Adrenocorticotropic hormone stimulation tests in healthy foals from birth to 12 weeks of age

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Abstract

The purpose of this study was to investigate total baseline plasma cortisol and adrenocorticotropic hormone (ACTH) concentrations, and ACTH-stimulated cortisol concentrations in foals from birth to 12 wk of age. Plasma (baseline) cortisol and ACTH concentrations were measured in 13 healthy foals at birth and at 1, 2, 3, 4, 5, 7, 10, 14, 21, 28, 42, 56, and 84 d of age. Each foal received cosyntropin (0.1 μg/kg) intravenously. Plasma cortisol concentrations were measured before (baseline), and 30, and 60 min after cosyntropin administration at birth and at 3, 5, 7, 10, 14, 21, 28, 42, 56, and 84 d of age. Compared with baseline, cortisol concentration increased significantly 30 min after administration of cosyntropin on all days. Cortisol concentration was highest at birth, measured at 30 and 60 min after cosyntropin administration, compared with all other days. With the exception of birth measurements, cortisol concentration was significantly higher on day 84, measured at 30 and 60 min after cosyntropin administration, when compared with all other days. Baseline plasma ACTH was lowest at birth when compared with concentrations on days 2, 3, 4, 5, 7, 10, 14, 42, 56, and 84. Administration of 0.1 μg/kg of cosyntropin, IV, reliably induces cortisol secretion in healthy foals. Differences in the magnitude of response to cosyntropin are observed depending on the age of the foal. These data should serve as a reference for the ACTH stimulation test in foals and should be useful in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in healthy and critically ill foals.

Résumé

L'objectif de la présente étude était d'étudier les concentrations du niveau de base du cortisol plasmatique total, de l'hormone adrénocorticotropique (ACTH) et les concentrations de cortisol suite à une stimulation par l'ACTH chez des poulains de la naissance jusqu'à l'âge de 12 sem. Les concentrations de base du cortisol plasmatique et de l'ACTH ont été mesurées chez 13 poulains en santé à la naissance et à 1, 2, 3, 4, 5, 7, 10, 14, 21, 28, 42, 56 et 84 jours d'âge. Les concentrations de cortisol plasmatique ont été mesurées avant (niveau de base), 30 et 60 min après administration de cosyntropine à tous les jours. En comparaison avec le niveau de base, les concentrations de cortisol augmentèrent significativement 30 min après l'administration de cosyntropine à tous les jours. La concentration de cortisol était à son maximum le jour de la naissance, 30 et 60 min après administration de cosyntropine, comparativement à tous les autres jours. À l'exception des mesures prises à la naissance, la concentration de cortisol était supérieure d'une manière significative au jour 84, 30 et 60 min après administration de cosyntropine, lorsque comparée aux autres journées. La valeur de base de l'ACTH plasmatique était à son plus bas au moment de la naissance lorsque comparée aux niveaux aux jours 2, 3, 4, 5, 7, 10, 14, 42, 56, et 84. L'administration de 0,1 μg/kg de cosyntropine par voie intraveineuse, a induit d'une manière fiable la sécrétion de cortisol chez des poulains en santé. Les différences dans l'ampleur de la réponse à la cosyntropine sont observées en fonction de l'âge du poulain. Ces données devraient servir de référence pour les tests de stimulation à l'ACTH chez les poulains et devraient être utiles dans les études subséquentes visant à évaluer l'axe hypothalamus-pitutaire-surrénale chez des poulains en santé et ceux sévèrement malades.

(Traduit par Docteur Serge Messier)

Introduction

Cortisol is the primary corticosteroid secreted in a diurnal pattern from the adrenal cortex of healthy, nonstressed horses and foals (1,2). Secretion of cortisol is under the control of the hypothalamus and anterior pituitary gland via corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), respectively. The central nervous system senses signals from the body, such as tissue injury, pain, hypotension, hypoxemia, and cytokine release, which are relayed to and integrated by the hypothalamus (3). In turn, release of CRH is increased or decreased based on the information received by the hypothalamus. Both CRH and ACTH are subject to negative feedback control by increased concentrations of circulating cortisol. During illness, increases in serum cortisol concentration are believed to be a vital component of the physiologic stress response (4). The effects of cortisol are numerous and necessary for normal function and homeostasis in the body. Cortisol is essential for provision of nutrients to tissues via carbohydrate, protein, and lipid metabolism; regulation of immune function; synthesis and action of catecholamines and adrenergic receptors; cardiac contractility; vascular tone and maintenance of blood pressure; wound healing; endothelial integrity; and various other functions (5,6). Factors, such

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as prolonged transport, colic, laminitis, anesthesia, and surgery, have been associated with increased serum cortisol concentrations in horses (7–10). As well, in times of stress, severe infection, trauma, or illness, secretion of cortisol can increase as much as 6-fold in humans and is roughly proportional to the severity of illness (11–13). Diurnal variation in cortisol secretion is lost during these instances as a result of increased production of CRH and ACTH and a reduction in negative feedback from cortisol (3,13).

Elevations in serum cortisol concentrations have been documented with illness, infection, and stress (13). However, a transient deficiency in circulating cortisol concentrations, characterized by inadequate production of cortisol in relation to increased demand during acute severe illness, has been documented in multiple species and has been termed relative adrenal insufficiency (RAI) (5,6,14,15). Sepsis and systemic inflammatory response syndrome (SIRS) are 2 of the most common causes of RAI in humans (3,5). Further evidence of adrenal dysfunction during severe illness in humans and small animals has been demonstrated by blunted cortisol production in response to exogenous ACTH stimulation testing (6,16,17). This syndrome has been associated with various non-specific clinical features, such as weakness, anorexia, depression, tachycardia, and fever. Hyponatremia, hyperkalemia, hypoglycemia, or any combination of these 3 may also be evident (13). Additionally, RAI is associated with hypotension, hemodynamic instability despite adequate fluid resuscitation, and ongoing inflammation coupled with increased morbidity and mortality in humans with septic shock (3,13).

Some controversy exists over the diagnostic approach to RAI in critically ill humans. Although the ACTH stimulation test remains the most practical diagnostic tool, the most appropriate dose of synthetic ACTH is controversial (18). Historically, a single dose of 250 μg of ACTH (high-dose ACTH test) has been administered intravenously or intramuscularly to evaluate the adrenal cortex response in humans (11,19). However, this dose results in much higher plasma ACTH levels than typically observed in sick patients and may produce misleading results due to this supraphysiologic dose resulting in a false positive cortisol response (4,19,20). A lower ACTH dose (1 μg; low-dose ACTH test) has been advocated as providing a dose that parallels normal physiologic concentrations of ACTH in response to illness or stress in humans (19,20).

Very little information is available in regard to relative adrenal insufficiency in veterinary species. Recent reports have investigated RAI in septic dogs and 1 cat (6,16). As septicemia is commonly documented in foals, one would suspect that RAI may occur in some of these foals (21). However, only 1 report in a septic neonatal foal has documented adrenal insufficiency (14). Because of this lack of information, investigation into the normal physiologic response to the low-dose ACTH stimulation test in healthy foals is necessary to establish a reference for this test at various ages. This, in turn, may help further investigations on the incidence of RAI in critically ill foals in the future. The purpose of the study reported here was to evaluate total plasma cortisol and ACTH concentrations in healthy foals from birth to 12 wk of age. Additionally, a low-dose ACTH stimulation test was utilized to evaluate the adrenal cortex and cortisol response to exogenous ACTH in healthy foals during this time period.

Materials and methods

Animals

Thirteen university-owned foals were included in this study. The foals were considered healthy based on physical examination findings; results of serial complete blood (cell) counts (CBC) evaluated at 12 h and 7, 14, and 30 d of age; serum immunoglobulin concentration of ≥ 800 mg/dL evaluated 24 h after birth; and lack of maternal abnormalities during gestation or parturition. Breeds included Thoroughbred (n = 6), and quarter horse and associated breeds (n = 7); at birth, the foals' weight ranged from 33.2 to 60.9 kg (73 to 134 lb). There were 9 males and 4 females. All foals were full-term and were born and housed in a large box stall for the first 7 d of life. Subsequently, each mare and foal pair was housed outside and brought into a stall prior to blood collection. The study was approved by the University Animal Use and Care Committee of Iowa State University.

Experimental design

The ACTH dosage in this study was based on previously published data in adult horses and a preliminary study involving 8 neonatal foals (2 to 12 d of age) performed by one of the authors (AS) (22,23). In the preliminary study, an intravenous ACTH stimulation test was performed every 2 d on 5 occasions using 1 of 4 dosages (0.02, 0.1, 0.25, and 0.5 μg/kg) of cosyntropin (Cortrosyn; Amphastar Pharmaceuticals, Rancho Cucamonga, California, USA) and saline as a negative control in random order. Blood was collected via a jugular catheter immediately prior (time 0) and 30, 60, 90, 120, 180, and 240 min after cosyntropin administration. Serum cortisol was measured and statistical analysis was performed using analysis of variance (ANOVA) and computer software (SAS, version 8.01; SAS Institute, Cary, North Carolina, USA). Stewart et al (23) showed that the lowest dose to significantly increase serum cortisol concentrations, when compared with saline, was 0.1 μg/kg; this was observed 30 min after administration of cosyntropin.

In this study, a dose of 0.1 μg/kg of cosyntropin was used for all foals. Cosyntropin was acquired as a lyophilized powder containing 250 μg of α1-24 corticotropin per vial (Cortrosyn; Amphastar Pharmaceuticals, Rancho Cucamonga, California, USA). Each vial was reconstituted according to the manufacturer's recommendations resulting in a solution with a concentration of 250 μg/mL of cosyntropin. This was further diluted 50-fold with sterile saline to produce a 5 μg/mL solution of cosyntropin. Individual aliquots of 2 mL of cosyntropin were stored frozen, for less than 1 mo, at -20°C for single use.

At < 24 h of age (range: 6 to 16 h; mean 11 h), each foal's body weight was measured. An individual dose of cosyntropin was thawed at room temperature for each foal immediately prior to blood collection. Each foal was manually restrained and 5 mL of blood was collected by direct venipuncture (baseline) and placed in an evacuated glass ethylenediamine tetra-acetic acid (EDTA) tube. Prior to removal of the venipuncture needle, cosyntropin was administered at a dose of 0.1 μg/kg, IV. Another 5 mL of blood was collected in evacuated glass EDTA tubes 30 and 60 min after administration of cosyntropin; the investigators left the stall between blood collection

Table I. Mean baseline plasma cortisol (ng/mL) concentrations (± 95% confidence interval) at various times between birth and 12 wk of age in 13 healthy foals and mean plasma cortisol (ng/mL) concentrations (± 95% confidence interval) 30 and 60 min after intravenous administration of 0.1 μg/kg cosyntropin. The mean plasma adrenocorticotropic hormone (ACTH) (pg/mL) concentrations (± 95% confidence interval) and the mean ACTH-to-baseline cortisol (ACTH/Cortisol) ratio (± 95% confidence interval) at various times between birth and 12 wk of age in healthy foals is also presented

	Time (day)													
	Birth	1	2	3	4	5	7	10	14	21	28	42	56	84
Baseline cortisol	32.0 (± 9.6)	27.2 (± 7.8)	29.8 (± 6.0)	28.5 (± 7.0)	26.1 (± 4.9)	26.5 (± 3.9)	26.3 (± 5.5)	25.1 (± 5.7)	24.9 (± 6.2)	24.3 (± 4.9)	24.8 (± 6.1)	27.1 (± 6.3)	28.8 (± 5.9)	32.5 (± 5.4)
30-min cortisol	83.6[a] (± 14.2)	N/P	N/P	36.8[a,c] (± 6.4)	N/P	34.9[a,c] (± 6.6)	34.2[a,c] (± 5.1)	32.7[a,c] (± 6.4)	33.7[a,c] (± 6.6)	35.3[a,c] (± 4.5)	36.4[a] (± 4.2)	39.4[a] (± 4.8)	40.9[a] (± 4.7)	47.7[a] (± 4.1)
60-minute cortisol	58.0[b] (± 11.1)	N/P	N/P	22.9[c] (± 4.0)	N/P	24.1[c] (± 3.3)	22.7[c] (± 4.8)	23.6[c] (± 5.5)	22.4[c] (± 4.6)	23.8[c] (± 3.9)	25.0[c] (± 4.2)	27.3[c] (± 3.8)	29.9 (± 3.5)	37.9 (± 4.5)
Baseline ACTH	16.0 (± 7.1)	23.7 (± 5.7)	34.7[d] (± 7.2)	37.9[d] (± 13.5)	35.9[d] (± 7.8)	36.4[d] (± 7.8)	36.1[d] (± 13.1)	26.7[d] (± 4.5)	26.8[d] (± 5.3)	25.0 (± 5.6)	26.0 (± 7.2)	32.7[d] (± 7.4)	33.28[d] (± 9.0)	38.9[d] (± 8.6)
ACTH/ cortisol ratio	0.602 (± 0.25)	0.983 (± 0.27)	1.251[e] (± 0.33)	1.427[e] (± 0.30)	1.441[e] (± 0.33)	1.420[e] (± 0.30)	1.527[e] (± 0.65)	1.238[e] (± 0.31)	1.408[e] (± 0.73)	1.088 (± 0.25)	1.173 (± 0.33)	1.499[e] (± 0.67)	1.239[e] (± 0.37)	1.244[e] (± 0.28)

Cortisol units: 1 ng/mL = 0.1 μg/dL = 2.76 nmol/L.

N/P — Not Performed.

[a] Within the 30-min time period, cortisol concentration differs significantly from baseline (adjusted $P \leq 0.03$) and 60 min (adjusted $P \leq 0.001$) on the same day.

[b] Within the 60-min time period, cortisol concentration differs significantly (adjusted $P < 0.0001$) from baseline cortisol concentration on the same day.

[c] Within a time period, cortisol concentration differs significantly from cortisol response measured on day 84 at 30 min (adjusted $P \leq 0.009$) or 60 min (adjusted $P \leq 0.001$).

[d] Baseline ACTH concentration differs significantly (adjusted $P \leq 0.03$) from ACTH concentration at birth.

[e] ACTH/Cortisol ratio differs significantly (adjusted $P \leq 0.02$) from ratio at birth.

times. Blood samples were immediately refrigerated until all samples were collected. Plasma was obtained within 90 min of collection via low-speed centrifugation (1500 rpm) for 10 min. Collected plasma was subsequently placed in plastic tubes and frozen at -80°C until assays were performed. This procedure was repeated at 3, 5, 7, 10, 14, 21, 28, 42, 56, and 84 d of age. Additional blood was collected in glass EDTA tubes at 1 (24 h), 2, and 4 d of age to determine baseline cortisol and ACTH concentrations at those respective time points. All blood collection and testing occurred between the hours of 8:00 and 10:00 AM and between the months of March and July.

Measurement of blood hormone concentrations

Total plasma cortisol concentrations (hereafter referred to as cortisol concentration) were determined using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California, USA) previously validated for equine cortisol (24). Each sample was analyzed in duplicate and the average between the duplicates at any specific time point was reported. The limit of sensitivity of the assay was 10 ng/mL. Both the intra-assay and inter-assay coefficients of variation were < 5%. All plasma cortisol measurements were expressed as nanograms per milliliter; the conversion to other units may be accomplished as follows: 1 ng/mL = 0.1 μg/dL = 2.76 nmol/L. Plasma ACTH concentrations (hereafter referred to as ACTH concentration) were determined on an automated analyzer by means of an enzyme immunometric assay using chemiluminescent detection (Immulite; Diagnostic Products Corporation) previously validated for use in horses (25,26). The mean reported intra- and inter-assay coefficients of variation were 9.3% and 8.1%, respectively (25,26). The limit of sensitivity of the assay was 9 pg/mL.

Statistical analysis

All computations were performed using a statistical software program (R version 2.4.0; The R Foundation for Statistical Computing, Vienna, Austria). Means and the 95% confidence intervals were calculated for all data. To detect time differences in cortisol concentrations at baseline compared with 30 or 60 min after administration of cosyntropin, the data were analyzed using pairwise *t*-tests. The *t*-tests compared overall differences as well as the percentage change in cortisol concentration between baseline and 30 or 60 min after cosyntroptin administration. To detect specific differences between baseline cortisol concentration and cortisol concentrations 30 and 60 min after cosyntropin administration at specific horse ages, pairwise *t*-test comparisons also were made. Similarly, to detect time differences within baseline ACTH concentrations and the ACTH-to-cortisol ratio across time, data were analyzed using pairwise *t*-tests. Baseline cortisol concentration and cortisol concentrations measured 30 and 60 min after cosyntropin administration were compared using a repeated-measures ANOVA. For all statistical analysis, a *P*-value of

Table II. Mean change in plasma cortisol concentrations, expressed as a change in nanograms from baseline (ng Δ-cortisol) and as a percentage change from baseline (% Δ-cortisol), at 30 or 60 min after administration of 0.1 μg/kg of cosyntropin in 13 healthy foals

	Foal age (day)										
	Birth	3	5	7	10	14	21	28	42	56	84
30 min (ng)	51.6[a] (± 9.8)	8.3[a,b] (± 4.9)	8.5[a,b] (± 2.6)	7.9[a,b] (± 3.7)	7.6[a,b] (± 3.6)	8.8[a,b] (± 4.6)	11.0[a,b] (± 4.6)	11.0[a] (± 4.1)	12.3[a] (± 4.2)	12.1[a] (± 2.7)	15.2[a] (± 4.0)
60 min (ng)	25.9[a] (± 5.0)	-5.6[b] (± 4.4)	-2.4[b] (± 3.1)	-3.6[b] (± 4.1)	-1.4[b] (± 3.8)	-2.4[b] (± 3.5)	-0.5[b] (± 3.7)	0.3[b] (± 3.0)	0.3[b] (± 4.7)	1.2 (± 3.6)	5.5 (± 4.0)
30 min (%)	192.5[a] (± 63.1)	36.7[a,b] (± 23.5)	37.2[a,b] (± 16.9)	37.2[a,b] (± 21.6)	36.1[a,b] (± 16.9)	54.8 (± 40.9)	54.2[a,b] (± 23.4)	71.4[a] (± 44.9)	63[a] (± 38.5)	9.5[a] (± 16.6)	52.5[a] (± 18.7)
60 min (%)	97.5[a] (± 32.4)	-15.7[b] (± 12.2)	-5.5[b] (± 14.6)	-10.4[b] (± 14.6)	-2.3[b] (± 14)	1.4[b] (± 25.2)	3.3[b] (± 18.3)	10.9[b] (± 18.6)	12.6[b] (± 28.8)	10.7 (± 10.7)	20 (± 12.4)

[a] Within a time period, cortisol concentration differs significantly (adjusted $P \leq 0.04$) from baseline cortisol concentration on the same day.

[b] Within a time period, cortisol concentration differs significantly (adjusted $P \leq 0.03$) from cortisol response measured at day 84.

≤ 0.05 was considered significant and all pairwise comparisons used a Bonferroni (all-pairwise) multiple comparison adjustment.

Results

No adverse effects were noted from administration of cosyntropin in any foal at any time. The mean baseline cortisol concentrations over the 12-wk period are presented in Table I. No statistical difference in the mean baseline cortisol concentration between any of the days was observed. There was, however, an overall effect of time after administration of cosyntropin on cortisol concentration ($P < 0.001$). Averaged over all days of the study, the mean baseline cortisol concentration and cortisol concentrations 30 and 60 min after administration of cosyntropin were 27.3, 41.4, and 28.9 ng/mL, respectively. More specifically, there was a statistically significant difference (adjusted $P \leq 0.03$) between baseline cortisol and cortisol measured 30 min after administration of cosyntropin at each respective day reported. Likewise, a significant difference (adjusted $P \leq 0.001$) between cortisol concentrations measured at 30 min when compared with 60 min was noted on all days. The plasma cortisol concentration was always higher at 30 min when compared with the baseline or 60 min cortisol concentration. After adjusting for multiple comparisons, a statistically significant difference (adjusted $P < 0.0001$) between the baseline cortisol and cortisol measured 60 min after administration of cosyntropin was only observed at birth, with a higher cortisol concentration noted at 60 min when compared with baseline. No difference in cortisol concentration was noted between baseline and 60 min on any other day.

Several differences were noted when comparing all of the cortisol responses at 30 min after administration of cosyntropin, over the 3-mo period, utilizing multiple comparison analyses. At birth, the cortisol concentration measured 30 min after administration of cosyntropin was statistically higher (adjusted $P \leq 0.004$) than all other days at this time. Additionally, when evaluating the cortisol response to cosyntropin between days 3 and 84, a significantly higher (adjusted $P \leq 0.009$) cortisol concentration was measured at 30 min on day 84 when compared with this time point on days 3 through 21 (Table I). As well, the cortisol response 60 min after administration of cosyntropin was significantly higher (adjusted $P \leq 0.03$) at birth when compared with all other days at this time. Excluding the cortisol response observed at birth, a significantly higher (adjusted $P \leq 0.001$) cortisol response was measured at 60 min on day 84 when compared to this time point on days 3 through 42 (Table I).

The differences between the cortisol concentrations at 30 or 60 min after administration of cosyntropin compared with the baseline cortisol concentration are presented in Table II. These cortisol concentrations are expressed as a numerical change in nanograms per milliliter from baseline (ng Δ-cortisol) and as a percentage change from baseline (% Δ-cortisol). A significant difference (adjusted $P \leq 0.03$) in ng Δ-cortisol between baseline and 30 min after administration of cosyntropin was present on all days. A significant difference (adjusted $P \leq 0.04$) % Δ-cortisol was observed between baseline and 30 min on all days evaluated except day 14. At 60 min after administration of cosyntropin, only at birth was the cortisol measurement significantly increased (adjusted $P < 0.004$) when compared with baseline for both ng Δ-cortisol and % Δ-cortisol.

Over the entire 3-mo study period, when evaluating for differences (ng Δ-cortisol, % Δ-cortisol) between baseline cortisol concentration and those measured 30 or 60 min after cosyntropin administration, the measurements at birth were significantly higher (adjusted $P \leq 0.03$) than on any other day. Excluding the birth measurements, ng Δ-cortisol was significantly higher (adjusted $P \leq 0.009$) on day 84 when compared with days 3 through 21 at 30 min. Similarly, ng Δ-cortisol was significantly higher (adjusted $P \leq 0.001$) on day 84 when compared with days 3 through 42 at 60 min after administration of cosyntropin over the 3 mo. Expressed as the % Δ-cortisol at 30 min, a significantly higher (adjusted $P \leq 0.03$) percentage change in cortisol was observed on day 84 when compared with days 3, 5, 7, 10, and 21. A significantly higher (adjusted $P \leq 0.008$) % Δ-cortisol also was observed on day 84 when compared with days 3 through 42 at 60 min.

The mean baseline ACTH concentrations and the ACTH/cortisol ratios were calculated over the 12-wk period and are presented in Table I. A statistically lower (adjusted $P \leq 0.03$) mean baseline ACTH concentration and lower (adjusted $P \leq 0.02$) ACTH/cortisol ratio were measured at birth when compared to days 2, 3, 4, 5, 7, 10, 14,

Table III. Mean (± 95% confidence interval) plasma cortisol and adrenocorticotropic hormone (ACTH) concentrations measured in 13 healthy foals at birth, between 3 to 56 d of age and at 84 d of age. Measurements reported include baseline cortisol (ng/mL) concentrations; cortisol (ng/mL) concentrations 30 and 60 min after administration of 0.1 μg/kg cosyntropin, IV; change in cortisol concentrations, expressed as a change in ng/mL from baseline (ng Δ-cortisol) and as percentage change from baseline (% Δ-cortisol), at 30 or 60 min after administration of 0.1 μg/kg of cosyntropin, IV; and baseline ACTH (pg/mL) concentrations and the ACTH-to-baseline cortisol (ACTH/Cortisol) ratio

	Baseline cortisol	30-min cortisol	60-min cortisol	30-minute ng Δ-cortisol	60-min ng Δ-cortisol	30-min % Δ-cortisol	60-min % Δ-cortisol	Baseline ACTH	ACTH/cortisol ratio
Birth	32.0 (± 9.6)	83.6 (± 14.2)	58.0 (± 11.1)	51.6 (± 9.8)	25.9 (± 5.0)	192.5 (± 63.1)	97.5 (± 32.4)	16.0 (± 7.1)	0.602 (± 0.25)
3 to 56 d	26.5 (± 3.5)	36.0 (± 3.7)	24.7 (± 3.0)	9.5 (± 2.1)	-1.9 (± 1.7)	37.7 (± 9.4)	-6.4 (± 5.3)	31.1 (± 4.7)	1.229 (± 0.26)
84 d	32.5 (± 5.4)	47.7 (± 4.1)	38.0 (± 4.5)	15.2 (± 4.0)	5.5 (± 4.0)	52.5 (± 18.7)	20.0 (± 12.4)	38.9 (± 8.6)	1.244 (± 0.28)

42, 56, and 84. No other significant differences were detected in the mean ACTH concentrations or the ACTH/cortisol ratio between any other days using comparison of multiple measures.

Discussion

In the study reported here, the response to exogenously administered ACTH in healthy foals from birth to 3 months of age was examined. The main results of this study suggest that the adrenal cortices of foals demonstrate consistent responsiveness to a low-dose ACTH stimulation test over this period of time, as a significant increase in cortisol concentration between baseline and 30 min after cosyntropin administration was observed at all times. The most consistent elevation in cortisol concentration was measured 30 min after administration of cosyntropin. However, no statistically significant difference in cortisol concentration was noted between baseline and 60 min post-ACTH stimulation test, with the exception of the measurements taken at birth, for the cosyntropin dose used in this study. This finding is similar to trends noted in humans, in which lower mean serum cortisol concentrations were present at 60 min compared with 30 min after a low-dose (1 μg) ACTH stimulation test (18,27). The lack of difference in plasma cortisol concentrations between the baseline and 60-min measurements was likely related to the dosage. Previous reports on humans and foals have demonstrated greater serum cortisol concentrations at 60 min when higher doses (3 to 6 μg/kg) of cosyntropin were used (18,27–29). Furthermore, when a high-dose (250 μg) ACTH stimulation test was performed in 3 to 4-day-old foals, the duration of increased cortisol concentrations was prolonged to 150 min and higher peak cortisol concentrations were observed (29).

The optimum dosage for the ACTH stimulation test to investigate the presence of RAI in foals and horses has not yet been established. However, in septic infant and adult humans, the low-dose (1 μg) ACTH test was more discriminatory in the diagnosis of RAI, when compared with the high-dose (250 μg) test (4,20). Therefore, in critically ill humans, the low-dose ACTH test is believed to be a better diagnostic tool for RAI and may be able to detect early or mild cases of adrenal suppression more accurately than the high-dose ACTH test (30). In a previous report, a significant cortisol response to cosyntropin was not observed in foals after intravenous administration of 1 μg cosyntropin (0.023 to 0.015 μg/kg) (31); however, a consistent increase in serum cortisol was observed in foals after the administration of 0.1 μg/kg of cosyntropin in a preliminary study performed by one of the authors of this study (23). Likewise, a study on adult horses concluded that a dose of 0.1 μg/kg of cosyntropin will provide sufficient stimulation to the adrenal glands to evaluate function (22). Clinically, the information from this study suggests that an intravenous dose of 0.1 μg/kg of cosyntropin is adequate to evaluate the responsiveness of the adrenal cortex in foals. Moreover, this study suggests that measurement of cortisol 30 min post-ACTH administration, compared with baseline, will provide the most diagnostic information.

A previous report investigated different doses used in the ACTH stimulation test in 3 to 4-day-old foals (29). However, considering the dynamic nature of the growing foal, we believed it was important to evaluate the ACTH stimulation test at different ages. The selected ages at which ACTH testing was performed encompassed a broad period of time, including the neonatal period (< 7 d of age) up to 3 mo of age. It was speculated that more frequent measurements were necessary early in the post-parturient period (< 14 d of age) when the most dramatic changes in neonatal adaptation to extrauterine life may occur. Conversely, later time points were evaluated less frequently, as we believed that healthy foals would demonstrate less fluctuation at older ages. The time points at which ACTH testing was performed are similar to other clinicopathologic investigations in healthy foals (31–34).

A significant difference in cortisol response to administration of cosyntropin was observed at birth when compared with later days. The higher cortisol concentrations noted in response to cosyntropin at birth may be related to a heightened sensitivity of the equine adrenal gland to ACTH that occurs in the prepartum fetus, within a few days of birth (35,36). It has been previously demonstrated that equine fetuses that are less than 300 d in gestation and challenged with 1 to 2 μg/kg of ACTH in utero have minimal cortisol response (35). Conversely, after 300 d gestation, the equine fetal response gradually increases with the greatest increase being observed shortly

before birth (35). It is likely that the exaggerated cortisol response noted in foals < 16 h old in this study may be related to a heightened response to endogenous or exogenous ACTH necessary to achieve high levels of cortisol in healthy foals in the immediate pre- and postpartum periods. This finding was also noted in another study in which cortisol response to ACTH on days 2 to 3 was not as great as day 0 (birth) (37).

Excluding the measurements at birth, a greater ng Δ-cortisol and % Δ-cortisol was observed between baseline and 30 or 60 min after administration of cosyntropin on day 84 when compared with earlier time points (Table II). These data suggest that the cortisol response to the ACTH stimulation test is greater on day 84, when compared with younger ages. Retrospectively, it would have been informative to perform ACTH stimulation tests, using a dose of 0.1 μg/kg cosyntropin, in foals between the ages of 56 and 84 days to identify any potential differences in adrenocortical response. Furthermore, testing beyond day 84 would be interesting as it has been noted that the cortisol concentration in adult horses (~9 y old) was approximately 90 ng/mL 25 min after administration of cosyntropin (0.1 μg/kg) (22). This is compared to a mean peak cortisol of 47.7 ng/mL 30 min after administration of cosyntropin to the foals in this study. It is possible that the response to cosyntropin may increase substantially through the first year(s) of life, but further studies are needed to better evaluate this time period. Alternatively, the heightened cortisol response observed on day 84 may have been related to stress of the foal in anticipation of restraint, repeated venipuncture, or other confounding environmental stressors. However, a previous study found that repeated venipuncture in foals undergoing ACTH stimulation testing did not affect their results (29). Additionally, the baseline cortisol concentration on day 84 did not significantly differ from other days in the study presented here.

Another objective of this study was to determine if any variability was present in the response to intravenous cosyntropin in different aged foals. Importantly, the information presented here indicates that healthy foals < 16 h old will respond to exogenously administered ACTH in a heightened fashion, when compared with older foals. In addition, foals between 3 and 56 d of age respond to cosyntropin in a relatively similar fashion, with an approximate mean increase of 7.5 to 12 ng/mL (mean 9.5 ng/mL) in plasma cortisol at 30 min. Furthermore, the information from this study suggests that healthy foals may have an increased response to the ACTH stimulation test at 3 mo of age when compared with earlier ages. Retrospectively, evaluation of the ACTH stimulation test on 2 d old healthy foals would have been informative; however, previous studies have suggested that the cortisol response is exaggerated on day 1 (16 to 29 h old) compared to later ages and is less on day 2 (31 to 48 h old) (37,38). The authors of the study reported here suggest that foals between birth and 3 mo of age can be separated into 3 groups with regard to their cortisol response to 0.1 μg/kg of cosyntropin: foals < 24 h old, foals 3 to 56 d of age, and foals approximately 3 mo of age. The mean values for various parameters involving cortisol and ACTH concentrations for these 3 groups are presented in Table III. Of note, the authors caution the use of % Δ-cortisol when evaluating response to ACTH administration. Specifically, when using % Δ-cortisol, if a high baseline cortisol concentration is measured (such as, 40 ng/mL), a higher numeric increase in ACTH-stimulated cortisol concentration would be anticipated (% Δ-cortisol of 35% predicts cortisol to increase by 14 ng/mL post-ACTH); conversely, if a lower baseline cortisol concentration is measured (such as, 25 ng/mL), a smaller numeric increase in ACTH-stimulated cortisol is predicted (% Δ-cortisol of 35% would predict cortisol to increase by 8.75 ng/mL). Thus, if a foal's baseline cortisol is elevated, the response to ACTH administration may be interpreted as being decreased, even though the absolute change in cortisol concentration may be the same as that measured in a foal with a lower baseline cortisol. Because of this mathematical tenet, results of ACTH stimulation tests utilizing % Δ-cortisol can be misleading.

The baseline cortsiol concentrations noted in this study were consistent with other reported measurements in age-matched foals (1,29). However, a statistically significant difference was not noted in the baseline cortisol concentrations between foals at birth and all other time points throughout the study. This is in contrast to other studies in which the cortisol concentration at birth was significantly higher than foals that were more than 1 d old (28,37). The difference between the study reported here and previous studies is likely a result of the time at which the first sample was collected. In the study herein, the first (birth) samples were collected between 6 and 16 h of age. Equine fetal cortisol demonstrates a notable increase within the last 24 to 48 h prior to parturition when compared to earlier times in gestation (35,36). Cortisol concentrations subsequently reach peak levels approximately 30 min after birth and decrease thenceforth in healthy neonatal foals (37,38). Previous data have demonstrated relatively high serum cortisol concentrations (70 to 80 ng/mL) in foals at the time of birth, which then rise to 120 to 150 ng/mL during the 1st hour of life, and decrease over the next 6 h of life to comparatively baseline concentrations (~ 30 ng/mL) by 24 h of age (28,37–39). The prepartum rise in blood cortisol concentrations may serve to promote final maturation of lungs and other body systems, as well as orchestrate developmental changes necessary for survival of the neonate (35). Unfortunately, the late increase in equine fetal cortisol places the premature fetus at a disadvantage for perinatal survival (36). With regard to the goals of this study, the ACTH stimulation test was not performed immediately at birth because of the aforementioned marked variation in cortisol concentration during the first 6 h of life. A high degree of variability in the response to the ACTH stimulation test is suspected during this period due to the fluctuations in cortisol and a previous report has suggested that the adrenal cortex can be evaluated via stimulation test from 6 hours of age and older (28). Moreover, clinically ill foals are typically not presented to veterinarians immediately at birth but rather after several hours, or longer, of observation by the owner.

Plasma ACTH concentrations in healthy adult horses range from 6.5 to 30 pg/mL (mean 18.68 pg/mL) (40). However, in a recent study in healthy adult horses and ponies, the mean ACTH concentration ranged from 17 to 60.5 pg/mL (41). Seasonal variations in plasma ACTH concentrations were observed in that study, with markedly higher ACTH concentrations reported in September when compared to values reported during January or May (41). Previous studies evaluating catheterized equine fetuses have demonstrated an increase in ACTH from a mean value of 159 pg/mL increasing to 246 pg/mL, 1 d prior to parturition (36). Subsequently, the ACTH concentration remains high at birth (250 to 300 pg/mL) and

rapidly declines over 60 to 120 minutes to similar levels as a 1-day-old foal (37). Adrenocorticotropic hormone concentrations have been reported to range from 38 to 48 pg/mL between 6 and 14 d of age (39,42). Seasonal variation in ACTH concentrations have not been evaluated in foals but previously reported values for foals were measured in the spring and summer. In the study reported here, ACTH concentrations were similar to previously published concentrations (37,38); however, in the study here, a significantly lower ACTH concentration was observed at birth (6 to 16 h of age) when compared to days 2, 3, 4, 5, 7, 10, 42, 56, and 84. It is possible that season may have affected plasma ACTH concentrations in foals, but evidence is lacking.

The ACTH/cortisol ratio has been used in various species to help distinguish individuals with primary adrenocortical insufficiency (Addison's disease) from normal adrenocortical function; in this instance, a high ACTH/cortisol ratio would suggest adrenal dysfunction or insufficiency (43,44). A recent report in septic foals showed a significant increase in the ACTH/cortisol ratio in septic foals when compared with healthy foals, which suggests that RAI exists in foals (43). Furthermore, the authors of that study suggested that the dysfunction of the hypothalamic-pituitary-adrenal axis occurred at the level of the adrenal gland based on the high concentrations of ACTH compared with the relatively low cortisol concentrations (43). The information from this study suggests that the ACTH/cortisol ratio is lower at birth when compared with older foals. Additionally, the information here provides a confidence interval for the ACTH/cortisol ratio in healthy foals of various ages.

Controversy exists in regard to the ideal dosage of cosyntropin to evaluate the adrenal cortical response for conditions such as RAI. Studies on critically ill humans suggest that a lower, physiologic, dose of ACTH is more appropriate in determining the adrenal response (3,4). The primary objectives of this study were to determine if a dose of 0.1 μg/kg of cosyntropin will consistently produce a change in cortisol from baseline in healthy foals and subsequently evaluate trends or differences associated with ACTH stimulation tests as the healthy neonatal foal ages. Through this study, it was determined that a dose of 0.1 μg/kg of cosyntropin is adequate to evaluate the responsiveness of the foal's adrenal cortex. Specifically, evaluating cortisol concentrations 30 min after intravenous administration of cosyntropin provides the most diagnostic information. Additionally, a confidence interval for a low-dose ACTH stimulation test and ACTH/cortisol ratio at various ages was established for foals. Further research is necessary to investigate the response to ACTH stimulation test and RAI in critically ill foals.

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