Variation in Plasma Adrenocorticotropic Hormone Concentration and Dexamethasone Suppression Test Results with Season, Age, and Sex in Healthy Ponies and Horses

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The purpose of this study was to evaluate the variation in plasma adrenocorticotropic hormone (ACTH) concentration and dexamethasone suppression test (DST) results with season, age, and sex in healthy, pony mares (n = 15) and pony stallions (n = 14) living under semiferal conditions and horse mares (n = 10) living at pasture. Plasma ACTH concentrations were measured in September 2002, and in January, May, and September 2003. DSTs were performed in January and September 2003. Plasma ACTH concentrations in September 2002 and September 2003 were similar and were significantly greater than in January and May (P < .001). Plasma ACTH concentration was within the reference range for 38 (97%) of 39 subjects in January, for 39 (100%) of 39 subjects in May, for 2 (5%) of 39 subjects in September 2002, and for 3 (8%) of 39 subjects in September 2003. DST results were within the reference range in all subjects in January and were within the reference range for 29 (74%) of 39 subjects in September 2003. Plasma cortisol concentration at the end of the DST was significantly greater in September than in January (P = .002). Age was positively correlated with plasma ACTH and plasma cortisol concentration at the beginning and end of the DST. Within the same season, plasma ACTH concentration in pony mares, pony stallions, and horse mares was not significantly different (P > .05). Seasonal changes in plasma ACTH concentration and DST results should be considered when interpreting endocrine test results.

Key words: Corticotropin; Cushing’s disease; Pituitary; Pregnancy; Semiferal.

Equine Cushing’s disease (ECD) is the most common equine endocrinopathy. Loss of dopaminergic inhibition of the pituitary pars intermedia (PI) is associated with an increase in plasma adrenocorticotropic hormone (ACTH) concentration.1-3 Plasma ACTH concentration and the dexamethasone suppression test (DST) are sensitive and specific diagnostic tests for ECD; however, little is known about factors that affect results of these tests.3-6

Ponies are endocrinologically and metabolically different from horses. It has been reported that plasma ACTH concentration in ponies is lower than in horses.7 Ponies are frequently affected with laminitis, hyperlipemia, hyperinsulinemia, and obesity.7-12 The frequency of these problems in association with ECD would suggest the possibility that pituitary dysfunction may be an important predisposing factor.7-12

The aging process is associated with a deterioration in immune function and an increased risk of laminitis, both of which could be attributed to abnormal function of the pituitary-adrenal (PA) axis.8-13-15 Because ECD is a slowly progressive disease of aged equids, it is important to understand the effects of aging in healthy horses and ponies on endocrine function tests. Although there are seasonal effects on the PA axis in other species, these effects have not been evaluated in equids. The presence of a seasonal variation may affect the sensitivity and specificity of endocrine function tests.

The purpose of this study was to evaluate the variation in plasma ACTH concentration and DST results in association with season, age, and sex in horses and semiferal ponies. We chose to evaluate semiferal ponies because they were not exposed to management factors such as diet, nonnatural photoperiod, medication, and social isolation that may disrupt the PA axis in domestic horses and ponies.16

Materials and Methods

Subjects

The study sample included 15 mature pony mares and 14 mature pony stallions from a semiferal herd, and 10 mature nonpregnant horse mares from a teaching herd maintained at New Bolton Center, University of Pennsylvania. The median age of ponies and horses was 5 years (range 2-24 years) and 15 years (range 8-26 years), respectively.

The semiferal herd is located in Chester County, Pennsylvania, at 39.9°N, 75.8°W. The climate is temperate, with 4 distinct seasons. The semiferal herd has been maintained on the same property since 1994, when 13 mature males and 13 mature females were assembled and allowed to organize and breed undisturbed. The herd is kept on approximately 50 acres of old pasture grasses and browse that support excellent nutritional condition for the herd for most of the year. Supplemental hay of similar grasses is provided when pasture becomes dormant during winter (mid-December through late February in most years). Water is available in wetlands, streams, and a pond. Trace mineral and salt licks are provided. Natural brush and light forest are available for shelter. To maintain herd size to between 50 and 70 animals, 1 or 2 harem families are removed from the herd approximately every 2 years. During this study, herd size ranged from 47 to 65 individuals, with 6-9 harem bands, 1 bachelor band, and 1 solitary bachelor. In this herd, mares foal annually in March-August, with most foaling and breeding in April and May. These animals are gentled at pasture to achieve quiet compliance with blood sampling with no or minimal restraint. The semiferal ponies included in this study were well acclimated to, and compliant with, blood sampling procedures and representative of the major adult social classes, including harem stallions, harem mares, and bachelor stallions.
The horse mares were not part of the semiferal herd. They were maintained in groups at pasture with access to run-in sheds and were supplemented with hay in the winter months. These were Thoroughbred, Standardbred, and warmblood mares retired from training or competition. This herd is subjected to a photoperiod of 16 hours of light per day beginning in December with artificial lighting in their sheds in the evening to advance the onset of ovarian cycling in spring. They were all nonpregnant, and none was on treatment during the study. All horses were accustomed to venipuncture. Animal management and sampling procedures were preapproved by the University of Pennsylvania Institutional Animal Care and Use Committee.

**Sampling**

Plasma ACTH concentration was measured in September 2002, and January, May, and September 2003. DSTs were performed in January and September 2003. All pony mares were pregnant when tested in September 2002, January 2003, and September 2003. In May 2003, 6 of 15 pony mares were pregnant; the remaining pony mares had recently foaled and were nursing a foal. None of the horse mares were pregnant during the study. None of the subjects had clinical signs of ECD and all had a DST result within the reference range in January 2003.

For hormone assay, blood was collected from the jugular vein into evacuated polyethylene terephthalate vials containing ethylenediaminetetraacetic acid for ACTH assay and heparin for cortisol assay. Vials were placed on ice. Plasma was separated within 2 hours of collection and stored in polypropylene tubes at −80°C for ACTH and −20°C for cortisol until assayed. All hormone assays were performed at the New York State Animal Diagnostic Laboratory. Plasma cortisol and ACTH concentration were determined by chemiluminescent immunnoassay previously validated for horses. The Shapiro-Wilk test revealed that plasma ACTH concentration was normally distributed. Plasma ACTH concentration of all subjects and were within the reference range for 29 (74%) of 39 subjects in September 2003 (Table 1). Plasma cortisol concentration in September was significantly greater than in January and May (P < .001). The horizontal dashed line represents the upper limit of the previously established reference range for plasma ACTH concentration in horses and ponies.

**Statistical Analysis**

The Shapiro-Wilk test revealed that plasma ACTH concentration was not normally distributed. Plasma ACTH concentration of all subjects was compared at each season with the Friedman nonparametric repeated measures test and the Dunn multiple comparisons post-test.\(^4\) The Wilcoxon signed ranks test was used to compare plasma cortisol concentration in January and September. Subsequent comparison was performed on data within each season because a seasonal variation in plasma ACTH was detected. Plasma ACTH concentration in pony mares, pony stallions, and nonpregnant horse mares was compared by performing the Kruskal-Wallis analysis of variance and the Dunn multiple comparisons post test to determine if sex or breed were associated with variation in plasma ACTH concentration. To evaluate the association between age and variation in the PA axis, data from horses and ponies were combined. The Spearman correlation was used to evaluate the association between age and cortisol concentration at the beginning and end of the DST and the association between age and plasma ACTH concentration. The Mann-Whitney U-test was used to compare the ages of equids with DST results within and above the reference range. Statistical calculations were performed by using commercially available software.\(^5\) For all tests, \(P < .05\) was considered significant.

**Results**

**Season**

Plasma ACTH concentrations in September 2002 and September 2003 were similar and were significantly greater than in January and May (\(P < .001\); Fig 1). Plasma ACTH concentration was within the reference range for 38 (97%) of 39 subjects in January, for 39 (100%) of 39 subjects in September 2002, and for 3 (8%) of 39 subjects in September 2003 (Table 1). DST results were within the reference range in January for all subjects and were within the reference range for 29 (74%) of 39 subjects in September 2003 (Table 1). Plasma cortisol concentration at the end of the DST was significantly greater in September than January (\(P < .001\); Table 1). Plasma cortisol concentration at the beginning of the DST in January was not significantly different than in September (\(P = .96\)).

**Age**

Age and ACTH concentration were positively correlated in January (\(r = 0.35, 95\%\) confidence interval [CI] 0.03–0.60, \(P = .03\)), September 2002 (\(r = 0.43, 95\%\) CI 0.12–0.66, \(P = .007\); Fig 2), and September 2003 (\(r = 0.35, 95\%\) CI 0.03–0.61, \(P = .03\)), but not in May (\(P = .15\)). In January, age and plasma cortisol concentration were positively correlated at the end of the DST (\(r = 0.42, 95\%\) CI 0.11–0.65, \(P = .008\); Fig 3), but not at the beginning of the DST (\(r = 0.30, 95\%\) CI −0.02–0.57, \(P = .06\)). In September, age and plasma cortisol concentration were positively correlated at the beginning (\(r = 0.32, 95\%\) CI 0.12–0.58, \(P = .05\)) and at the end of the DST (\(r = 0.43, 95\%\) CI 0.12–0.66, \(P = .006\)). Equids with DST results above the reference range were significantly older than equids with DST results within the reference range (16 years, range 2–25 years, versus 5 years, range 2–22 years; \(P = .002\)).

**Sex and Breed**

Within season, plasma ACTH concentrations of pony mares, pony stallions, and horse mares were not significantly different (\(P > .05\)).
Table 1. Seasonal variation in plasma adrenocorticotropic hormone (ACTH) concentration and dexamethasone suppression test (DST) results in healthy horses and ponies.*

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<tr>
<td>Median (range) plasma ACTH concentration (pg/mL)</td>
<td>9–35</td>
<td>17.1 (13.4–32.4)</td>
<td>16.1 (13.1–22.2)</td>
<td>60.5 (25.6–140.0)</td>
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<td>Animals with plasma ACTH concentration within the reference range</td>
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<tr>
<td>January 2003</td>
<td>Horses</td>
<td>Ponies</td>
<td>Horses</td>
<td>Ponies</td>
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<tr>
<td>100%</td>
<td>17.0 (8.1–36.9)</td>
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<tr>
<td>May 2003</td>
<td>(10/10)</td>
<td>100%</td>
<td>3%</td>
<td></td>
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<tr>
<td>20%</td>
<td>(29/29)</td>
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<td>September 2002</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>100%</td>
<td>16.1 (12.0–33.1)</td>
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<tr>
<td>September 2003</td>
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<tr>
<td>100%</td>
<td>19.0 (34.9–479.0)</td>
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<tr>
<td>60%</td>
<td>(10/10)</td>
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<tr>
<td>79%</td>
<td>(23/29)</td>
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<td>Animals with plasma cortisol concentration &lt; 1.0 μg/dL, 19 hours after dexamethasone administration</td>
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<tr>
<td>Median (range) plasma cortisol concentration at the beginning of the DST (μg/dL)</td>
<td>2–6</td>
<td>3.07 (1.88–5.49)</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>September 2003</td>
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<tr>
<td>Median (range) plasma cortisol concentration at the end of the DST (μg/dL)</td>
<td>&lt;1.0</td>
<td>0.15 (0.05–0.50)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>September 2003</td>
<td></td>
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<td>ND, not determined.</td>
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* Plasma ACTH concentrations in September 2002 and September 2003 were significantly greater than in January and May (P < .001). Plasma cortisol concentration at the end of the DST was significantly greater in September than in January (P = .001).

Discussion

Season

Seasonal differences in plasma ACTH concentrations and DST results were the major findings of the present study. A seasonal change in the PA axis has implications for endocrine diagnostic testing. One study suggested that the repeatability of the DST in horses with clinical signs of ECD was poor. This may be explained by the present study because there is seasonal variation in DST results in horses and ponies.

Seasonal variation in plasma ACTH concentration and sensitivity of the PA axis to dexamethasone in horses and ponies has not been reported. In the present study, plasma ACTH concentration and cortisol concentration at the end of the DST were highest in September. A seasonal effect on the PA axis is present in other species. In the Soay ram, plasma ACTH concentration and beta-endorphin concentration increase in summer and autumn, respectively. Furthermore, a seasonal variation in the ACTH response to corticotropin-releasing hormone (CRH) occurs in the Soay ram with the greatest response in the fall. In squirrels, serum
cortisol levels peak in August in association with an increase in body mass before hibernation.20

It is unclear whether the seasonal increase in plasma ACTH concentration and decrease in sensitivity of the PA axis to dexamethasone are responsible for the increased risk of chronic laminitis in horses and ponies in the autumn.13,21 Corticosteroids have been associated with laminitis, possibly by their ability to alter vascular tone and glucose metabolism.22-24 The pathogenesis of laminitis is complex and the onset of laminitis and changes in the PA axis could occur simultaneously because of another event such as a change in diet or photoperiod.

The reason for an increase in plasma ACTH concentration and decrease in sensitivity of the PA axis to dexamethasone in autumn is unclear. It would be evolutionarily adaptive for equids to alter their metabolism in preparation for a decrease in availability of forage during winter. Seasonal change in lipid metabolism in horses and ponies has been studied. Nonesterified fatty acid concentration increases during cold months (December–March) in ponies. In Morgan and Thoroughbred horses, triglyceride concentration decreases during cold months.25 Seasonal changes in the PA axis may have been related to changes in reproductive function because all horses and ponies in the present study were sexually intact. However, plasma ACTH concentration and DST results in stallions and mares were not different. Unfortunately, the present study did not include geldings.

Because horses and ponies were maintained primarily at pasture during most of the year, seasonal changes in pasture quality may have influenced the results. Carbohydrate content of pasture increases greatly during the autumn and moderately in the spring in the mid-Atlantic United States.26 Seasonal increase in ergot alkaloid content of endophyte-infected fescue grass also occurs in the autumn.27 However, one would expect that ergot alkaloids would cause a decrease in activity of the PA axis rather than an increase in activity. Evaluation of the PA axis at shorter intervals would have better described the extent and frequency of changes. The results of the present study do not allow one to determine the duration of the autumn increase in plasma ACTH concentration and the decrease in sensitivity of the PA axis to dexamethasone. Furthermore, peaks in PA activity may have been undetected.

**Age**

The positive correlation between age and plasma cortisol concentration at the beginning and end of the DST suggests that the sensitivity of the PA axis to dexamethasone decreases with age in horses and ponies. This is consistent with similar studies in rhesus monkeys,8,28 dogs,29 and rats.30 Serum cortisol concentration 19 hours after dexamethasone treatment was higher in aged rhesus monkeys when compared to young controls.28 Aged dogs have higher resting plasma ACTH and cortisol concentration but no difference in the PA axis sensitivity to dexamethasone. Aged dogs also have higher peak plasma ACTH and cortisol responses to CRH.29 Aged rats are more resistant to dexamethasone suppression of circulating corticosterone than are young rats. Furthermore, aged rats have a greater ACTH response to CRH than do young rats.30

The decrease in sensitivity of the PA axis that occurs during aging in horses and ponies is consistent with the pathogenesis of ECD. One hypothesis on both aging and development of ECD is exposure to oxidative stress. The presence of oxidative stress in the PI of horses was investigated by using antibodies against a product of oxidative stress, 3-nitrotyrosine (3NT). A progressive increase in 3NT-positive cells was demonstrated when young horses were compared to healthy aged horses and horses with ECD.31 Although it may be common for aged horses to experience loss of PI function, it should not be considered normal. In the present study, age-related changes in PA sensitivity to dexamethasone support the concept that the diagnosis of ECD is not a “yes” or “no” diagnosis. ECD may be a slow loss of PI function, which may frequently accompany the aging process.

The decrease in sensitivity of the PA axis that occurs during aging in horses is also consistent with the increased risk of chronic laminitis in older horses. Three epidemiological studies on laminitis have reported that chronic laminitis is more common in older horses; unfortunately, the etiology was not investigated.8,13,15 In a survey of geriatric horses, those with ECD were significantly more likely to have a history of laminitis.31 It is possible that subclinical pituitary dysfunction is more common than previously recognized and may be part of the pathogenesis of laminitis in older horses.

Age-related changes in the PA axis might also explain the decrease in immune function in aged horses. Aged horses have lower lymphocyte concentrations and lower in vitro lymphoproliferative response to mitogens, which are consistent with an increased exposure to corticosteroids.14,32 However, aged horses have a greater CD4:CD8 ratio, which is consistent with an inflammatory rather than immunosuppressive process.33

**Sex**

Significant sex-associated differences in test results were not detected. During the last week of gestation, plasma cortisol concentration in pregnant mares is significantly higher than in nonpregnant mares.34 CRH is made by the placenta of many species, including sheep, and increases during late gestation; however, this does not appear to occur in horses.35,36 It would appear that if there is a difference between plasma ACTH concentrations in pregnant and nonpregnant horses and ponies, it is small.

**Horses and Ponies**

Although it has been reported4 that ponies have lower plasma ACTH concentration than horses, a difference was not detected in the present study. There are several possibilities for this discrepancy. First, we compared pregnant pony mares and pony stallions to nonpregnant horse mares. It is possible that the previous study compared horse and pony geldings. The PA axis may be different in geldings. Previous studies may have tested horses in the autumn and tested ponies at another time of year. This would reveal an apparent difference in plasma ACTH concentration between...
ponies and horses. Evaluation of the original data from a previous study\(^1\) revealed that plasma ACTH concentrations in ponies and horses in the autumn were greater than in winter, spring, or summer (Couetil, personal communication).

In summary, plasma ACTH concentration was markedly higher in September than in January and May. Clinically important seasonal differences in DST results were present. The positive correlations between age and plasma ACTH concentration and plasma cortisol concentration suggests deterioration in PA function with age. Plasma ACTH concentrations and DST results were similar in pony stallions, pony mares, and nonpregnant horses. Seasonal changes in plasma ACTH concentration and DST results should be considered when interpreting test results.

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**Footnotes**

\(^1\) Immulite ACTH, DPC-Cirrus, Los Angeles, CA

\(^2\) InStat version 2.0, GraphPad Software, San Diego, CA

\(^3\) Stata Statistical Software: Release 8.0, Stata Corporation, College Station, TX


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**References**


