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Serum cortisol concentrations associated with artificial insemination events in an African elephant (*Loxodonta africana*)

Brent C. White[1], Steven R. Taylor[2], Zoltan S. Gyimesi[2], Cristin L. Rieskamp[1], William Sarros[1], Steven D. Burton II[2]

[1]*Centre College, Danville, Kentucky, USA*
[2]*Louisville Zoological Garden, Louisville, Kentucky, USA*

[*]*For correspondence: Brent White, brent.white@centre.edu*

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Abstract

Elephants are among the most charismatic and controversial species in modern zoo collections. Advanced cognitive and emotional capabilities have been attributed to elephants. As a result, good zoo management includes monitoring and enhancing the well-being of individual animals. To this purpose, we have assessed the serum cortisol concentration (SCC) of an adult female African elephant (*Loxodonta africana*) as artificial insemination (AI) procedures were performed. Fifteen AI procedures spanning four years showed a statistically significant decline in SCC from the morning on the day of the AI to the afternoon sample, which followed the AI within 30 minutes. A similar decline was found on 15 control days when the afternoon samples did not follow an AI, indicating that the decline was most likely due to circadian variation common to many species. There was no statistically significant difference between the afternoon SCC measurements associated with the AI events and the afternoon control samples. Six of the AI procedures occurred after the transition from free contact management of the elephants to restricted contact. There was no significant difference between SCC after AI's during free and restricted contact management, suggesting that for this elephant under these conditions, AI is not stressful.

Introduction

Elephants are among the most charismatic and controversial animals in the modern zoo. Their charisma comes from their size and complex social behaviours, which imply that they have significant cognitive and emotional capabilities. Unfortunately, in their remnant habitats, elephants face continuing threats from poaching, diminishing open ranges, and increasing conflict with local human populations. The well-being of wild populations may be enhanced by detailed knowledge of the species obtained from the management and study of zoo elephants. Well-managed zoo elephants provide opportunities to further our understanding of health, reproduction, basic physiology, and behaviour.

A sustainable managed program necessitates successful reproduction. Due to the logistics of moving male elephants, artificial insemination (AI) is another technology that affords the facilitation of reproduction and genetic management in managed elephant populations. Artificial insemination in elephants was developed in the 1990's and has resulted in the birth of numerous calves worldwide (Hildebrandt et al. 1999, Brown et al. 2004). Since the AI procedure involves hands-on manipulation of the elephant cow, it is important to understand the physiological effects of this assisted reproduction technique on the animal. The Indianapolis Zoo was the first to successfully use extended semen in AI with an African elephant (*Loxodonta africana*) (Hildebrandt et al. 1999). The calf was born in 2000. Several years later the Louisville Zoo used AI to produce a healthy male calf with the African cow that is the subject of the present paper (unpublished source, zoo records).

In the interest of monitoring the welfare and well-being of the elephants at the Louisville Zoo, we have collected serum samples to assess cortisol concentration (SCC), a hormone that

is secreted in response to stressful situations and also contributes to metabolic regulation. This paper reports SCC measurements associated with AI procedures. For a control comparison, a similar number of instances with afternoon serum samples that did not follow an AI procedure were examined. The AI incidents spanned the transition from free contact to restricted contact management of the elephants. AI in a restricted contact system involves staff sharing space with a tethered elephant. This husbandry transition provided the opportunity to evaluate SCC changes during AI under both management systems.

The initial hypothesis was that the manipulations associated with AI, particularly in restricted contact (tethered elephant), would result in an elevation of SCC. Given the normal circadian decline in cortisol from the morning to the afternoon (Brown et al. 2010), it was expected for SCC following an afternoon AI to be similar to or higher than the morning SCC.

Methods

The subject was a 32-year-old female African elephant housed with a 47-year-old female Asian elephant (*Elephas maximus*) in a barn and yard designed for restricted contact management. Structural changes in the barn and yard were made during the study period to accommodate the transition to restricted contact husbandry. From December 2013 to January 2016, nine AI procedures were performed using free contact management. Beginning in April 2016, AI procedures (6 of 15) were performed with the cow tethered, as required by the Association of Zoos and Aquariums when staff is in shared space with an elephant. As with training for AI under free contact, successive approximations involved several steps whereby the elephant was exposed to the equipment and veterinary staff while her movement was restricted. During this process, she was positively reinforced with food rewards and interaction with familiar keepers.

The AI procedure, always performed in the afternoon, involved manual removal of faeces and an enema followed by insertion of a large animal endotracheal tube into the vertical urogenital canal to serve as a channel for a 2.0 m long flexible endoscope. Insemination was either intrauterine or, in most cases, at the external cervical orifice. The duration of the manipulation was approximately 90 minutes from the enema to the withdrawal of the endoscope.

Approximately, 5.0 mL were obtained in a vacuum sampling syringe and spun in a centrifuge to separate the serum. The serum was frozen at −80̊ C until assayed for cortisol. On days when an AI was performed, a blood sample was obtained within 30 minutes after the completion of the procedure. This was always in the afternoon or evening (mean hour=18:24). On other occasions, an afternoon sample was collected to monitor hormonal status. These latter afternoon samples provided control samples (mean hour=16:06) for comparison with the AI samples. They were not timed to correspond with the AI procedures and did not bear any systematic temporal relationship to the AI procedures. The 15 control samples were selected to approximate the circadian timing of the AI samples.

Cortisol assay

The Corti-Cote (MP Biomedicals, LLC, Diagnostics Division, 13 Mountain View Ave., Orangeburg, N.Y. 10962-1294, USA) radioimmunoassay was used to determine SCC. The range of the assay was adjusted by diluting the lowest standard (1.0 µg/dL) by ¼ and eliminating the highest (60 µg/dL), providing an appropriate range for elephant SCC (Proctor et al. 2010). Otherwise, the assay was conducted according to the manufacturer's protocol. Serum cortisol concentration was read from an Isocomp-I gamma counter (MGM Instruments, Hamden, CT, USA).

Quality control

Samples were processed in paired replicates (mean CV=2.7). Precision was assessed using six, five, and seven replications of a sample at early, middle, and late stages of the study (mean CV=6.6). Interassay reliability was determined from moderate and high concentration pooled samples that were included in each run of the assay as control samples (moderate concentration CV=13.2; high concentration CV=10.7). Five replications of serial dilution distributed over 3 years of the study yielded close approximations to linear decline with mean deviation from expected concentrations of 4.1%. Mean recovery from a high concentration spike was 116% and 104% from a low spike with five replications at each concentration.

Statistical Analysis

The Schapiro-Wilk Normality test indicated SCC measurements were not normally distributed; hence a log transformation was used in the statistical analyses. A general model analysis of variance/covariance (AOV/CV) evaluated the four daily SCC results as repeated measurements, the AI and control as a between subjects comparison, and the restricted contact as a cofactor. Post-hoc pair-wise comparisons followed the significant main effect, with the significance level set at 0.05. Data manipulation and statistical analyses employed Statistix 9 (Analytical Software, Tallahassee, FL, USA).

Consequences

The solid columns in Figure 1 show the mean SCC for the morning of the day before, the morning of, the afternoon of, and the morning following AI or the control afternoon sample, respectively. A repeated measures AOV/CV yielded a statistically significant F-ratio ($F=11.36$; $df=3,84$; $p<0.0001$). Post hoc comparisons indicated that the mean of the samples taken 30 min after AI and the afternoon control samples were lower ($p<0.05$) than the other three conditions, which did not differ from each other ($p>0.05$).

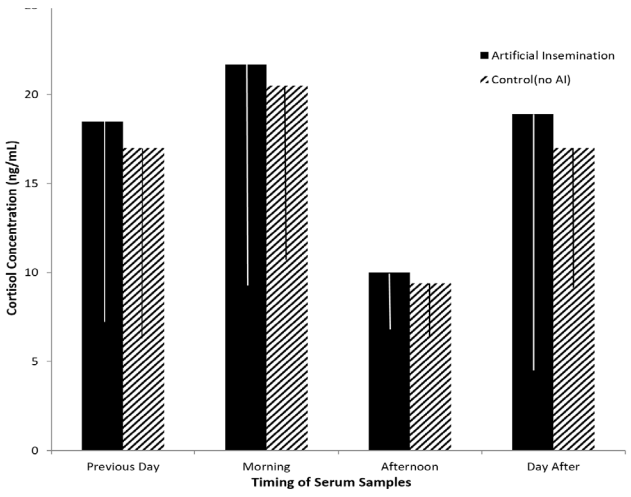

Figure 1. Serum cortisol concentration associated with artificial insemination in an African elephant. Solid columns represent mean-SD serum cortisol concentrations the morning before, the morning of, within 30 minutes of and the morning after AI events. Hatched columns indicate mean serum cortisol concentrations for similar times surrounding control afternoon serum samples that did not follow AI events. Afternoon samples were significantly lower than the other times, which did not differ from each other.

Figure 2. Change in serum cortisol concentration from morning to AI events during free contact and restricted contact procedures. The sequence of samples from left to right is the order in which the AI events occurred.

There was also no difference between the samples 30 minutes after AI and the afternoon control samples.

The most recent six AI's were conducted with the elephant tethered. The difference between the mean SCC of these six events and the mean of the earlier nine was not statistically significant. However, the first two AIs in restricted contact produced an increase in SCC from the morning sample to the sample taken 30 minutes after the procedure (see Figure 2). The remaining four restricted contact AIs had the decline from morning to after the event (mean=73.4%) that was larger than the decline during the free contact series (mean=48.8%).

The assay's biological validity is supported by evidence for a significant (paired T=5.59, df=41, p<0.0001) circadian decline in SCC from morning (mean±SD=18.9±10.7 ng/mL) to afternoon samples (10.1±6.2). In this analysis, afternoon samples (n=42) were paired with morning samples (n=42) from the same day when available or from the previous morning.

Discussion

The results show a decline in SCC from morning to afternoon on occasions when the afternoon blood sample followed the AI procedure and also when there was no AI. This decline is consistent with reported circadian declines in cortisol in the afternoon for elephants maintained in zoos (Brown et al. 2010; Cacares et al. 2016; Kelling 2008) and many other species (Van Cauter 1990; White et al. 2010). The fact that a similar decline was observed, regardless of whether an AI was performed, and that there were no statistically significant differences between AI and control SCC, suggests that the procedure does not stress this elephant as measured by SCC.

In domestic species, AI appears to have mixed impact on cortisol secretion. In dairy cattle, Nakao et al. (1994) reported a significant increase in plasma cortisol following rectal palpation and AI, but state that the pathophysiologic effect of this cortisol spike is unknown. Macalay et al. (1986) and Norrby et al. (2007) compared cortisol response following AI and natural breeding in dairy cows and sows, respectively. Both studies showed higher plasma cortisol concentrations following natural breeding than after AI. In mares, Berghold et al. (2007) concluded that gynecologic procedures can act as stressors based on faecal cortisol metabolite results. However, cortisol elevation did not influence fertility, and cortisol was not elevated in mares familiar with the AI procedure.

In the present study, it appears that during the transition from free contact to restricted contact there was an initial impact as seen by the increase in SCC during the first two events while the cow was tethered. After this initial increase, there was habituation to the restraint and the manipulation of the elephant for the AI procedure. Suedmeyer (2001) described a similar habituation in serum hydrocortisone in an African bull elephant during a series of 25 restrained manual semen collections. Conditioning was used to facilitate habituation to the restraint.

In stressful situations, cortisol is secreted into the blood by the adrenal cortex when it is stimulated by adrenocorticotrophic hormone (ACTH) from the anterior pituitary. Circulating cortisol promotes release of stored energy resources and delays inflammation and healing mechanisms as it contributes to the organism's capacity for action. In our measurement of stress-related changes in cortisol concentration, we expect to see a short latency between the stressful event and a rise in SCC. In ACTH challenge tests with elephants (Brown et al. 1995), SCC increased four-fold or more within 30 minutes and was elevated for eight hours after the last of three injections. Kelling (2008) reported an increase in salivary cortisol after an elephant was tethered in training for AI. This was training for the restraint and did not include the AI procedure. The author did not report whether this was the elephant's first experience with the restraint.

The elephant in the present study has lived at the Louisville Zoo since it was two years old and is considered to have a 'good temperament' and be very tractable by elephant staff. Nevertheless, the cow was carefully trained using positive reinforcement in gradual stages to accustom the animal to the manipulations, which likely explains the lack of cortisol response by the end of the procedure. The present study demonstrates that with systematic training, an AI can be performed without elevation of SCC and apparently without stress.

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References

Brown J.L., Göritz F, Pratt-Hawkes N., Hermes R., Galloway M., Graham L.H., Gray C., Walker S.L., Gomez A., Moreland R., Murray S., Schmitt D.L., Howard J., Lehnhardt J., Beck B., Bellem A., Montali R., Hildebrandt T.B. (2004) Successful artificial insemination of an Asian elephant at the National Zoological Park. *Zoo Biology* 23: 45-63.

Brown J.L., Kersey D.C., Freeman E.W., Wagener T. (2010) Assessment of diurnal urinary cortisol excretion in Asian and African elephants using different endocrine methods. *Zoo Biology* 29: 274-283.

Brown J.L., Wemmer C.M., Lehnhardt J. (1995) Urinary cortisol analysis for monitoring adrenal activity in elephants. *Zoo Biology* 14: 533-542.

Casares M., Silván G., Carbonell M.D., Gerique C., Martinez-Fernandez L., Cáceres S., Illera J.C. (2016) Circadian rhythm of salivary cortisol secretion in female zoo-kept African elephants (*Loxodonta africana*). *Zoo Biology* 35: 65-69.

Hildebrandt T.B., Göritz F., Hermes R., Schmitt D., Brown J.L., Schwammer H., Lostkutoff N., Pratt N.C., Lehnhardt J.L., Montali R.J., Olsen D. (1999) *Artificial insemination of African (Loxodonta africana) and Asian (Elephas maximus) elephants.* Proceedings AAZV Conference 83-86.

Kelling A.S. (2008) *An examination of salivary cortisol concentrations and behavior in three captive African elephants (Loxodonta africana) at Zoo Atlanta.* Dissertation Georgia Institute of Technology.

Macalay A.S., Roussel J.D., Seybt S.H. (1986) Cortisol response in heifers to artificial insemination, natural mating, and no mating at estrus. *Theriogenology* 26: 117-123.

Nakao T., Sato T., Moriyoshi M., Kawata K. (1994) Plasma cortisol response in dairy cows to vaginoscopy, genital palpation per rectum and artificial insemination. *Zentralbl Veterinarmed A* 41: 16-21.

Norrby M., Madsen M.T., Alexanderson C.B., Kindahl H., Madej A. (2007) Plasma concentrations of cortisol and PGF metabolite in Danish sows during mating, and intrauterine and conventional insemination. *Acta Veterinaria Scandinavica* 49: 36-42.

Proctor C.M., Freeman E.W., Brown J.L. (2010) Influence of dominance status on adrenal activity and ovarian cyclicity status in captive African elephants. *Zoo Biology* 29: 168-178.

Suedmeyer W.K. (2001) *Serum hydrocortisone levels in a manually restrained African elephant (Loxodonta africana) pre- and post-semen collection.* Proceedings AAZV, AAWV, ARAV, NAZWV Joint Conference 388-389.

Van Cauter E. (1990) Diurnal and ultradian rhythms in endocrine function in humans: a minireview. *Hormone Research* 34: 45-53.

White B.C., Jamison K.M., Grieb C., Lally L., Luckett C., Kramer K.S., Phillips J. (2010) Specific gravity and creatinine as corrections for variation in urine concentration in humans, gorillas and woolly monkeys. *American Journal of Primatology* 72: 1082-1091.