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Comparison of Serum and Hair Testosterone Concentrations in Free-Roaming Stallions

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Abstract

In many species, male social rank has been found to correlate with testosterone concentration ([TEST]), with more dominant males having higher circulating [TEST]. However, the influence of [TEST] on social ranking has not yet been evaluated in free-roaming horse (*Equus caballus*) herds. The purpose of this study was to compare [TEST] and cortisol concentrations ([CORT]) in serum and hair in stallions from different social ranks to understanding the behavior of free-roaming horses. Mane hair and serum samples were collected from each stallion along with behavioral evaluations. The behavioral evaluations where categorized into three groups and [TEST] and [CORT] were compared. There were no significant differences found among hair and serum [TEST] or [CORT] between the three groups. There was also no correlation found between serum and hair [TEST] or [CORT] (R^2= 0.0257, and 0.0025, respectively). While it was unexpected that [TEST] and [CORT] did not differ between social ranks, these results are significant in understanding the social hierarchy of free-roaming stallions before and after castration occurs of which the latter should be studied further. This information could be used across various free-roaming horse ranges where range managers are making decisions about population control tactics with the most regard to animal welfare.

Keywords: Behavior; Cortisol; Dominant; Stallion; Submissive; Testosterone

Introduction

In many species, such as rock hyrax (*Procavia capensis*) and white-browed sparrow-weavers (*Plocepasser mahali*), male social rank is correlated with testosterone concentrations ([TEST]) such that dominant males have higher circulating [TEST] [1]. Glucocorticoid stress hormones, such as cortisol ([CORT]) and corticosterone increase in primates and rodents that belong to lower social ranks [2]. Although [TEST] have been measured in free-roaming stallions previously, it has never been correlated to social hierarchy [3-5].

When analyzing hormone concentrations in wild animals, a variety of different samples can be tested, such as blood, saliva, feces, or urine [6]. Unlike the other types of samples, hair is stable at room temperature for long periods and represents average hormone concentrations over time [7].

The purpose of this study was to compare [TEST] and [CORT] in serum and hair from stallions of different social ranks. This study could benefit behavioral research in wildlife by showing that noninvasive sample collection can be done to evaluate behavior correlations with physiological changes.

Materials and Methods

Stallions (n=15) used in this study were part of the free-roaming horse herds, located in Central Oregon and varied in age (Table 1). These horses were managed by the Confederated Tribes of Warm Springs Reservation who approved the animal care procedures. The horses were gathered mid-May 2015 for the purpose of castration. The stallions to be castrated were sorted from the herd and put into holding pens before being individually moved to a chute for castration. Behavioral evaluations were conducted over a two-

Behavior	Age (years)
Submissive	8 ± 3
Neutral	4.5 ± 3.62
Dominant	7 ± 1.41

Table 1: Mean age ± standard deviation in years of stallions sampled. There was no correlation between age of stallions and social rank

day observation period while horses were in various holding pens prior to castration. A 5-point behavior score was created with 1 being most submissive and 5 being most dominant. A horse that received a score of 1 exhibited tense behaviors with a high flight response. While a horse that received a score of 5 exhibited behaviors such as fighting, rearing, and lunging toward other stallions.

Samples were collected when each stallion was anesthetized for castration. Blood samples were collected prior to castration from the jugular vein using a Vacutainer® tube without an anticoagulant in the serum tubes and a 19-gauge needle. Blood samples were allowed to clot and then centrifuged at 3000 rpm for 15 minutes. Serum was pipetted off and stored at -20 °C. A mane hair sample was pulled out by the root, 100 ± 20 mg of hair was weighed, minced into 3-4 mm pieces, and sonicated in methanol (2 mL) at 20 °C for 30 minutes. The samples were then incubated overnight at 50 °C in a water bath with gentle shaking. The methanol was pipetted off into a new glass vial and evaporated to dryness under nitrogen. The samples were then reconstituted with 125 µL of assay buffer (#80-0170, Assay Designs, Inc., Ann Arbor, MI).

Chemiluminescence (Immulite 1000, Siemens Healthcare Diagnostics, Tarrytown, NY) was used to measure [TEST] and [CORT] from serum and extracted hair samples according to the manufacturer's protocol. For [TEST], the Total Testosterone Kit (#LKTW1) by Immulite was used with an assay sensitivity of 0.15 ng/mL. The serum intra-assay CV was at 12.8% and the hair intra-assay CV was at 10.3%. For [CORT], the Cortisol Kit (#LKCO1) by Immulite was used with an assay sensitivity of 1 ng/mL. The serum intra-assay CV was 5.1% and the hair intra-assay CV was 9.4%.

Behavior scores for each stallion over two days were averaged and then stallions were categorized based upon their average behavior score as either submissive (with a score of <2.5), neutral (2.5-3.5), or dominant (>3.5). Serum and hair [TEST] and [CORT] were compared to the average behavior scores using an analysis of variance (ANOVA). Significance was defined as $p<0.05$.

Results

Of the stallions sampled, 20% exhibited behaviors that were classified as submissive, 53% were neutral, and 27% were dominant. Serum [TEST] was not significantly higher in dominant stallions and serum [CORT] was not significantly higher in submissive stallions (Table 1). Hair [TEST] was higher than serum [TEST] in all stallions regardless of social dominance. On the other hand, serum [CORT] was higher than hair [CORT]. Although the magnitude of these results is modest, they lay the foundation for additional research to be carried out after castration that could have significant implications on animal welfare in regard to herd social dominance (Table 2).

Behavior	Serum [TEST]	Hair [TEST]	Serum [CORT]	Hair [CORT]
Submissive	35.3 ± 26.5	18.86 ± 8.96	10.2 ± 5.4	0.10 ± 0.0
Neutral	35.9 ± 26.0	17.34 ± 4.30	9.4 ± 3.0	0.10 ± 0.0
Dominant	71.0 ± 54.2	18.35 ± 4.26	10.9 ± 1.4	0.13 ± 0.6

Serum units (ng/mL) were converted to (ng/g) to be comparable to hair concentration units that were measured in (mg/g).
Table 2: Mean ± standard deviation in serum and hair testosterone ([TEST]) and cortisol concentrations ([CORT]) taken from free-roaming stallions managed by the Confederated Tribes of Warm Springs Reservation in May 2015. Hair and serum where measured in ng/g. There were no significant differences between the behavior groups

Discussion

Free-roaming horse management is a complex issue incorporating social, economic, emotional, political, and environmental factors. On federally owned lands free-roaming horses are covered under the provisions of the Wild Free-Roaming Horses and Burro Act of 1971 [8]. However, on Native American owned lands, each tribe can have a different management policy based on its sovereignty. The horse herd reduction program for the Confederated Tribes of Warm Springs includes castration and either the release of geldings back to the range or the selling of geldings to be trained as ranch and rodeo horses. Castration has not been widely studied as a contraceptive tool for free-roaming horses due to concerns about behavior alterations, as seen with porcine zona pellucida immunocontraceptive in mares [9,10]. If castrated stallions are returned to the range, it is important to consider the effect of castration on herd hierarchy and animal welfare.

This is the first study to report on the use of hair samples to measure [TEST] or [CORT] in horses. Hair hormone concentrations differed significantly from serum hormone concentrations. Hormone concentrations deposit into hair over weeks or months and are not subject to fluctuations seen in serum hormone concentrations [1]. In free-roaming stallions, serum [TEST] and [CORT] have been found to be highest at 0800 h, lowest at 2300 h, with oscillations in between [3]. Free-roaming horses are also seasonal breeders with peak breeding season occurring from March-August [11]. Because of this seasonality, [TEST] change throughout the year with peak concentrations occurring in May and the lowest in October [4]. In addition, when stallions are housed with other stallions (as the stallions were in the current study prior to castration) serum [TEST] significantly decreases compared to when stallions are housed with mares [11]. Either may explain the difference observed between hair and serum [TEST].

Cortisol concentrations were low in both serum and hair as well as numerically similar between all three behavior categories. Because stallions were separated from their individual harems and housed together in pens for two days, it is likely that their social

dominance was disrupted. Literature reveals that correlations between [CORT] and social rank becomes nonexistent when the social dominance is disrupted [12]. This may explain why no difference in serum [CORT] was observed across the various social ranks in the present study. In other species, such as the mongoose, [CORT] were similarly found to have no correlation between various males with different social ranks [13].

While it was unexpected that [TEST] and [CORT] did not differ between social ranks, these results are important in understanding the social dominance of free-roaming stallions before castration occurs and lays the foundation for further studies into the effect of castration after release back onto rangelands.

The effects of castrating and releasing stallions back on to the range, needs to be observed and studied in the future to evaluate how castration affects harem distribution and the potential success of bachelor stallions. Also, due to samples being opportunistically collected at time of castration, the long-term endocrine concentrations retrieved from hair from gathered free-roaming stallions needs to be assessed further to understand if the concentrations are representative of observed behavior of social dominance while on the range.

Conclusion

It has been concluded from this study that [TEST] and [CORT] did not differ significantly between social ranks among free-roaming stallions. It is not known if the results yielded in this study would be seen in a larger group of horses from various locations and across various seasons.

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