response to oCRH. Our findings agreed with other studies in humans and horses, where plasma AVP concentrations are unchanged after the administration of oCRH.[37,40] In critical illness, inflammatory cytokines and endotoxin are stimulatory factors for the increase of AVP concentrations in horses.[6] Experimentally administered exogenous CRH appears to be a stimulatory factor for the release of circulating AVP concentrations in healthy dogs and rats.[40] It is possible that in some species (including foals), either oCRH does not stimulate AVP secretion, the dose used in this study was too low to stimulate AVP, or a positive feedback loop occurs but might not be functional at all times and is activated in certain pathologic or physiologic conditions.[8,44]

This study reports eCRH values in foals. A key finding was eCRH concentrations increased above baseline after administration of oCRH. This finding was not expected given cortisol's negative feedback on the pituitary and hypothalamus. The exact reason for the increase in eCRH concentrations is unknown, but there are possible explanations. oCRH might act as a stimulatory hormone causing a feed-forward effect on the hypothalamus to secrete eCRH. Similar

findings were reported in adult horses where plasma CRH levels were increased for over an hour after oCRH was administered.[37] Another potential cause is the reciprocal interactions of the locus ceruleus/norepinephrine (LC/NE)-sympathetic system with CRH, in that norepinephrine stimulates CRH release.[41] However, this is more likely to occur under pathologic stressful conditions (eg, hypoglycemia or hypotension), rather than solely after exogenous oCRH stimulation. Environmental stress of performing the oCRH stimulation could have influenced eCRH concentrations; however, basal CRH concentrations would have been high and decreased over time rather than increased. Additionally, there is minimal influence of venipuncture on HPA axis function.[10] It is proposed that severe stress and large amounts of CRH can break through the feedback inhibition because of high cortisol concentration.[42] Studies in other species revealed much more complex, distributed control network of neuroendocrine HPA factors than what can be explained by simple negative feedback.[42] Finally, although, the hCRH RIA had a low (1.8%) cross-reactivity between ovine and human CRH and final concentrations of eCRH was calculated taking into account cross-reactivity, it is possible that the increase in eCRH observed in this study was because of cross-reactivity with oCRH. Future studies are needed to determine the HPA axis response to oCRH in equine neonates.

An optimum dose for oCRH stimulation in foals has not been established. Based on our data we propose that a 1 μg/kg dose of oCRH appears to be optimal for the assessment of the HPA axis in foals and should be safe. After administration of 1 μg/kg oCRH, there was a significantly greater magnitude of change (absolute or percent delta) in hormone concentrations, and higher mean peak eCRH, ACTH, and cortisol concentrations compared with the lower doses (0.1 and 0.3 μg/kg). These findings agree with studies in people and other species in that 1 μg/kg oCRH was safe, demonstrated ACTH and cortisol responses in a reproducible fashion, and significantly increased ACTH and cortisol concentrations above baseline.[23,38,39]

These are preliminary data on oCRH stimulation tests in neonatal foals, and more studies in a larger sample are needed. Because of the limited numbers of foal, a placebo group was not possible; however, future studies with a placebo group could further our understanding of the effects of oCRH on the HPA axis. oCRH stimulation tests were performed at different times of day. Foals <5 days of age have shown to lack a circadian rhythm, and this was not considered to influence the results.[32] The age variation between the healthy and hospitalized foals might have influenced the cortisol response because of the continued maturation in the HPA axis during the first week of life. However, there was no significant difference in age between the two groups of foals. Additionally, by 24 hours of age, cortisol concentrations are stable.[29,31,35] The hospitalized foals had low severity of disease and conclusions from this study should not be extrapolated to critically ill foals. Furthermore, the HPA axis response to oCRH in premature foals remains to be elucidated.

ACKNOWLEDGMENT

Funding provided by CVM Seed Grant 2019, Iowa State University and the 2020 VCS Research Incentive Grant, Iowa State University.

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 Iowa State University.

HUMAN ETHICS APPROVAL DECLARATION

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

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How to cite this article: Johnson K, Peterson J, Kopper J, Dembek K. The hypothalamic-pituitary-adrenal axis response to ovine corticotropin-releasing-hormone stimulation tests in healthy and hospitalized foals. *J Vet Intern Med*. 2023;37(1):292-301. doi:10.1111/jvim.16604