Adrenocorticotropic Hormone Levels in Equine Plasma After Centrifugation at Multiple Time Points from Collection[1]

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Introduction

Pituitary pars intermedia dysfunction (PPID) has been considered the most common endocrinologic disorder of aged horses. The diagnosis of PPID is typically based on history, clinical signs and demonstration of endocrinologic dysfunction via diagnostic assay results. The most common diagnostic test utilized by veterinary practitioners for the diagnosis of PPID is currently measurement of basal plasma adrenocorticotropic hormone (ACTH) concentration. Likely, due to the ease of collection and the availability of seasonal reference ranges that allow for testing any time of the year, determination of resting plasma ACTH concentration has been the most practical diagnostic test for PPID.[2] Multiple recommendations exist concerning the in vitro stability of ACTH. Most all recommendations state samples should be chilled promptly after collection. Multiple recommendations exist for the timing of centrifugation of chilled samples from sample collection. In the US, discordance exists between laboratory recommendations concerning centrifugation time from sample collection; anywhere from 2 hours up to 4 hours from sample collection. Realistically, many equine ambulatory practitioners do not have the ability to centrifuge the sample within 2 to 4 hours.

Study Purpose

Based on this dilemma, the purpose of this study was to determine the stability of ACTH levels in blood samples with increasing duration from collection prior to centrifugation.

Materials and Methods

Ten horses, 5 PPID+ and 5 PPID- (non-PPID), were enrolled in the study during the autumn time period. On Day 0, 5 mL of whole blood was collected from each horse into each of six EDTA tubes and immediately chilled. One tube was centrifuged within 15 minutes of collection (time 0) followed by centrifugation of one tube from each horse at 4, 8, 12, 24 and 36 hours following collection. At each time point, plasma was centrifuged and separated into 1.5 mL polypropylene tubes and stored at -80^{o}C. Plasma was shipped frozen with cold packs overnight to the Animal Health Diagnostic Center, Cornell University, Ithaca, NY for analysis.

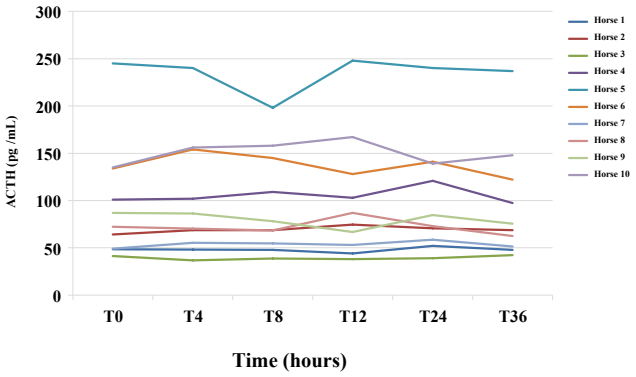
Statistical Analysis

Due to differing baseline values, the percent change from baseline (PCFB) was reported to standardize the data. Mean PCFB, standard deviation and 95% confidence intervals (CI) were reported. The null hypothesis that there was no time effect was tested with a mixed model repeated measures methodology with subject as a random effect. P-values <0.05 were considered statistically significant.

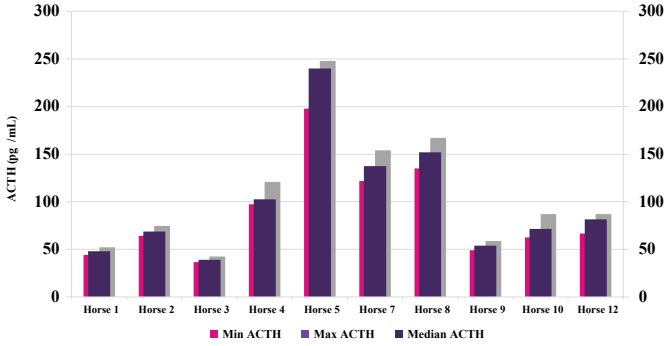
Results

The absolute changes over time revealed no pattern of change over time. The mean PFCB was 2.8% ± 7.96% (CI -2.88%, 6.99%). There was not significant evidence ($p=0.5056$) of a time effect using the repeated measures model. There was no evidence of any significant time effect on the level of ACTH in PPID+ or PPID- (non-PPID) horses. There did not appear to be any systematic degradation of ACTH level due to delay in measurement through 36 hours post collection.

Absolute changes in adrenocorticotropin hormone (ACTH) concentration from baseline in pg/mL maintained at 35° F. Lines represent the ACTH concentration over time (0-36 hours; T0 to T36) for each horse.



The Minimum, Median, and Maximum Changes in Adrenocorticotropin Hormone (ACTH) in pg/mL Over Time (0-36 hours)



Mean, Standard Deviation and 95% Confidence Intervals by Horse for Percent Change of ACTH Concentration (pg/mL) from Baseline.

Horse Number	Mean	Standard Deviation	95% Confidence Interval
Overall	2.8	7.96	-2.88, 6.99

Discussion

The results of this study support the recommendation that immediately chilled EDTA blood samples (properly stored) may be centrifuged and plasma separated within 36 hours of collection for shipment to the respective laboratory for analysis without degradation of the ACTH level. Further research is needed to determine ACTH stability in a larger number of horses (PPID+ and PPID-) from separate sites and different geographic regions.

Take Home Message

Even though the results of the study support properly stored EDTA blood samples may be centrifuged and plasma separated within 36 hours of collection for shipment to the respective laboratory; it is advisable for veterinarians to centrifuge EDTA blood samples and separate plasma the same day of collection.

Acknowledgments

A special thank you to Middle Tennessee State University that enrolled horses in this study. This project was supported by Boehringer Ingelheim Animal Health.

References

1. Shepard K, Haffner J, Grubbs S, Neal D, Pierce G. Adrenocorticotropic hormone levels in equine plasma after centrifugation at multiple time points from collection. *J Vet Intern Med* 2017; 31: 1352.

2. Equine Endocrinology Group. 2017 Recommendations for the diagnosis and treatment of pituitary pars intermedia dysfunction (PPID).