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Plasma Adrenocorticotropin, Cortisol, and Adrenocorticotropin/Cortisol Ratios in Septic and Normal-Term Foals

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Background: Little information exists on the hypothalamic-pituitary-adrenal axis in septic foals.

Hypothesis: The plasma concentrations of adrenocorticotropin (ACTH) and cortisol are expected to be higher in septic foals as compared to normal foals. The concentrations of hormones in septic foals also are expected to differ further depending upon survival.

Animals: Twenty-eight control foals and 46 septic foals <14 days of age were included in this study.

Methods: Blood was collected in EDTA once from 28 normal foals born in the University of Georgia or Cornell University equine research herds and from 46 septic foals within 12 hours after admission to 1 of the 3 tertiary care referral centers involved in the study. Septic foal selection was based on a sepsis score of >11 or a positive blood culture. The control foals were age matched to the septic foals in the study. ACTH and cortisol concentrations were measured by a chemiluminescent immunoassay system.

Results: Cortisol concentrations in control foals did not vary with age. Septic foals had significantly higher mean ACTH, cortisol, and ACTH/cortisol ratios than did normal foals. Within the septic foal group, 28 foals survived to discharge, and 18 were euthanized or died. The mean age was not significantly different between the septic surviving and nonsurviving foals. The mean ACTH/cortisol ratio was significantly higher in the septic nonsurviving foals as compared to the septic surviving foals.

Conclusions and Clinical Importance: Septic foals had higher hormone concentrations as compared to normal foals, which is an expected endocrine response to critical illness. The increased ACTH/cortisol ratio in nonsurviving septic foals in comparison to surviving septic foals could indicate hypothalamic-pituitary-adrenal axis dysfunction at the level of the adrenal gland in critically ill septic foals.

Key words: Corticosteroids; Equine; Hypothalamic-pituitary-adrenal axis; Neonates; Relative adrenal insufficiency.

The hypothalamic-pituitary-adrenal axis (HPA axis) plays an instrumental role in health and during critical illness by maintaining homeostasis, cardiovascular integrity, and immune and metabolic functions. In periods of stress, secretion of hypothalamic hormones, corticotropin-releasing hormone, and arginine vasopressin[1] (released from the neurohypophysis) stimulate adrenocorticotropin (ACTH) release from the adenohypophysis. ACTH travels via blood to the adrenal cortex to stimulate the production and release of cortisol. In critical illness such as sepsis, inflammatory cytokines, principally interleukins (IL) IL-1β, IL-6, and tumor necrosis factor (TNF)-α initially act upon the HPA axis to increase concentrations of these hormones.[2–6] However, with prolonged or excessive cytokine activity, blunting of the adrenal axis may occur in response especially to TNF-α, which may impair both pituitary and adrenal function.[1–3,5,7,8] This effect is thought to contribute to relative adrenal insufficiency (RAI), which has been identified in septic human patients. RAI is defined as inadequate production of cortisol in relation to increased demand during periods of severe stress.[9] The incidence of RAI in critically ill adult humans appears to be about 40–45% although the exact mechanism remains unclear.[2–7] Most studies have indicated the level of dysfunction in humans to be the adrenal gland but in a study involving septic children 4–7 days of age, ACTH concentrations were less than those of normal age-matched children, suggesting the hypothalamus or the pituitary gland also may be a sites of dysfunction.[10] RAI in septic or highly stressed humans has been diagnosed by either a single cortisol concentration with cortisol values <15 or 25 μg/dL or an ACTH stimulation test with an absolute incremental increase of ≤9 μg/dL 30–60 minutes after a 250-μg dose of corticotropin is given.[2–8] Although controversy about the site of dysfunction and criteria for diagnosis continues, the clinical importance of RAI is exemplified by the fact that refractory hypotension frequently resolves and survival rates improve in septic human patients treated with physiologic doses of corticosteroids.[4–6,8,11] Not every critically ill patient with RAI, however, responds positively to steroid therapy, and some critically ill patients without RAI respond favorably to corticosteroid administration.[6,7,11]

Little information exists on the HPA axis in septic foals. Rossdale et al showed that full-term thoroughbred foals experienced an increase in cortisol concentration between birth and 30 minutes later, which then declined to lower concentrations (4.2 ug/dL) by 6 hours after birth and remained near this concentration for the remainder of the monitoring period (24–60 hours).[12] Silver and Rossdale also showed that premature pony foals have lower cortisol at birth and less response to exogenous ACTH than term pony foals.[13] The term pony foals had a 200% increase in cortisol concentrations after ACTH stimulation. The relative lack of response to ACTH in the premature foals was thought

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to be because of an immature HPA axis.[13] Murray et al evaluated cortisol, gastrin, and somatastatin concentrations in foals from birth to 28 days. The cortisol concentrations increased (2.65–12.65 μg/dL) in the first 2 days of life, but then remained stable thereafter, ranging from 2.16–3.02. μg/dL.[14] These studies showed that full-term foals have functioning HPA axes with enhanced activity during the first hours of life that then stabilizes during the first day of life so that cortisol concentrations are similar to those of normal adult horses.[15–17]

The purpose of this study was to quantify ACTH and cortisol concentrations and ACTH/cortisol ratios in healthy and septic term foals, to determine if there is a difference in hormone concentrations between the 2 groups, and if a correlation exists between these concentrations and survival in septic foals. Because of the lack of response of the HPA axis in premature foals, only term foals were included in the study. The hypotheses were that the serum ACTH and cortisol concentrations and ACTH/cortisol ratios would be greater in septic foals in comparison to normal foals and that the concentrations of these hormones in septic foals would differ depending upon survival.

Methods and Materials

Case Selection

Two groups of foals aged 10 hours to 14 days were studied: healthy term (control) foals at either Cornell University or University of Georgia research farms and septic foals admitted to Cornell Hospital for Animals or Hagyard Equine Medical Institute during the 2004 and 2005 foaling seasons and from the University of Georgia Large Animal Veterinary Teaching Hospital during the 2003 and 2004 foaling seasons. Septic foals were included in the study if they were mature (>330 days gestational age) and had a sepsis score >11[18] or a positive blood culture at the time of admission. A sepsis score was calculated for each foal by the attending veterinarian; immunoglobulin G (IgG) was determined in all foals using Snap Foal IgG.[a] Each septic foal had blood collected into ethylenediaminetetraacetic acid (EDTA) tubes within 12 hours of hospitalization. Blood was centrifuged at 1,200 × *g* at room temperature, plasma transferred to a sterile plastic container (3 mL), and frozen at –80°C until analyzed. Data recorded for the study were gestational and actual age of the foal, breed, presence or absence of a positive sepsis score or positive blood culture, and IgG concentration. No effort was made to compare fibrinogen concentration, glucose concentration, and neutrophil count among the foals because the methodology for these tests differed between hospitals. Foals were considered nonsurvivors if they died or were euthanized during hospitalization. Three foals were euthanized at the owners request because of financial constraints. Owner consent was obtained for patient inclusion in the study.

Control foals (n = 14) were born at Cornell University Equine Research Park during the spring of 2005 and at the University of Georgia in the spring of 2003 and 2004 (n = 14). Foals were included if they were >330-days gestation; more than 10-hours old; had normal vaginal delivery, physical examination, CBC, serum chemistry, and fibrinogen concentration; and an IgG concentration >800 mg/dL (Snap foal IgG).[a] Blood for ACTH and cortisol concentrations was collected into ETDA tubes once between 10 hours and 14 days of age from all control foals to be included in the study. The control foals were selected so that their distribution of age matched that of the septic foals. Blood collection from the control foals was performed at the research parks and not in the hospital. All experimental procedures were approved by the Animal Care Committee of Cornell University and were in accordance with guidelines established by the National Institutes of Health.

ACTH and Cortisol Concentrations

Plasma ACTH and cortisol concentrations were determined using Immunlite, a solid phase chemiluminescent immunoassay system manufactured by Diagnostic Products Corporation.[b] Both immunoassay methods were previously validated for use in horses.[19,20] The mean intra- and interassay coefficients of variation (% CV) for ACTH concentrations were 9.3% and 8.1%, respectively. The detection limit defined as the concentration 2 standard deviations above the response at zero dose was 9 pg/mL.[19]

Cortisol intra-assay precision was determined by testing 3 pooled equine specimens 10 times consecutively each within the same run. The mean CV (% CV) was 4.8% for 3 samples with mean concentrations of 3.80, 4.41, and 5.67 μg/dL. The interassay precision for 2 pools of equine specimens tested 28 times across several months with mean cortisol concentrations of 4.98 and 9.65 μg/dL was 10.7% and 6.7%. Analytical sensitivity determined similarly to that of ACTH by the manufacturer was 0.2 μg/dL.

Statistical Analysis

All computations were performed with a statistical software program.[c] Means and standard deviations were computed on all data. ACTH, cortisol, and ACTH/cortisol ratios were compared between groups with 2-sample test and analysis of variance (ANOVA) with significance set at $P < .05$. The Pearson rank (r) correlation coefficient was used to define if a relationship existed between the age of control foals and cortisol concentrations.

Results

Twenty-eight control foals were included in the study. The breeds of the control foals consisted of 10 Warmbloods, 2 Thoroughbreds, and 16 Quarter Horses. The age ranges of control and septic foals were from 10 hours to 14 days. The mean age of control foals was 4 ± 3.5 days. The sepsis score of the control foals was 0. The control foals were born at the university research facilities and were not given any prophylactic plasma products or antimicrobials.

Forty-six foals with sepsis scores[18] >11 or a positive blood culture were included in the septic foal group: 28 were survivors, 18 were nonsurvivors. The septic foals all had IgG concentrations <800 mg/dL. Various breeds were represented in the study: 18 Thoroughbred, 9 Quarter Horse, 5 Warmbloods, 4 American Saddlebred horses, 4 Tennessee Walking Horses, 3 Standardbreds, 1 Arabian, 1 Miniature horse, and 1 Paint horse. The mean age of the septic foals was 2.5 ± 2.6 days. The mean sepsis score of all septic foals was 14. The septic foals were treated with antimicrobials, plasma, and fluids. Some of the septic foals required vasopressors including norepinephrine, dopamine, and dobutamine. The foals were considered survivors if they were discharged from the hospital. Most of the nonsurviving foals appeared to have minimal or no response to fluids and vasopressors although monitoring varied considerably among the septic foals. Some of the septic foals died

Table 1. Mean, standard deviation, minimum, and maximum of all measured variables in control, surviving septic foals, and nonsurviving septic foals.

Variable	N	Mean	SD	Min	Max
Control-ACTH (pg/mL)	28	44.16	42.75	9.96	182.00
Cortisol (μg/dL)	28	4.05	3.02	1.12	15.00
ACTH/cortisol ratio	28	16.85	22.77	1.16	110.12
Age (days)	28	4.24	3.55	10 hours	13
Sepsis score	28	0	0	0	0
Survivor-ACTH (pg/mL)	28	237.69	368.20	13.5	1,250.00
Cortisol (μg/dL)	28	10.45	11.46	1.11	50.00
ACTH/cortisol ratio	28	27.26	48.01	2.72	232.00
Age (days)	28	2.4	2.06	10 hours	9
Sepsis score	28	13.6	3.5	11	23
Nonsurvivor-ACTH (pg/mL)	18	495.39	464.59	13.5	1,251.00
Cortisol (μg/dL)	18	13.29	13.20	1.39	45.70
ACTH/cortisol ratio	18	59.00	79.01	2.84	260.30
Age (days)	18	2.7	3.3	12 hours	14
Sepsis score	18	14	3.2	11	20

ACTH, adrenocorticotropin. Normal reference hormone concentrations at Cornell University. Animal Health Diagnostic Endocrinology Laboratory; ACTH, 9–35 pg/mL; cortisol, 2–6 μg/dL.

before therapy. The mean values and standard deviations for ACTH concentration, cortisol concentration, and ACTH/cortisol ratio in control and septic foals (combined surviving and nonsurviving) are presented in Table 1.

All hormone concentrations in the septic foal group were significantly ($P < .05$) different compared to those of the control foals. Comparison of cortisol concentration, ACTH concentration, and ACTH/cortisol ratio among the 3 groups (control, surviving septic, and nonsurviving septic foals) showed all 3 measured variables to be different between control foals and both septic foal groups (Figs 1–3). Only the mean ACTH/cortisol ratio was significantly different (higher in the nonsurvivors than survivors) between the 2 septic foal groups. Twenty-three surviving septic foals and 14 nonsurviving septic foals had plasma cortisol concentrations <15 μg/dL.

Discussion

The extremely high mean concentrations of ACTH and ACTH/cortisol ratios in the septic foals was somewhat surprising and differed from some reports on septicemic human patients with RAI in whom both ACTH and cortisol concentrations were relatively low.[2–6] This difference could be a result of species differences, ages of patients, degrees of illness, duration of illness, and study limitations including single sample interpretation.[9–11,23–25] Comparisons to reports of RAI in humans

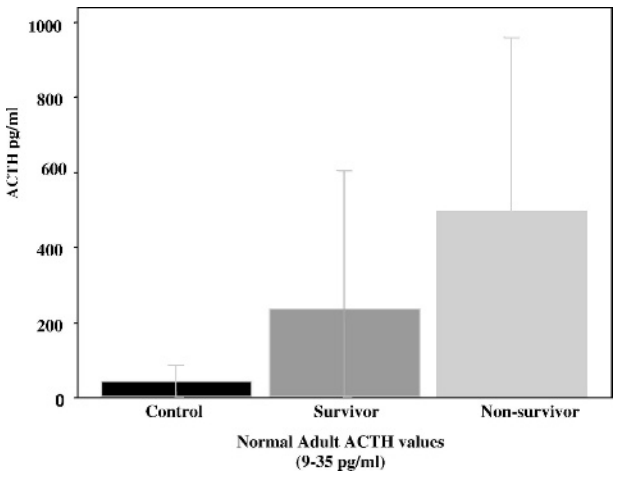

Fig 1. Adrenocorticotropin mean and standard deviation for control, septic surviving, and nonsurviving foals. There was significant difference ($P < .05$) between the control group and both of the 2 septic foal groups. There was no significant difference between the septic foal groups.

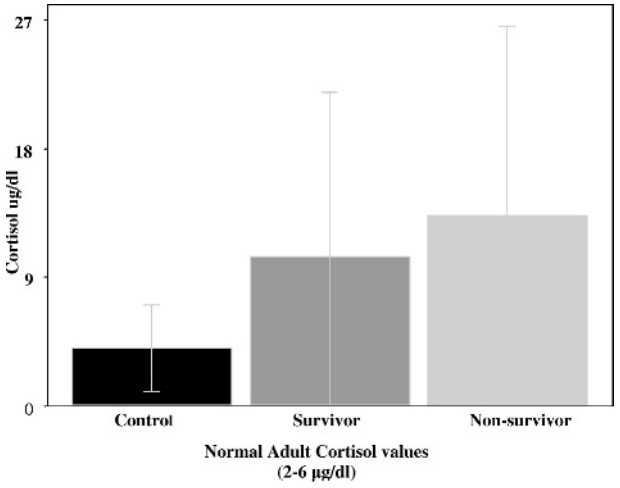

Fig 2. Cortisol mean and standard deviation for control, septic surviving, and septic nonsurviving foals. There was significant difference ($P < .05$) between the control group and both of the 2 septic foal groups. There was no significant difference between the septic foal groups.

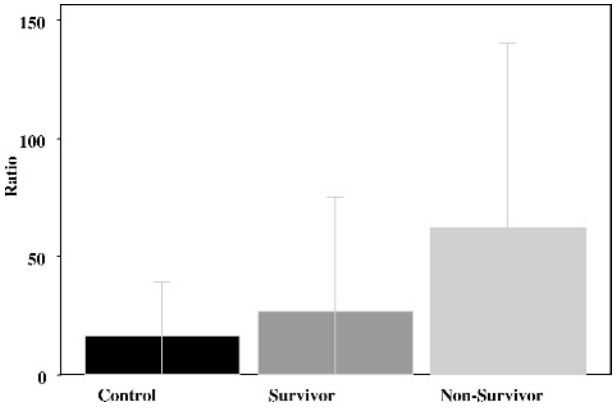

Fig 3. Mean and standard deviation adrenocorticotropin/cortisol ratio for control and septic surviving and nonsurviving foals. Significant differences ($P < .05$) existed between the control group and both septic foal groups and between the septic foal groups.

are further limited because an exact definition, site, and mechanism of dysfunction and even clinical relevance of RAI in humans remain controversial. There are no established and accepted criteria to define RAI in critically ill humans.[8] A study on 57 children of various ages found that the incidence of RAI could be as high as 44% or as low as 9% depending upon which published criteria for diagnosis were used.[9] The terminology of relative versus absolute adrenal insufficiency also is controversial because most studies simply refer to patients who fail either test (baseline cortisol concentration or inadequate response to ACTH) as having RAI, whereas in 1 study baseline cortisol concentrations <20 μg/mL in children with shock was considered absolute adrenal insufficiency and those having baseline cortisol concentrations >20 μg/mL but having <9 ug/mL response to ACTH were considered to have RAI.[9]

The extremely high concentrations of ACTH and significantly different ACTH/cortisol ratios in nonsurviving septic foals versus surviving septic foals suggest that if RAI exists in the equine neonate the dysfunction most likely is at the level of the adrenal gland. Septic human patients are classified with RAI using either a single cortisol concentration or a corticotropin stimulation test.[5–8] Recent publications in critically ill people show a low-dose (1 μg) challenge with ACTH to be more discriminatory in classifying RAI than the 25-μg dose of ACTH.[5,21,22] Critically ill human infants are thought to have some degree of adrenal insufficiency when individual cortisol concentrations are <15 μg/dL.[10,25,26] In most of these studies, basal ACTH concentrations were not obtained.[9,10,25,26] In the study by Soliman et al, however, basal ACTH concentrations were reported from full term normal, septic, or respiratory distress children 4–7 days of age (which is similar to the age range of these foals), and ACTH concentrations were lower in septic children than in either normal or those in respiratory distress suggesting a suppressive effect of sepsis on the hypothalamic-pituitary axis.[10] Based on this study and the previous report that term foals have normal baseline cortisol and ACTH concentrations by 7 hours after birth, some degree of adrenal insufficiency might exist in foals (>12 hours of age) with marked increases in endogenous ACTH concentrations or evidence of severe sepsis associated with physiologic stress (ie, hypotension but normal or only marginally increased plasma cortisol concentrations). Using the general definition for RAI in human medicine (ie, inadequate production of cortisol in relation to increased demand during periods of severe stress[8]), these foals could be considered to have RAI. Exogenous ACTH administration may not be needed to diagnose RAI in septic and severely ill foals when the endogenous plasma ACTH concentration is abnormally high and plasma cortisol concentration remains within normal range. On the other hand, critically ill foals with only modest increases in plasma cortisol concentrations (8–15 μg/dL as occurred in 22% of septic foals in this study) despite increased endogenous ACTH concentration would likely require exogenous ACTH testing to rule out RAI. These modest increases in cortisol concentrations, despite marked increases in endogenous ACTH concentrations, could be normal for the foal whose adrenal gland may not respond to ACTH with the same magnitude as does the human adrenal gland. In fact, this study suggests that the use of the most common cortisol cut-point (15 μg/dL) for diagnosing RAI in humans would likely be inappropriate in septic foals or else 35 out of 48 septic foals would have been classified as having RAI. Absolute adrenal insufficiency should be considered if cortisol concentrations are below the normal range in septic foals.[28] A much larger septic foal study that monitors the hormone concentrations, degree of illness, and adrenal response to exogenous ACTH would be needed before a specific cortisol concentration or ACTH/cortisol ratio can be used to determine if RAI exists in septic foals. Although an effect of age on cortisol concentration was not found in this group of 10-hours to 14-day-old control foals, age and maturation may affect responsiveness of the HPA axis during sepsis. Although white blood cell counts and fibrinogen concentrations were measured in all of the foals in this study to determine sepsis score, there was no attempt to correlate these results with the degree of illness because normal values varied between hospitals and there was no available evidence suggesting that these variables would correlate with the degree of hypotension or severity of sepsis.

The current recommendation for septic human patients with RAI is to treat with physiologic doses of corticosteroids.[2–7] In 1 study involving 59 adult patients in septic shock that received corticosteroid treatment, 37% were steroid-responsive and 95% of the steroid responsive group had RAI (baseline cortisol <25 μg/dL).[27] Infants diagnosed with RAI and treated with cortisone had responses similar to adult human patients with RAI.[22,23,25,26] In a study investigating RAI in septic children, RAI was diagnosed by laboratory testing in 26% of all patients, in 80% of those with catecholamine-resistant shock, and in 0% of children with fluid-responsive shock.[9] Therefore, the diagnosis of RAI

and the use of steroids in septic neonates that are responsive to fluid therapy would seem inappropriate. In this study, no effort was made to determine the response to corticosteroids or other specific treatments (eg, vasopressor drugs), because the goal was only to determine by laboratory testing whether RAI might exist in septic foals.

This study shows that ACTH concentrations are extremely high in most septic foals. Moreover, in some of the septic foals cortisol concentrations remained in the normal range or lower than expected given the magnitude of the ACTH concentrations, particularly in nonsurviving septic foals. This study provides some evidence that RAI may exist in some critically ill and septic foals. Additional research is needed to better define adrenal insufficiency in critically ill neonatal foals using hemodynamic monitoring and responsiveness to corticosteroids when both fluid and vasopressor treatments have failed.

Footnotes

[a] Snap Foal IgG, Idexx, Laboratories, Westbrook ME
[b] Immunolite, Diagnostic Products Corporation (DPC), Los Angeles, CA
[c] Analytical software, Statistix 8, Tallahassee, FL

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