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Effects of season, age, sex, and housing on salivary cortisol concentrations in horses

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ABSTRACT

Analysis of salivary cortisol is increasingly used to assess stress responses in horses. Because spontaneous or experimentally induced increases in cortisol concentrations are often relatively small for stress studies, proper controls are needed. This requires an understanding of the factors affecting salivary cortisol over longer times. In this study, we have analyzed salivary cortisol concentration for 6 mo in horses (n = 94) differing in age, sex, reproductive state, and housing. Salivary cortisol followed a diurnal rhythm with the highest concentrations in the morning and a decrease throughout the day ($P < 0.001$). This rhythm was disrupted in individual groups on individual days; however, alterations remained within the range of diurnal changes. Comparison between months showed highest cortisol concentrations in December ($P < 0.001$). Cortisol concentrations increased in breeding stallions during the breeding season ($P < 0.001$). No differences in salivary cortisol concentrations between nonpregnant mares with and without a corpus luteum existed. In stallions, mean daily salivary cortisol and plasma testosterone concentrations were weakly correlated ($r = 0.251$, $P < 0.01$). No differences in salivary cortisol between female and male young horses and no consistent differences between horses of different age existed. Group housing and individual stabling did not affect salivary cortisol. In conclusion, salivary cortisol concentrations in horses follow a diurnal rhythm and are increased in active breeding sires. Time of the day and reproductive state of the horses are thus important for experiments that include analysis of cortisol in saliva.

1. Introduction

Analysis of salivary cortisol concentrations is increasingly used to assess the adrenocortical response of horses to potentially stressful situations, and several cortisol assays have been validated for equine saliva [1–4]. From horses accustomed to routine handling by humans, saliva can be collected easily, repeatedly, and without restraint of the animal. Salivary cortisol mirrors the unbound, that is, biologically active fraction of total plasma cortisol while plasma cortisol is largely bound to carrier proteins [5].

In horses, as in other species including humans [6] and rhesus monkeys [7], cortisol release into blood follows a diurnal rhythm with the highest concentrations in the morning and a nadir in the late afternoon and evening [8–10]. This rhythm can be disrupted by even minor perturbations resulting in a damping of the daily oscillations and elevated cortisol concentrations, especially around the time of the daily nadir [10]. The diurnal rhythm in cortisol release is well reflected by concentrations in saliva [11–14]. However,

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salivary cortisol concentrations have previously been determined in horses during specific experiments only, not allowing the comparison of undisturbed cortisol release patterns among different horse groups or times of the year. Cortisol release into blood or saliva may be physiologically influenced also by reproductive state and the sex of the individuals (humans [15] and dog [16]). Although most phases of pregnancy were without effect on plasma cortisol in horses [17], salivary cortisol increased in mares shortly before foaling [18]. Elevated plasma cortisol concentrations have also been demonstrated in pregnant women [19].

Marked increases in salivary cortisol concentrations as occurring in mares at foaling [20], in horses transported by road [21–23], or in foals at weaning [11] clearly indicate increased adrenocortical activity. However, experimentally induced increases in salivary cortisol concentrations are often relatively small and hardly exceed the range of physiological diurnal changes. This is especially true for studies aimed at assessing stress in horses submitted to equestrian training [3,24–26]. Whether such increases, although statistically significant, indicate that horses perceive a particular challenge or procedure as an acute stressor often can be doubted, thus, for stress studies, proper controls are required. The accuracy of studies evaluating adrenocortical activity in horses could be improved by a better understanding of the factors affecting salivary cortisol concentrations.

In this study, we have analyzed salivary cortisol concentration in horses of the same breed and genetic background and kept on the same premises over a 6-mo period from December to May. Horses have a seasonal sexual activity (reviewed in [27]), and the study period included the nonbreeding season and the breeding season. Horses were divided by age, sex, sexual activity, and reproductive state. We hypothesized that basal cortisol concentrations not only follow a diurnal but also a circannual rhythm. We further tested if salivary cortisol is affected by age of the horses, their reproductive state, and by the housing system with increased cortisol release in single housed vs group-stabled horses.

2. Materials and methods

2.1. Animals

A total of 94 warmblood sport horses of the Brandenburg State Stud at Neustadt (Dosse), Germany, were included into the study. They were kept either in group stables on straw or in individual loose boxes on straw or wood chippings. Horses were fed oats, concentrates, and mineral supplements 3 times daily and hay twice daily. Water was freely available at all times. From December to April, all group-housed horses had daily access to a paddock for 4 to 5 h. Horses housed in individual boxes had either access to individual paddocks or were ridden for approximately 1 h every day. Except adult breeding stallions and adult geldings, all groups were turned out on pasture in May. On pasture, broodmares received oats, concentrates, and mineral supplements twice daily, whereas all other groups were not given any additional feed. Details on horses included into the study are summarized in Table 1. Adult breeding stallions were used exclusively for artificial insemination, and semen was collected once daily 4 to 6 times per week. Geldings had been orchidectomized at least 8 mo before the start of the study.

2.2. Experimental procedures

Saliva for cortisol analysis was taken from December to May on 1 d/mo at 6 AM, 12 noon, and 6 PM (sampling days: 17–20 December, 14–17 January, 11–14 February, 18–21 March, 15–18 April, and 13–16 May, all horses in 1 group were sampled on the same day). The sampling day was always kept apart from any management-related changes in the husbandry system (eg, start of pasture period). In contrast to all other groups, late pregnant mares were sampled only until February (19 December, 16 January, and 13 February). Mares foaled between February 16 and April 18; and thus, no homogenous group of late pregnant mares was available after February.

Saliva was collected with a cotton-based swab (Salivette cortisol; Sarstedt, Nümbrecht-Rommelsdorf, Germany). The Salivette was inserted at the angle of the lips into the mouth of the horse and placed gently onto the tongue for 1 min until it was well soaked. After centrifugation for 10 min at 1,000*g*, 1 mL of saliva was aspirated, transferred into polypropylene tubes (Sarstedt), and frozen at −20°C until analysis. Collection of saliva was tolerated by the horses of all groups without resistance. In addition, after the last saliva sample on each sampling day, 1 blood sample was taken from a jugular vein into

Table 1
Horse groups included in the study.

Group	Number (n)	Age (yr)	Housing	Remarks
1. Adult breeding sires	11	6.8 ± 0.7	Individual	Breeding via AI only
2. Nonpregnant, adult mares	6	9.5 ± 1.5	Group	
3. Pregnant, adult mares	7	8.8 ± 1.8	Group	
4. Adult geldings	12	12.8 ± 1.6	Individual	
5. 1-yr-old stallions	8	1[a]	Group	
6. 1-yr-old stallions	8	2[a]	Group	
7. 3-yr-old geldings	8	3[a]	Group	Castrated at 2 yr of age
8. 1-yr-old mares	11	1[a]	Group	
9. 2-yr-old mares	11	2[a]	Group	
10. 3-yr-old mares	12	3[a]	Group	

Abbreviations: AI, artificial insemination; SEM, standard error of the mean.
Values are mean ± SEM.
[a] Horses of groups 5 to 10 were born between January and May of their respective years of birth.