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Comparative endocrinology of testicular, adrenal and thyroid function in captive Asian and African elephant bulls

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Abstract

Concentrations of serum testosterone, cortisol, thyroxine (free and total T4), triiodothyronine (free and total T3) and thyroid stimulating hormone (TSH) were measured to assess adrenal and thyroid function as they relate to testicular activity and musth in captive elephants. Blood samples were collected approximately weekly from Asian ($n = 8$) and African ($n = 12$) bulls at seven facilities for periods of 4 months to 9.5 years. Age ranges at study onset were 8–50 years for Asian and 10–21 years for African elephants. Based on keeper logs, seven Asian and three African bulls exhibited behavioral and/or physical (temporal gland secretion, TGS, or urine dribbling, UD) signs of musth, which lasted 2.8 ± 2.5 months in duration. Serum testosterone was elevated during musth, with concentrations often exceeding 100 ng/ml. Patterns of testosterone secretion and musth varied among bulls with no evidence of seasonality ($P > 0.05$). Only three bulls at one facility exhibited classic, well-defined yearly musth cycles. Others exhibited more irregular cycles, with musth symptoms often occurring more than once a year. A number of bulls (1 Asian, 9 African) had consistently low testosterone (<10 ng/ml) and never exhibited significant TGS or UD. At facilities with multiple bulls ($n = 3$), testosterone concentrations were highest in the oldest, most dominant male. There were positive correlations between testosterone and cortisol for six of seven Asian and all three African males that exhibited musth (range, $r = 0.23$–0.52; $P < 0.05$), but no significant correlations for bulls that did not ($P > 0.05$). For the three bulls that exhibited yearly musth cycles, TSH was positively correlated (range, $r = 0.22$–0.28; $P < 0.05$) and thyroid hormones (T3, T4) were negatively correlated (range, $r = -0.25$ to -0.47; $P < 0.05$) to testosterone secretion. In the remaining bulls, there were no clear relationships between thyroid activity and musth status. Overall mean testosterone and cortisol concentrations increased with age for all bulls combined, whereas thyroid activity declined. In summary, a number of bulls did not exhibit musth despite being of adequate physical maturity. Cortisol and testosterone were correlated in most bulls exhibiting musth, indicating a possible role for the adrenal gland in modulating or facilitating downstream responses. Data were generally inconclusive as to a role for thyroid hormones in male reproduction, but the finding of discrete patterns in bulls showing clear testosterone cycles suggests they may facilitate expression or control of musth in some individuals.

Keywords: Asian elephant; African elephant; Hormones; Musth; Bulls; Testosterone; Cortisol; TSH; Thyroid hormones

1. Introduction

Captive elephant populations in North American zoos are not self-sustaining because of low reproductive rates (Wiese and Willis, 2006), a problem exacerbated by the small number of available bulls and limited breeding

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facilities. Proportionately, males make up <20% of the captive population in North America, and many are still sexually immature (Keele, 2005; Olson, 2006). Few zoos have the required space and reinforced enclosures to hold adult bulls, which can be difficult to handle during periods of musth. If we knew more about the biology of bull elephants and what controls musth, perhaps better strategies could be developed to mitigate management problems.

In general, musth is a period of heightened aggressive and sexual behavior in association with increased temporal gland secretion (TGS) and urine dribbling (UD). Elevated androgens, up to 50-fold higher than baseline, are believed to be at least partly responsible for physical and behavioral changes (Jainudeen et al., 1972a; Hall-Martin and VanDer Walt, 1984; Rasmussen et al., 1984; Cooper et al., 1990; Niemuller and Liptrap, 1991; Brown et al., 1993; Lincoln and Ratnasooriya, 1996). Although well characterized, controlling mechanisms have yet to be identified. One factor linked to the occurrence and/or intensity of musth is nutritional status. Both wild (Poole, 1989) and 'domesticated' (Jainudeen et al., 1972b) elephants drop out of musth in response to loss of body condition. Consequently, one method of decreasing unwanted symptoms is to withhold food and water (Cooper et al., 1990; Lincoln and Ratnasooriya, 1996; Ganswindt et al., 2005a).

By what means nutrition impacts musth has not been determined, but changes in metabolic function could affect testicular steroidogenesis. In some species, thyroid hormone activity decreases during fasting to lower metabolism and conserve energy (St. Aubin et al., 1996; Ortiz et al., 2001). Zoo personnel note it is not uncommon for feed intake to diminish voluntarily in bull elephants during musth, which can result in a loss of body condition. Regulation of metabolic activity in most species is driven by the hypothalamo-pituitary-thyroid axis, the control of which involves classic negative feedback (see review, Greenspan, 2004). Among ungulates, changes in thyroid activity also are associated with the reproductive cycle of seasonal breeders. Duration of the breeding season can be extended by thyroidectomy, whereas the transition from a reproductive to quiescent state is shortened by thyroid hormone administration (Parkinson and Follett, 1995; Zucker and Prendergast, 1999). Similarly, increased thyroid hormone concentrations have been observed at the seasonal transition to a nonbreeding state (Ryg and Lagnvatn, 1982; Loudon et al., 1989; Shi and Barrell, 1994). These findings suggest thyroid hormones play some role in regulating seasonal reproduction. Although musth is not seasonal, thyroid hormones might play a similar role in altering gonadal steroidogenesis through regulation of metabolic rate and the partitioning of energy resources.

Finally, cortisol is not a reproductive hormone per se, but it has been shown to increase during rut and in response to mating stimuli (Liptrap and Raeside, 1978; Howland et al., 1984; Borg et al., 1991). As reviewed by Sands and Creel (2004), increased cortisol can be associated with aggressive behavior and elevated dominance status among males. Thus, it is possible increased distraction and aggression in bull elephants during musth could be associated with heightened adrenal activity and an increase in cortisol secretion.

The objective of this study was to evaluate individual and species variability in musth characteristics and associated endocrine activity of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephant bulls in North America. Longitudinal analyses of testosterone, cortisol, thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) were conducted to determine if changes in adrenal or thyroid activity are related to testicular function and musth status.

2. Materials and methods

2.1. Animals and sample collection

This study was approved by Institutional Animal Use and Care Committees at the National Zoological Park and Riddle's Elephant and Wildlife Sanctuary. Serum samples were collected from eight Asian and 12 African elephant bulls housed at seven facilities across the United States (Table 1). At the beginning of the study, ages ranged from 8 to 50 years for Asian (mean, 27.4 ± 0.4 years), and 10–21 years for African (18.1 ± 0.1 years) bulls. Maximal ages at study end were 52 years for Asian and 24 years for African elephants. In general, bulls had olfactory and visual contact with females, and occasional physical contact. Four facilities housed more than one adult bull, three of which were same species. Bulls were housed separately with only visual and olfactory contact at Facilities A, B and C, whereas bulls at Facility D often were in physical contact. Several of the bulls were proven breeders. Three African bulls were castrated. Facilities provided animals with standard diets and *ad libitum* water, which were not altered during musth. All elephants were conditioned to blood sampling procedures as part of the management routine. Blood was collected weekly, biweekly or monthly for periods of 4 months to 9.5 years from a vein on the caudal aspect of the ear or from the saphenous vein in the leg. Blood was centrifuged (~1500*g*) within 1–3 h of collection and the serum stored at −20 °C or colder until analysis.

Musth logs were provided by each facility based on subjective observations of TGS and UD, accompanied to a greater or lesser degree by aggressive behavior. At multi-bull facilities, dominance status was subjectively determined based on behavior. At Facility D, where bulls had occassional physical contact, interactions consisted mainly of deference by subordinates to the dominant bull, but at times also included intimidation, pushing or mild aggression by the dominant individual. At Facility A and C, where bulls were kept separate, hierarchy was established by observations of behavioral posturing (bull assuming a dominant, neutral or submissive posture) and physical location within adjacent yards in reaction to cohort visual contact or scents in the enclosures. Although Facility B had two bulls (one African, one Asian), they were kept in different parts of the zoo ~0.15 km apart, so dominance status was not evaluated.

2.2. Radioimmunoassays

Each sample was analyzed for concentrations of thyroid (free and total T4, free and total T3), adrenal (cortisol), and reproductive (testosterone) hormones, and TSH using assay methods validated for elephants (Brown et al., 1993, 2004). Serum TSH was measured by heterologous ^{125}I double-antibody radioimmunoassay. The TSH assay employed an anti-ovine TSH antiserum (NIDDK-anti-oTSH-1) and human TSH label (NIDDK-hTSH-I-8) and standards (NIDDK-hTSH-RP-2) in a phosphate-buffered saline system (0.01 M PO_4, 0.5% BSA, 2 mM EDTA, 0.9% NaCl, 0.01% thimerosal, pH 7.4). Serum steroids (testosterone, cortisol) and thyroid hormones (free and total T3 and T4) were measured using solid-phase ^{125}I radioimmunoassays (Coat-A-Count; Diagnostic Products Corporation,

Table 1
Summary of bulls, sampling dates and number of serum samples analyzed in this study

Species	Facility	Animal number	Birth year	Exhibited musth	Proven breeder	Sample dates	Number of samples
Asian	A	1	1960	Yes	Yes	1/94-1/03	250
	A	2	1962	Yes	Yes	5/94-1/03	232
	A	3	1983	Yes	No	5/95-1/03	217
	B	4	1966	Yes	Yes	6/01-8/03	92
	C	5	1978	Yes	No	1/96-2/99	75
	C	6	1962	Yes	Yes	1/96-1/00	89
	D	7	1988	Yes	Yes	6/96-4/03	258
	E	8	1944	No	Yes	7/94-2/96	62
African	B	9	1980	Yes	Yes	5/01-3/03	115
	D	10	1979	No	No	8/94-12/94	12
	D	11	1979	Yes	Yes	11/93-4/03	240
	D	12	1980	No	No	10/93-4/03	383
	D	13[a]	1980	No	No	4/94-12/94	27
	D	14[a]	1980	No	No	5/94-10/94	12
	D	15	1983	No	No	4/99-7/02	107
	D	16	1983	No	No	6/96-4/03	259
	D	17	1983	No	No	11/93-4/03	381
	D	18	1984	No	No	4/01-4/03	104
	F	19	1978	Yes	No	1/97-2/00	70
	G	20[a]	1981	No	No	1/98-12/03	130

[a] Castrated.

Los Angeles, CA). All assays were validated for bull elephant serum by demonstrating: (1) parallelism between dilutions of pooled serum samples and the respective standard curve hormones; and (2) significant (>90%) recovery of exogenous standard hormone added to pooled samples before analysis. Assay sensitivities were: 0.10 ng/ml for testosterone, 0.25 ng/ml for TSH, 2.5 ng/ml for cortisol, 20 ng/dl for total T3, 1 μg/dl for total T4, 0.25 pg/ml for free T3, and 0.25 ng/dl for free T4. For all assays, intra- and interassay coefficients of variation were <10% and <15%, respectively.

2.3. Statistical analysis

Analyses were carried out using Excel and Sigma Stat (v2.03, SPSS, Inc.). Data were tested for normality using a Shapiro-Wilk test for goodness-of-fit. Data that were not normal were log transformed. Time series analyses were used to determine if there were cyclical patterns in hormone secretion. Baseline hormonal values for each individual were determined using an iterative analysis (Brown et al., 2004) where the mean and standard deviation were calculated followed by removal of all values above the mean plus two times the standard deviation (SD). This process was repeated until only those values that no longer exceeded the mean plus 2 SD remained. Comparisons between species were done using ANOVA. Changes in hormone secretory patterns throughout the year were evaluated based on seasonal means (December–February, March–May, June–August, September–November) by ANOVA, followed by pairwise comparisons using Tukey's tests. Average hormone concentrations were calculated for each bull at each year of age. Pearson Product Moment Correlation analyses were used to determine relationships between hormone concentrations within each bull. For hormones where there were no significant species differences ($P > 0.05$), data were pooled for further analyses (Brown et al., 2004). Pearson Product Moment Correlation analyses were conducted to examine bull age and hormone concentrations using overall yearly mean values for ages where $n => 2$ bulls. Boxplot analyses were conducted to identify outliers. Mean data are presented ± SEM.

3. Results

Mean concentrations of all hormones varied within and among individual bulls (Tables 2 and 3). Seven of eight Asian and three of 12 African bulls exhibited physical and behavioral signs indicative of musth, which lasted 2.8 ± 2.5 months in duration on average (range, 1–10 months per episode). There were no differences ($P > 0.05$) in hormone concentrations between species, with the exception of higher overall mean testosterone concentrations in Asian bulls due to the higher incidence of musth ($P < 0.05$). There was no species difference in baseline testosterone concentrations ($P > 0.05$). In general, there was no effect of castration on concentrations of TSH, thyroid hormones or cortisol ($P > 0.05$).

Table 2
Overall mean (±SEM) concentrations of testicular, adrenal and thyroid hormones and baseline testosterone in individual Asian elephant bulls

Elephant	Testosterone (ng/ml)	Baseline testosterone (ng/ml)	Cortisol (ng/ml)	Total T4 (μg/dl)	Free T4 (ng/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)
1	33.40 ± 1.88	1.12 ± 0.39	17.21 ± 1.52	7.78 ± 0.73	0.82 ± 0.03	105.65 ± 4.68	1.53 ± 0.12	0.56 ± 0.02
2	29.36 ± 3.30	6.79 ± 1.36	12.04 ± 1.61	7.51 ± 0.51	0.49 ± 0.02	109.19 ± 3.50	1.46 ± 0.11	0.94 ± 0.05
3	15.82 ± 1.42	1.77 ± 0.69	12.63 ± 1.25	9.97 ± 0.57	0.89 ± 0.04	139.40 ± 4.35	2.41 ± 0.09	0.58 ± 0.04
4	17.80 ± 1.44	1.14 ± 0.45	20.03 ± 1.45	5.85 ± 0.47	0.57 ± 0.03	64.81 ± 2.14	0.41 ± 0.03	0.30 ± 0.03
5	13.77 ± 2.41	0.75 ± 0.12	12.98 ± 1.56	10.08 ± 0.60	0.58 ± 0.02	95.78 ± 2.10	2.17 ± 0.10	0.99 ± 0.06
6	35.63 ± 2.86	8.83 ± 1.32	18.41 ± 1.28	7.06 ± 0.21	0.51 ± 0.01	113.13 ± 4.97	1.62 ± 0.08	0.75 ± 0.04
7	11.32 ± 0.54	6.87 ± 1.25	10.16 ± 0.54	11.01 ± 0.22	0.72 ± 0.01	113.71 ± 2.12	2.22 ± 0.04	0.90 ± 0.03
8	0.11 ± 0.01	0.10 ± 0.01	19.25 ± 1.54	10.14 ± 0.59	1.21 ± 0.04	121.86 ± 3.11	1.43 ± 0.07	0.31 ± 0.01
Overall	19.65 ± 4.31	3.42 ± 1.22	14.63 ± 1.16	8.68 ± 0.65	0.72 ± 0.09	112.15 ± 4.85	1.72 ± 0.18	0.67 ± 0.10

Table 3
Overall mean (±SEM) concentrations of testicular, adrenal and thyroid hormones and baseline testosterone in individual African elephant bulls

Elephant	Testosterone (ng/ml)	Baseline testosterone (ng/ml)	Cortisol (ng/ml)	Total T4 (μg/dl)	Free T4 (ng/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)
9	63.58 ± 3.86	8.68 ± 2.43	30.21 ± 3.34	6.36 ± 0.71	0.32 ± 0.02	99.23 ± 4.05	1.35 ± 0.12	0.34 ± 0.03
10	0.15 ± 0.03	0.10 ± 0.01	56.62 ± 6.63	12.72 ± 1.39	1.04 ± 0.06	127.93 ± 6.27	1.58 ± 0.14	0.60 ± 0.02
11	9.71 ± 1.41	4.32 ± 0.64	10.71 ± 0.55	8.07 ± 0.13	0.66 ± 0.01	110.93 ± 1.63	2.15 ± 0.05	0.62 ± 0.02
12	0.16 ± 0.01	0.11 ± 0.01	10.60 ± 0.53	10.44 ± 0.15	0.76 ± 0.01	109.00 ± 1.35	2.19 ± 0.03	0.41 ± 0.01
13[a]	<0.10	<0.10	45.43 ± 13.95	10.99 ± 0.89	0.84 ± 0.04	84.33 ± 3.64	1.41 ± 0.11	0.61 ± 0.02
14[a]	<0.10	<0.10	38.04 ± 10.01	11.56 ± 0.44	0.93 ± 0.03	84.42 ± 3.31	1.74 ± 0.12	0.57 ± 0.14
15	1.00 ± 0.08	0.87 ± 0.06	15.98 ± 1.01	8.34 ± 0.19	0.82 ± 0.02	108.18 ± 1.63	2.25 ± 0.05	0.37 ± 0.02
16	1.78 ± 0.21	1.32 ± 0.20	10.07 ± 0.54	11.97 ± 0.17	0.87 ± 0.01	122.62 ± 1.74	2.13 ± 0.04	0.46 ± 0.01
17	1.64 ± 0.08	1.21 ± 0.05	9.23 ± 0.39	10.97 ± 0.15	0.82 ± 0.01	111.33 ± 1.03	2.10 ± 0.02	0.40 ± 0.01
18	2.25 ± 0.15	1.97 ± 0.13	24.30 ± 1.45	10.61 ± 0.32	0.71 ± 0.02	129.41 ± 3.51	6.11 ± 0.36	0.28 ± 0.03
19	5.76 ± 0.31	3.13 ± 0.28	11.21 ± 0.57	5.78 ± 0.16	0.48 ± 0.01	77.78 ± 1.25	0.96 ± 0.03	0.44 ± 0.02
20[a]	<0.10	<0.10	12.86 ± 0.98	12.71 ± 0.30	0.82 ± 0.02	128.07 ± 2.53	2.69 ± 0.06	0.92 ± 0.03
Overall	8.61 ± 5.66[b]	2.18 ± 0.77[b]	23.02 ± 4.86	10.04 ± 0.68	0.76 ± 0.06	107.77 ± 5.23	2.22 ± 0.38	0.54 ± 0.05

[a] Castrated.
[b] Data excluded for Bulls 13, 14 and 20.

Musth was associated with elevated testosterone concentrations, which generally exceeded 50 ng/ml (e.g., Figs. 1 and 2). By contrast, testosterone generally was <10 ng/ml in bulls not exhibiting musth or during inter-musth periods. Yearly patterns of testosterone and musth varied among bulls with no evidence of seasonality ($P > 0.05$). Only bulls at Facility A exhibited regular yearly musth cycles (e.g., Fig. 1), which occurred on average from late winter to mid summer in the two oldest bulls (Bulls 1 and 2), followed by Bull 3 from mid summer to late fall. Other bulls exhibited less clear patterns, with increases in TGS, UD or aggressive behavior associated with elevated testosterone occurring more than once a year ($n = 6$). For example, Bull 6 exhibited increases in testosterone for several month-long periods in both the spring and fall (Fig. 2a). The other bull at that facility (Bull 5) also exhibited more than one musth period per year, which often overlapped those of Bull 6. Bull 9 had the highest overall and baseline testosterone concentrations and was described as being in a nearly constant state of musth (Fig. 2b, Table 3). In general, UD and/or TGS was observed as testosterone began to increase or within a few weeks thereafter, but rarely before.

Bulls 1, 2, 4, 5, 6, 9 and 19 began exhibiting musth before study onset. In those bulls, there were no periods of increased testosterone that were not associated with recorded musth episodes. In younger bulls (Bulls 3, 7, 11, 17, 18), age-related increases in testosterone were observed, but concentrations rarely exceeded 20 ng/ml and were not related to musth signs. Three bulls began exhibiting musth during the study period. The age of first recorded musth was 12 years for Bull 7 (Fig. 2c), 15 years for Bull 3 and 21 years for Bull 11. Testosterone concentrations in Bulls 15, 16 and 17 went from near undetectable levels at the start of the study to values that occasionally exceeded 5 ng/ml, but none showed signs of musth even though they were nearly 20 years of age at study completion. One Asian and five African bulls had overall mean concentrations of testosterone that were consistently at or below the detection limit of the assay (Tables 2 and 3). Of these, Bull 8 was the oldest, Bull 10 was in poor health and Bulls 13, 14 and 20 were castrated. Bull 12 also had consistently low testosterone (Table 3) and, based on transrectal ultrasound examinations, was considered sexually immature with underdeveloped accessory sex organs and testes.

There were positive correlations between serum cortisol and testosterone for six of seven Asian and all three African elephants that exhibited musth (e.g., Figs. 1 and 2) (average $r = 0.35 \pm 0.02$; range, 0.23 –0.52; $P < 0.05$). There were no correlations ($P > 0.05$) between testosterone and cortisol for one Asian musth bull ($r = 0.15$; $P = 0.16$) and all other bulls not exhibiting musth (range, $r = -0.11$ to 0.05; $P > 0.05$). In addition to fluctuating with musth, there was an overall increase in testosterone and cortisol secretion with respect to age ($r = 0.69$ and 0.73, respectively; $P < 0.05$) (Fig. 3). Outliers for overall mean cortisol (Bulls 10, 13, 14) were associated with males that arrived at Facility D in poor health (colic, edema, sore legs) and were euthanized.

Relationships between thyroid and testicular hormone activity in bulls that exhibited musth varied. In all three bulls at Facility A, TSH was slightly, but positively correlated ($r = 0.12$, 0.18 and 0.13; $P < 0.05$) and thyroid hormones were negatively correlated (Total T4, $r = -0.33$, -0.26 and -0.47; Free T4, $r = -0.31$, -0.34 and -0.38; Total T3, $r = -0.25$, -0.30 and -0.39; Free T3, $r = -0.27$, $-.0.31$ and -0.31; $P < 0.05$) to testosterone ($P < 0.05$). Fig. 1 illustrates the relationships between testosterone and TSH and Total T4 in two of these bulls. In general, Total T4 concentrations peaked just before or as testosterone began to increase, and declined to baseline by about mid-musth. By contrast, TSH increased after the rise in testosterone, so that peak concentrations occurred at mid-musth or shortly thereafter. For all other bulls, patterns of thyroid activity were irregular throughout the year and did not correspond to changes in testosterone secretion. There was a significant seasonal effect on T3 and T4, with lower concentrations occurring during the summer months in African ($P < 0.05$), but not in Asian ($P = 0.08$) bulls. There also was a decrease ($P < 0.05$) in thyroid hormone secretion as a function of age

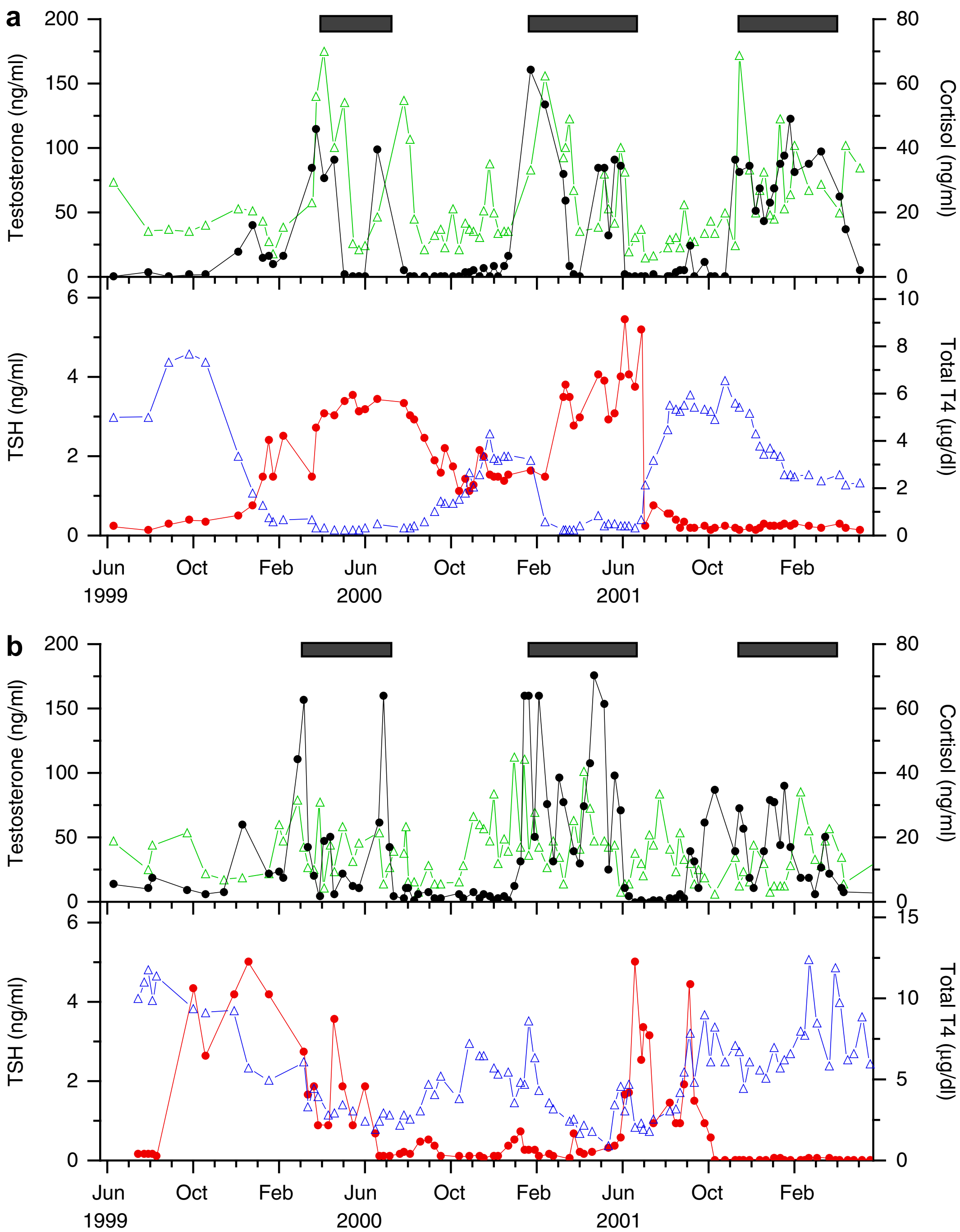

Fig. 1. Individual profiles of serum testosterone (●) and cortisol (△) concentrations in the upper panel, and TSH (●) and Total T4 (△) concentrations in the lower panel for Bull 1 (a) and Bull 2 (b) housed at Facility A showing cyclic changes in secretory hormone patterns. Black bars represent recorded periods of musth.

($r = -0.74$ for Total T4, -0.86 for Free T4, -0.47 for Total T3 and -0.49 for Free T3) (Fig. 3). TSH, however, did not vary significantly with age or season ($P > 0.05$). Positive correlations ($P < 0.05$) among thyroid hormones indicated co-secretory relationships between T3 and T4 and also between free and total hormone forms. Correlations (mean ± SEM, range) were as follows: Total T3 and Free T3 (0.58 ± 0.09, 0.14–0.88), Total T3 and Total T4 (0.29 ± 0.08, 0.19–0.53), Total T3 and Free T4 (0.38 ± 0.07, 0.26–0.59), Free T3 and Total T4 (0.32 ± 0.09, 0.23–0.57), Free T3 and Free T4 (0.31 ± 0.07, 0.29–0.59) and Total T4 and Free T4 (0.54 ± 0.06, 0.23–0.75).

Comparing same species bull groups within a facility ($n = 3$), the oldest, most dominant males had the highest ($P < 0.05$) concentrations of testosterone. For the Asians at Facility A, overall testosterone concentrations were in rank order to age and dominance status (Bull 1 ≈ Bull 2 > Bull 3) (Table 2). At Facility D, the dominant African bull (Bull 11) was the oldest and also had the highest overall and baseline testosterone concentrations (Table 3). At Facility

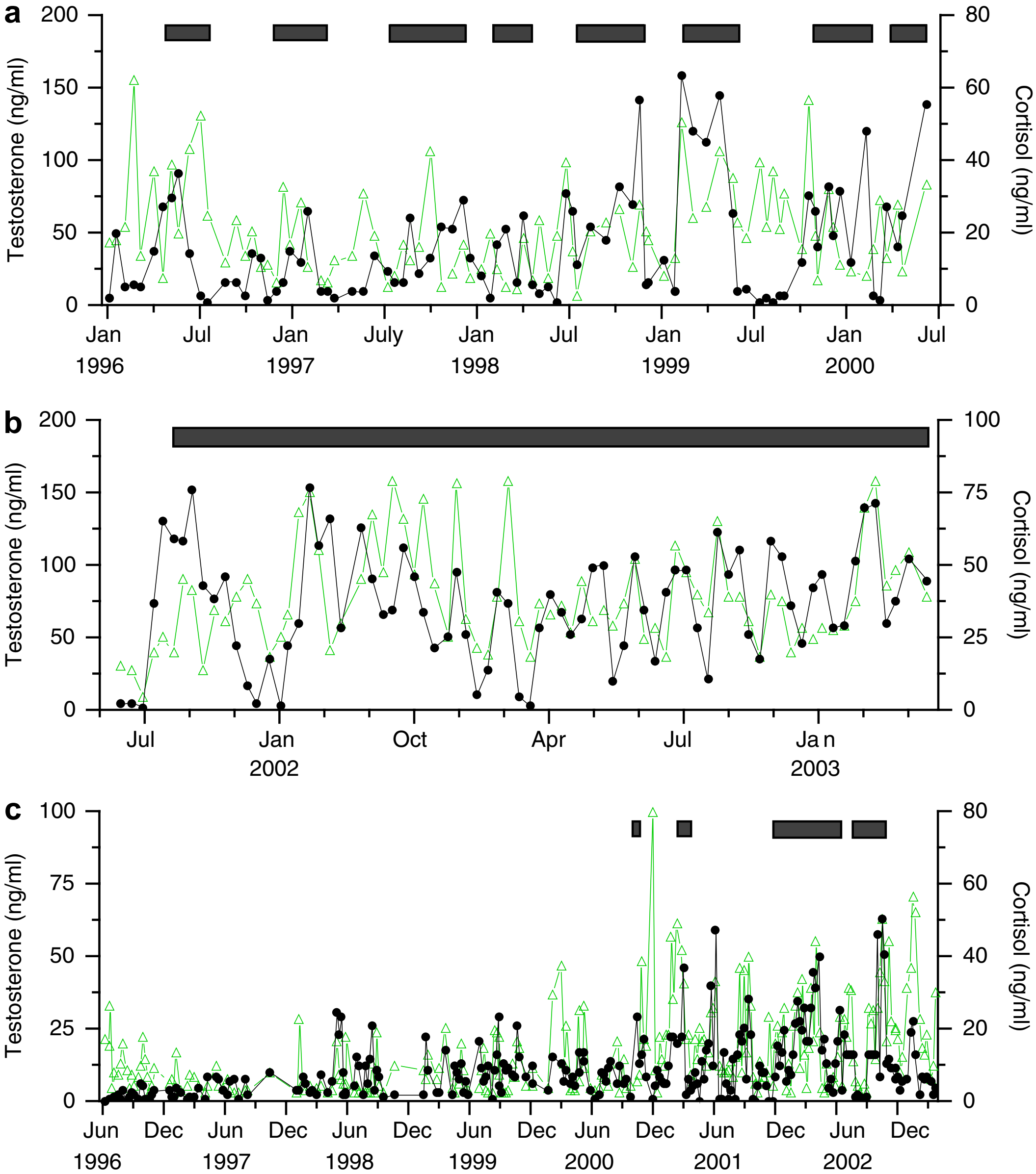

Fig. 2. Individual profiles of serum testosterone (●) and cortisol (△) concentrations for Bull 6 at Facility C, Bull 9 at Facility F and Bull 7 housed at Facility D showing among bull variations in hormone secretion and incidence of musth activity. Black bars represent recorded periods of musth.

C, overall and baseline testosterone concentrations in the dominant Bull 6 were over 2- and 10-fold greater, respectively, than those observed in the younger, subordinate Bull 5 (Table 2). There were no clear relationships between age or dominance status for the other hormones evaluated ($P > 0.05$).

4. Discussion

By examining a number of bulls over extended periods of time it was possible to begin assessing the degree of individual and species variability in testicular activity and musth, and how they relate to adrenal and thyroid function. Significant co-relationships between adrenal and testicular activity were identified, whereas findings related to thyroid function were more difficult to interpret. We were somewhat surprised to find only about half the bulls showed obvious physical (TGS and UD), physiological (increased testosterone) and/or behavioral (aggression) signs of musth, despite being of adequate age (⩾20 years) and physical maturity. For most of those bulls, musth occurrences were random, with many exhibiting multiple episodes per year. This differs from the more predictable, yearly musth cycle described for other groups of elephants, both captive and wild (Jainudeen et al., 1972a; Hall-Martin and VanDer Walt, 1984; Cooper et al., 1990; Lincoln and Ratnasooriya, 1996). Just three Asian bulls exhibited well-defined yearly cycles, and all were housed at the same facility. Two of these bulls were older (42 and 44 years at study onset), which may have been a factor, but the third was comparatively young (age 15 years at first musth). There were no obvious differences in environment, management, exposure

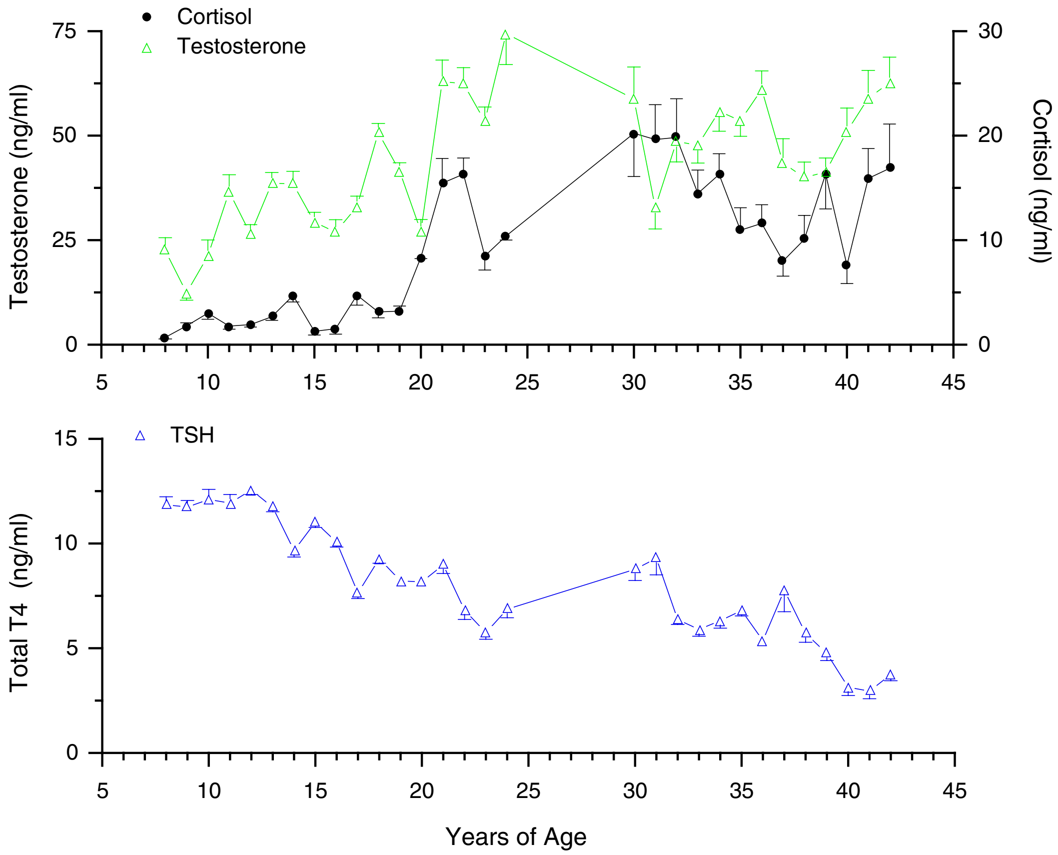

Fig. 3. Overall mean concentrations of serum testosterone (●) and cortisol (△) in the upper panel, and Total T4 (△) in the lower panel as a function of age for all Asian and African bulls combined, where $N > 2$.

to females for breeding or body condition, so there is no explanation for why only this group exhibited more traditional musth patterns and the other bulls did not. Rather these findings serve to indicate the extent of biological variation that exists among captive elephant bulls, which may be related to facility as well as individual differences.

Age, nutrition and social status all can impact the expression of musth. In terms of age, musth does not occur in wild bulls until after they reach their mid 20's, and bouts tend to be short and sporadic until the mid 30's (Poole, 1989, 1994). However, in captivity bulls often exhibit musth much earlier (Cooper et al., 1990; Niemuller and Liptrap, 1991; Ganswindt et al., 2005a). In this study, several bulls $\leqslant$15 years of age were exhibiting musth at study onset and three began during the study at 12, 15 and 21 years of age. Across all bulls, there was a marked increase in testosterone secretion at around 20 years of age, although age clearly was not the only factor determining if a bull exhibited musth. The early sexual maturation and multiple episodes of observed musth in some captive bulls presumably is due to overall better nutrition and perhaps a lack of environmental stressors (Jainudeen et al., 1972b; Poole, 1989). Cooper et al. (1990) reported that food restriction delayed the yearly cycle and reduced the intensity of musth in a captive Asian bull. In our study, zoos reported food intake often decreased voluntarily during musth, although weight logs were not available to determine if significant changes in body weight occurred.

Social factors also can influence musth expression. In comparing testosterone data for bulls in multi-bull facilities, there was a direct relationship between overall concentrations, age and social rank not unlike that reported for captive (Jainudeen et al., 1972b; Niemuller and Liptrap, 1991; Lincoln and Ratnasooriya, 1996) and wild (Hall-Martin and VanDer Walt, 1984) elephants. The higher proportion of Asian bulls exhibiting musth may be due to the age differential, but there is some indication, albeit mostly anecdotal, that captive African elephants may not exhibit musth as early as Asian elephants (Rasmussen et al., 1984). In one published comparison, an Asian bull began showing musth at 7 years of age, whereas an African bull at the same facility did not exhibit musth until 17 years of age (Cooper et al., 1990). There also may be species differences in bulls' responses to social factors. All Asians in multi-bull groups exhibited musth regardless of dominance status, whereas at the one facility with multiple Africans, it occurred only in the dominant bull. Ganswindt et al. (2005a) similarly reported social suppression by a dominant bull might have been responsible for lack of musth cycles in a captive 18-year-old African bull. Clearly more balanced studies are needed, but it is tempting to speculate that African elephants may be more sensitive to group pressures, at least in captivity. In females, ovarian acyclicity is related to social status, but only in African elephants, although in that example it is the dominant individual that does not cycle (Brown, 2006).

Temporally, TGS and/or UD occurred one to several weeks after the rise in testosterone with TGS almost always observed before UD, as previously described (Lincoln and Ratnasooriya, 1996; Ganswindt et al., 2005a,b). There was a comparable lag between peak testosterone and behavioral "attitude" changes as well, not unlike that described for Soay sheep, where maximal aggression occurred after androgens began to decrease (Lincoln and Davidson, 1977). Humans also exhibit increased aggression, anger and irritability after a temporary reduction in testosterone (Bagatell et al., 1994). Thus from a management standpoint, detection of elevated androgens may be useful as an early indicator of impending musth. However, not all increases in testosterone are associated with subsequent musth behaviors (Lincoln and Ratnasooriya, 1996; Ganswindt et al., 2005a,b; this study), so the predictive value may be limited.

Of the bulls that exhibited musth, there were modest, but significant positive correlations between circulating testosterone and cortisol in all but one. In a preliminary study, Wingate and Lasley (2002) reported similar correlations in captive Asian and African elephants. Positive relationships also have been reported in other mammalian species (Liptrap and Raeside, 1968, 1978; Howland et al., 1984; Borg et al., 1991). In boars, increases in testosterone secretion were observed after administration of ACTH (Liptrap and Raeside, 1978), whereas no such androgen responses were observed after ACTH in adrenalectomized males (Liptrap and Raeside, 1968), suggesting some androgenic activity might be of adrenal origin (Wingate and Lasley, 2002). Dexamethasone suppression tests might be useful in exploring a possible involvement of the adrenal-testicular axis in musth. However, a larger question is why these findings are in stark contrast to fecal hormone data where negative correlations were found between excreted corticoid and androgen metabolites in African elephant bulls (Ganswindt et al., 2003, 2005a,b). These researchers concluded that musth does not represent a physiological stress mediated by the HPA axis, and indeed suggested there may be a negative effect of elevated androgens and/or the musth condition on adrenal function (Ganswindt et al., 2005a,b). The only obvious difference between these studies is the measurement of circulating versus excreted steroids. One advantage to serum analyses is they measure native hormone, and there is no need to find antibodies that recognize biologically relevant excreted metabolites. The serum RIAs used in the present study measured bound and unbound hormones and were very specific; i.e., the cortisol antibody does not crossreact with testosterone and vice versa (company literature). In addition, there were a number of examples where surges in testosterone or cortisol were not correlated. But Ganswindt et al. (2003, 2005a,b) also were careful to use properly validated assays, specific for fecal corticoid and androgen metabolites. One possibility is that fecal corticoids may not be the best way to monitor adrenal activity. After radiolabelled infusion, only ~15% of corticoid metabolites were excreted in feces; the majority of which were found in urine (Ganswindt et al., 2003). HPLC analyses further showed that different EIAs detected varying proportions of several corticoid metabolites (Ganswindt et al., 2003). So, perhaps either not enough corticoid metabolites are excreted in feces to detect significant changes, or by having to use an EIA that does not crossreact with androgens, some biologically relevant metabolites are not accurately measured. Alternatively, there may be changes in the ratios of circulating free steroid to that bound to corticosteroid binding globulin (CBG). If so, that may impact the metabolism and/or excretion of corticoids in urine or feces at any given time. The only way to resolve this apparent conflict is to conduct studies that evaluate profiles of circulating and excreted (urine and feces) adrenal and testicular steroids concomitantly.

With regard to thyroid activity, relatively clear patterns were observed only in the three bulls that exhibited clearly defined yearly musth cycles. In those individuals, TSH was significantly correlated positively and T3 and T4 were correlated negatively to testosterone concentrations. Specifically, increases in thyroid hormones preceded the rise in testosterone associated with musth onset. Thereafter, thyroid hormone concentrations declined and reached nadir concentrations in conjunction with the return of testosterone to baseline. Negative feedback of thyroid hormones on TSH presumably were responsible for the inverse relationships observed among these hormones. Such relationships between T3 and/or T4 hormones and testicular function have been reported in other species, as males transition from a breeding to a nonbreeding state (Boissin-Agasse et al., 1980; Maurel and Boissin, 1981; Shi and Barrell, 1994). Temporally this was not unlike that observed in the three bulls where thyroid hormones decreased as bulls were coming out of musth. Thus, an increase in thyroid hormone hormones preceeding musth may be necessary for increased metabolic activity in preparation for other physical and physiological changes associated with musth. Such a relationship between thyroid hormones and testosterone in the other bulls, however, was not observed. Nevertheless, this intriguing finding suggests further investigations are warranted to examine possible cause and effect relationships related to the hypothalamo-thyroid-testicular axis in elephants. It is possible that alterations in thyroid activity may not occur without regular or predictable changes in testicular activity (or vice versa). Perhaps musth cycles occurring too close together do not allow the hypothalamo-pituitary-thyroid-gonadal axis to reset in time for the next wave of activity. Obviously, musth can occur without the involvement of thyroid hormones. As more bulls in the population age and mature, perhaps further examinations will lead to an explanation for the differing hormone patterns observed among these bulls.

Mean thyroid hormone concentrations varied seasonally in captive elephants, significantly for Africans, but only tending towards significance in Asians. In both species, total and free T3 and T4 concentrations decreased slightly during the summer months (June – August), not unlike that in other reports where seasonal decreases in metabolism and thyroid function occur in response to increased air and body temperature (Greenspan, 2004; Silva, 2006). Bulls have a relatively low surface to volume ratio and do not need a high metabolic rate to maintain body temperature in warmer summer months. Mean total and free thyroid hormone concentrations also decreased with age, which is supported by findings in other mammals (St. Aubin et al., 1996; Greenspan, 2004).

In summary, the variation in temporal hormone and musth patterns makes it clear there is no one profile that fits all bulls. However, data suggest that singleton or dominant individuals can exhibit musth as early as 7, or at least by 20 years of age. We also found that musth occurred when testosterone exceeded 50 ng/ml and that there was an overall good correlation between testosterone and cortisol concentrations in bulls exhibiting musth. Although the relationship is far from clear, thyroid hormones might play a role in testicular steroidogenic activity, but perhaps only in bulls with consistent yearly musth cycles. Despite this being among the largest captive study population to date, it is clear that even larger, more controlled studies are needed to determine the degree to which sexual, adrenal, thyroid and gonadal activity are related to species, age, social status, husbandry and nutrition in bull elephants. From these results, there does not appear to be any benefit of modulating musth symptoms through control of thyroid activity. Rather, suggested strategies could focus on treatments to reduce unwanted behaviors by suppressing testosterone action either directly (e.g., antiandrogens like flutamide or cyproterone acetate) or indirectly through the inhibition of LH secretion (e.g., GnRH analogs). Studies along this line have been limited to date, and it still is not clear if any of these approaches would be of practical value (Brown, 2006). Given the young age of the captive Asian (average, 20.7; median, 18.0, standard deviation, 13.9 years; $n = 43$) and African (average, 20.0; median, 23.0; standard deviation, 10.1 years; $n = 31$) bull populations in North America (Keele, 2005; Olson, 2006), a significant number will reach sexual maturity within the next decade. While this may prove challenging to managers, it undoubtedly will provide tremendous opportunities to do more comparative research on the physiology of musth.

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References

Bagatell, C.J., Heiman, J.R., Rivier, J.E., Bremmer, W.J., 1994. Effects of endogenous testosterone and estradiol on sexual behaviour in normal young men. J. Clin. Endocrinol. Metab. 78, 711–716.

Boissin-Agasse, L., Maurel, D., Boissin, J., 1980. Seasonal variations in thyroxine and testosterone levels in relation to the moult in the adult male mink (*Mustela vison*, Peale and Beauvois). Can. J. Zool. 59, 1062–1066.

Borg, K.E., Ebenshade, K.L., Johnson, B.H., 1991. Cortisol, growth hormone, and testosterone concentrations during mating behavior in the bull and boar. J. Anim. Sci. 69, 3230–3240.

Brown, J.L., Bush, M., Wildt, D.E., Raath, J.R., deVos, V., Howard, J.G., 1993. Effects of GnRH analogues on pituitary-testicular function in free-ranging Africn elephants (*Loxodonta africana*). J. Reprod. Fertil. 99, 627–634.

Brown, J.L., Walker, S.L., Moeller, T., 2004. Comparative endocrinology of cycling and noncycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Gen. Comp. Endocrinol. 136, 360–370.

Brown, J.L., 2006. Reproductive endocrinology. In: Mikota, S., Fowler, M. (Eds.), The Biology, Medicine and Surgery of Elephants. Blackwell Publishing, Ames, IA, pp. 377–388.

Cooper, K.A., Harder, J.D., Clawson, D.H., Frederick, D.L., Lodge, G.A., Peachey, H.C., Spellmire, T.J., Winstel, DP., 1990. Serum testosterone and musth in captive male African and Asian elephants. Zoo Biol. 9, 297–306.

Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., Hodges, J.K., 2003. Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. Gen. Comp. Endocrinol. 134, 156–166.

Ganswindt, A., Heistermann, M., Hodges, J.K., 2005a. Physical, physiological, and behavioral correlates of musth in captive African elephants (*Loxodonta africana*). Physiol. Biochem. Zool. 78, 505–514.

Ganswindt, A., Rasmussen, H.B., Heistermann, M., Hodges, J.K., 2005b. The sexually active states of free-ranging male African elephants (*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behavior. Horm. Behav. 47, 83–91.

Greenspan, F.S., 2004. The thyroid gland. In: Greenspan, F.S., Gardner, D.G. (Eds.), Basic & Clinical Endocrinology, 7th ed. Lange medical Books/McGraw-Hill, New York, pp. 215–294.

Hall-Martin, A.J., VanDer Walt, L.A., 1984. Plasma testosterone levels in relation to musth in the male African elephant. Koedoe 27, 147–149.

Howland, B.E., Sanford, L.M., Palmer, W.M., 1984. Changes in serum levels of LH, FSH, prolactin, testosterone, and cortisol associated with season and mating in male pygmy goats. J. Androl. 6, 89–96.

Jainudeen, M.R., Katongole, C.B., Short, R.V., 1972a. Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elephas maximus*. J. Reprod. Fertil. 29, 99–103.

Jainudeen, M.R., McKay, G.M., Eisenberg, J.F., 1972b. Obervations on musth in the domesticated Asiatic elephant (*Elephas maximus*). Mammalia 36, 247–261.

Keele, M., (ed). 2005. Asian Elephant North American Regional Studbook. Portland, OR.

Lincoln, G.A., Davidson, W., 1977. The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle in rams, and the influence of photoperiod. J. Reprod. Fertil. 49, 267–276.

Lincoln, G.A., Ratnasooriya, W.D., 1996. Testosterone secretion, musth behavior and social dominance in captive male Asian elephants living near the equator. J. Reprod. Fertil. 108, 107–113.

Liptrap, R.M., Raeside, J.I., 1968. Effect of corticotropin and corticosteroids on plasma interstitial cell-stimulating hormone and urinary steroids in the boar. J. Endocrinol. 42, 33–41.

Liptrap, R.M., Raeside, J.I., 1978. A relationship between plasma concentrations of testosterone and corticosteroids during sexual and aggressive behavior in the boar. J. Endocrinol. 76, 75–81.

Loudon, A.S.I., Milne, J.A., Curlewis, J.D., McNeilly, A.S., 1989. A comparison of the seasonal hormone changes and patterns of growth, voluntary food intake and reproduction in juvenile and adult red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*) hinds. J. Endocrinol. 122, 733–745.

Maurel, D., Boissin, J., 1981. Plasma thyroxine and testosterone levels in the red fox (*Vulpes vulpes* L.) during the annual cycle. Gen. Comp. Endocrinol. 43, 402–404.

Niemuller, C.A., Liptrap, R.M., 1991. Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (*Elephas maximus*). J. Reprod. Fertil. 91, 136–146.

Olson, D.J., 2006. African Elephant North American Regional Studbook. Indianapolis Zoo, Indianapolis, IN.

Ortiz, R.M., Wade, C.E., Ortiz, C.L., 2001. Effects of prolonged fasting on plasma cortisol and TSH in postweaned northern elephant seal pups. Am. J. Physiol. 280, 790–795.

Parkinson, T.J., Follett, B.K., 1995. Thyroidectomy abolishes seasonal testicular cycles of Soay rams. Proc. Royal Soc. Lond. 259, 1–6.

Poole, J.H., 1989. Announcing intent: the aggressive state of musth in African elephants. Anim. Behav. 37, 140–152.

Poole, J.H., 1994. Sex differences in the behavior of African elephants. In: Short, R.V., Balaban, E. (Eds.), The Differences Between the Sexes. Cambridge University Press, Cambridge, pp. 331–346.

Rasmussen, L.E., Buss, I.O., Hess, D.L., Schmidt, M.J., 1984. Testosterone and dihydrotestosterone concentrations in elephant serum and temporal gland secretions. Biol. Reprod. 30, 352–362.

Ryg, M., Lagnvatn, R., 1982. Seasonal changes in weight gain, growth hormone, and thyroid hormones in male red deer (*Cervus elaphus atlanticus*). Can. J. Zool. 60, 2577–25810.

Sands, J., Creel, S., 2004. Social dominance, aggression and faecal clucocorticoid levels in a wild population of wolves, *Canis lupis*. Anim. Behav. 67, 387–396.

Shi, Z.D., Barrell, G.K., 1994. Thyroid hormones are required for the expression of seasonal changes in red deer (*Cervus elaphus*) stags. Reprod. Fertil. Dev. 6, 187–192.

Silva, J.E., 2006. Thermogenic mechanisms and their hormonal regulation. Physiol. Rev. 86, 435–464.

St. Aubin, D.J., Ridgway, S.H., Wells, R.S., Rhinehart, H., 1996. Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. Marine Mamm. Sci. 12, 1–13.

Wiese, R.J., Willis, K., 2006. Population management of zoo elephants. Int. Zoo Yrbk. 40, 80–87.

Wingate, L., Lasley, B., 2002. Is musth a reproductive event: an examination of arguments for and against this view. In: Schwammer, H.M., Foose, T.J., Fouraker, M., Olson, D. (Eds.), International Elephant and Rhino Research Symposium. Schüling Verlag, Münster, pp. 150–156.

Zucker, I., Prendergast, B.J., 1999. Circannual rhythms. In: Knobil, E., Neill, J.D. (Eds.), Encyclopedia of Reproduction. Academic Press, New York, pp. 620–627.