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Comparative endocrinology of cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants

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Abstract

Up to 14% of Asian and 29% of African elephants in captivity are not cycling normally or exhibit irregular cycles based on progestin profiles. To determine if ovarian acyclicity is related to other disruptions in endocrine activity, serum pituitary, thyroid, adrenal, and ovarian hormones in weekly samples collected for 6–25 months were compared between normal cycling ($n = 22$ each species) and non-cycling ($n = 6$ Asian; $n = 30$ African) elephants. A subset of cycling females ($n = 4$ Asian, 7 African) also were blood sampled daily during the follicular phase to characterize the peri-ovulatory period. In normal cycling females, two leutinizing hormone (LH) surges were observed 3 weeks apart during a normal follicular phase, with the second inducing ovulation (ovLH). Serum FSH concentrations were highest at the beginning of the non-luteal phase, declining to nadir concentrations within 4 days of the ovLH surge. FSH remained low until after the ovLH surge and then increased during the luteal phase. A species difference was noted in prolactin secretion. In the African elephant, prolactin was increased during the follicular phase, but in Asian elephants concentrations remained stable throughout the cycle. Patterns of thyroid hormones (thyroid-stimulating hormone, TSH; free and total thyroxine, T4; free and total triiodothyronine, T3) and cortisol secretion were not affected by estrous cycle stage or season in cycling elephants. In non-cycling elephants, there were no fluctuating patterns of LH, FSH, or prolactin secretion. Overall mean concentrations of all hormones were similar to those in cycling animals, with the exception of FSH, prolactin, and estradiol. Mean serum FSH concentrations were lower due to females not exhibiting normal cyclic increases, whereas serum estradiol was higher overall in most acyclic females. Prolactin concentrations were significantly increased in 11 of 30 non-cycling females, all of which were African elephants. In sum, while there were no consistent endocrine anomalies associated with ovarian acyclicity, hyperprolactinemia may be one cause of ovarian dysfunction. The finding of elevated estrogens in some acyclic females also deserves further investigation, especially determining how it relates to reproductive tract pathologies.

Keywords: Ultrasound; Ovarian cyclicity; Reproductive dysfunction; Hormones; Progestins

1. Introduction

Despite the well-recognized need to establish self-sustaining populations of captive elephants (Olson and Wiese, 2000; Wiese, 2000), less than 20% of Asian and 10% of African elephants of reproductive age have produced offspring (Asian Elephant Studbook, 2000, 2001). The logistics and expense of transporting females to breeding facilities have hampered captive breeding efforts, but there also are reproductive problems of physiological origin. Through basic progestin monitoring encouraged by the Elephant Taxon Advisory Group/Species Survival Plan, many female elephants of reproductive age have been identified as 'flatliners,' a term given to describe the observation of stable, baseline concentrations of serum progestins indicative of ovarian inactivity (Brown, 2000). Based on a recent survey, up to 14% of Asian and 29% of African elephants in North America are not cycling normally (Brown et al., in press).

There are many possible causes for ovarian inactivity, including reproductive tract pathologies (hereditary or idiopathic) and neoplasias, hormone receptor dysfunction, metabolic or nutritional deficiencies, stress, and hypothalamic–pituitary disruptions (Knobil and Neill, 1998). The probability that the etiology of acyclicity is

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the same for all females is unlikely, however, there may be common symptoms that can be identified. Given the complexity of the endocrine control of reproduction, it is possible that ovarian acyclicity could be related to a hormonal imbalance. Thus, the objective of this study was to determine if there were differences in hormone secretory patterns related to pituitary function (luteinizing hormone, LH; follicle-stimulating hormone, FSH; thyroid-stimulating hormone, TSH; prolactin), ovarian activity (estradiol), thyroid function (free and total thyroxine, T4; free and total triiodothyronine, T3), and adrenal status (cortisol as an indicator of stress) between normal cycling and non-cycling Asian and African elephants.

2. Materials and methods

2.1. Animals and sample collection

Serum samples, collected weekly, biweekly or monthly for periods of 6–25 months, from normal cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephant females were obtained for this study (Table 1). A subset of females were sampled daily for characterization of follicular phase hormone dynamics (Asian: $n = 4$ females, 7 cycles; African: $n = 7$ females, 15 cycles). In general, samples were collected from a vein on the caudal aspect of the ear while the cow was in lateral recumbency, or from the saphenous vein in the leg. All elephants were well-conditioned to the blood sampling procedure which was part of the management routine. Blood was maintained at ~4 °C and centrifuged (~1500*g*) within a few hours of collection and the serum stored at −20 °C or colder until analysis. Samples were selected from those banked at the CRC (1991-present) or were obtained upon request. Attempts were made to analyze recently collected samples; however, for some animals samples up to 7 years old were used. Non-cycling elephants were categorized based on the lack of a cyclic progestin profile for a minimum 1-year period before initiation of this study. Overall, elephants were evaluated for 12.4 ± 1.2 months with an average of 52.3 ± 4.3 samples analyzed per female.

Each serum sample was analyzed for concentrations of thyroid (free and total T4, free and total T3), adrenal (cortisol), ovarian (estradiol, progestin), and pituitary (LH, FSH, TSH, and prolactin) hormones. Assays were previously validated for elephants (Brown and Lehnhardt, 1995, 1997; Brown et al., 1991, 1999a,b), with the exception of those for thyroid hormones and TSH which were validated in this study.

2.2. Hormone iodinations

Highly purified prolactin (NIDDK-oPRL-I-2), LH (LER-1374-A), FSH (LER-1976-A2), and TSH (NIDDK-hTSH-I-8) were iodinated using chloramine-T. For each iodination, 5 μg of hormone (in 20 μl of 0.5 M $NaPO_4$, pH 7.6) was incubated with 1 mCi carrier-free Na-^{125}I (10 μl) for 1 min in the presence of 2.5 μg chloramine-T (10 μl in 0.05 M $NaPO_4$, pH 7.6). The reaction was stopped with sodium metabisulfite (10 μg in 10 μl of 0.05 M $NaPO_4$, pH 7.6) and labeled hormone separated from free ^{125}I using anion-exchange chromatography (AG 2-X8, 100–200 mesh, chloride form; Bio-Rad Laboratories, Melville, NY). Columns (1×5 cm) were prepared by equilibrating resin in 0.05 M $NaPO_4$ (pH 7.6) followed by successive elution with 2 ml each of 0.5 M, 0.05 M with 5% bovine serum albumin (BSA), and 0.05 M $NaPO_4$ (pH 7.6). The iodination reaction mixture was layered onto the column and labeled hormone eluted with 2 ml of 0.05 M $NaPO_4$ into a tube containing 1 ml phosphate-buffered saline (PBS) with 0.1% BSA (pH 7.4).

2.3. Radioimmunoassays

Serum prolactin, LH, FSH, and TSH were measured by heterologous ^{125}I double-antibody radioimmunoassays (RIA). All assays were conducted using a PBS–BSA buffer system (0.01 M PO_4, 0.5% BSA, 2 mM EDTA,

Table 1
Mean (±SEM) and range for age, sampling period, and number of samples per female of individual cycling and non-cycling Asian and African elephant females

	Asian		African	
	Cycling ($n = 22$)[a]	Non-cycling ($n = 6$)	Cycling ($n = 22$)	Non-cycling ($n = 30$)
Age (years)	28.7 ± 2.5 (16–45)[b]	27.8 ± 4.0 (20–47)	23.2 ± 2.6 (15–40)	26.6 ± 2.0 (14–42)
Sampling period (months)	9.2 ± 1.1 (6–17)	10.2 ± 1.8 (6–12)	9.8 ± 0.9 (6–14)	10.1 ± 1.2 (6–25)
Number of samples per female[c]	46.4 ± 5.9 (15–102)	43.2 ± 9.2 (20–98)	77.7 ± 7.3 (24–136)	45.6 ± 7.5 (12–152)

[a] Number of individual animals.
[b] Data range.
[c] Values do not include LH surge data from daily samples collected during the follicular phase.

0.9% NaCl, and 0.01% thimerosal, pH 7.4), with the exception of second antibody where BSA was omitted. The prolactin assay employed an anti-human prolactin antiserum (NIDDK-anti-hPRL-3) and ovine prolactin label and standards (NIDDK-oPRL-I-2). The LH assay employed a monoclonal anti-bovine LH antiserum (518-B7) and ovine LH label and standards (NIH-oLH-26). The FSH assay employed an anti-ovine FSH antiserum (JADLER #178) and ovine FSH label and standards (NIDDK-FSH-S16). The TSH assay employed an anti-ovine TSH antiserum (NIDDK-anti-oTSH-1) and human TSH label and standards (NIDDK-hTSH-RP-2). The prolactin, LH, and FSH assays were incubated at room temperature over a 3-day period, whereas the TSH assay was conducted in a cold room (4 °C). Serum steroids (progesterone, cortisol, total T4, free T4, total T3, and free T3) were measured by solid-phase ^{125}I RIA (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). Estradiol was quantified using an ultrasensitive double-antibody ^{125}I RIA (DLS-39100; Diagnostic Systems Laboratories, Webster, TX). All assays were validated for elephant serum by demonstrating: (1) parallelism between dilutions of pooled serum samples to the respective standard curve preparations and (2) significant (>90%) recovery of exogenous standard hormone added to pooled samples before analysis. Assay sensitivities were as follows: 0.3 ng/ml for LH, 0.5 ng/ml for FSH, 1.0 ng/ml for PRL, 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, 0.25 ng/dl for free T4, 0.05 ng/ml for progesterone, and 5 pg/ml for estradiol. For all protein and steroid assays, intra- and interassay coefficients of variation were <10 and <15%, respectively.

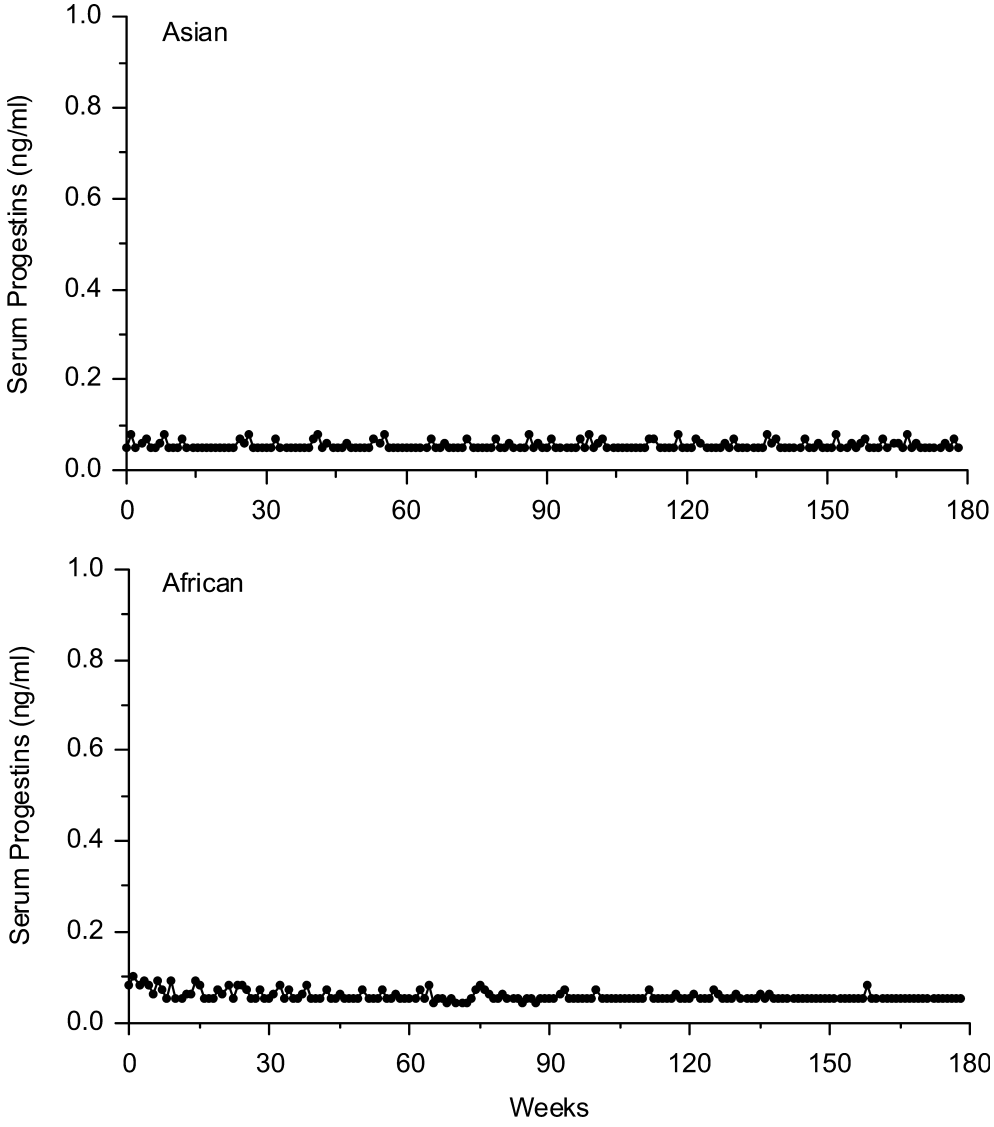

Fig. 1. Representative profiles of serum progestins in a non-cycling Asian (top panel) and African (bottom panel) elephant female.

2.4. Statistical analysis

Statistical analysis of data was performed using Sigma Stat version 2.03 (SPSS). Time series analysis was used to determine if there were cyclical patterns in hormone secretion. Overall mean values per individual were used for comparison between groups of elephants and were analyzed using one-way ANOVA. For elephants evaluated over a continuous 1-year period (Asian: 8 cycling, 4 non-cycling; African: 9 cycling, 11 non-cycling), means also were evaluated across seasons (December–February, March–May, June–August, and September–November). Data were tested for normality using a Shapiro-Wilk test for goodness-of-fit. For normally distributed data, pairwise comparisons were made using Tukey's tests. When normality tests failed, Kruskal–Wallis one-way Analysis of Ranks were employed and, subsequently, Dunn's method for pairwise comparisons. Baseline hormonal values were calculated using an iterative analysis (Brown et al., 1999b). Means and standard deviations were calculated followed by removal of all values above the mean plus two times the standard deviation. This process was repeated until only those values that did not exceed the mean plus two standard deviations remained. Mean values were considered outliers when values were greater than three interquartile ranges. Linear regressions were conducted to evaluate hormonal relationships within individual females. Comparisons among animals and between species were done using ANOVA on individual means. Mean data are ±SEM.

3. Results

Examples of progestin profiles in non-cycling Asian and African elephants are presented in Fig. 1. Concentrations generally remained below 0.1 ng/ml for the duration of the study. Figs. 2 and 3, respectively, show the patterns of progestins, FSH, prolactin, and LH in normal cycling Asian and African elephants. Daily sampling during the follicular phase of cycling females revealed two LH surges that occurred 3 weeks apart. Only the second LH surge induced ovulation (ovLH) and a subsequent rise in serum progestins. A cyclic pattern of FSH secretion also was identified in both species. Serum FSH concentrations were highest at the beginning of the non-luteal phase, declined to nadir concentrations within 4 days of the ovLH surge, remained low until after the ovLH surge, and then increased during the luteal phase. In the African elephant, prolactin secretion increased during the follicular phase (Fig. 3), but in Asian elephants prolactin remained stable throughout the cycle (Fig. 2). In normal cycling females, serum estradiol concentrations varied across the cycle, averaging less than 25 pg/ml with occasional spikes up to 65 pg/ml (data not shown). Increases in estradiol preceding LH surges were observed in females where daily samples were collected, but not consistently.

Overall mean hormone values for cycling and non-cycling Asian and African elephants are presented in Table 2. Although baseline LH concentrations were similar between species ($P > 0.05$), there was a difference

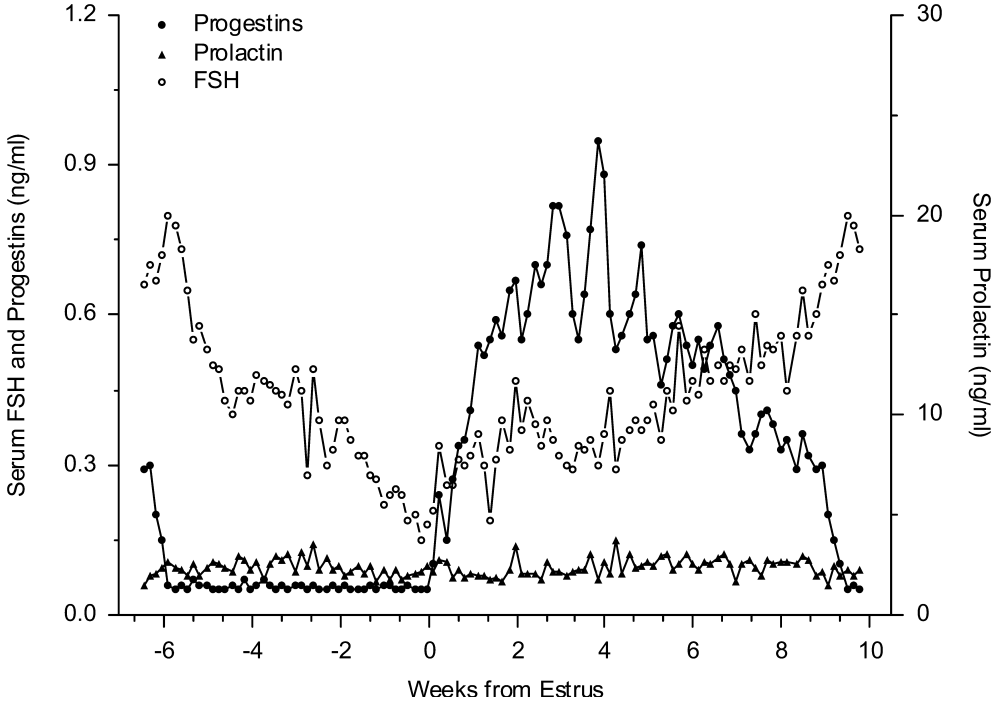

Fig. 2. Mean profiles of serum progestins, prolactin, and FSH in throughout the estrous cycle in reproductively normal Asian elephants ($n = 4$ females, 7 cycles). Week 0 designates estrus. The follicular phase is considered the period between successive luteal phases (Week −6 to Week 0).

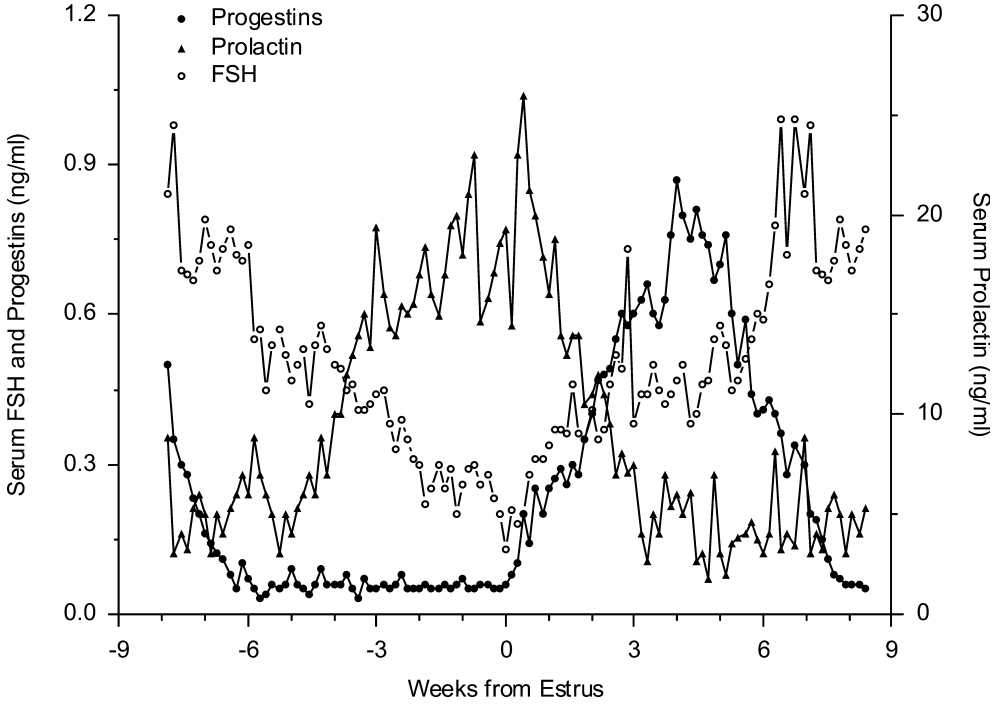

Fig. 3. Mean profiles of serum progestins, prolactin, and FSH in throughout the estrous cycle in reproductively normal African elephants ($n = 7$ females, 15 cycles). Week 0 designates estrus. The follicular phase is considered the period between successive luteal phases (Week −6 to Week 0).

in peak LH surge concentrations between species (Asian, 14.2 ± 2.1 ng/ml; African: 5.3 ± 1.1 ng/ml; $P < 0.05$). When compared to cycling females, no LH surges were observed in non-cycling individuals. Significant differences ($P < 0.05$) were found between cycling and non-cycling elephants in mean FSH

Table 2
Overall mean (±SEM) and average overall mean range concentrations of serum pituitary, ovarian, thyroid, and adrenal hormones in cycling and non-cycling Asian and African elephant females

Hormone	Asian		African		All Elephants[A]
	Cycling	Non-cycling	Cycling	Non-cycling	
LH[B]	0.82 ± 0.06	0.63 ± 0.09	0.67 ± 0.05	0.73 ± 0.06	0.71 ± 0.03
(ng/ml)	(0.65–1.02)	(0.39–0.93)	(0.34–0.98)	(0.34–1.19)	
FSH	4.32 ± 0.29^{a}	2.91 ± 0.32^{b}	4.41 ± 0.29^{a}	2.16 ± 0.23^{b}	
(ng/ml)	(1.92–3.40)	(1.51–3.51)	(0.56–6.41)	(0.83–3.49)	
Prolactin	4.85 ± 0.42^{a}	4.36 ± 0.45^{a}	7.81 ± 0.54^{b}	15.19 ± 2.74^{c}	
(ng/ml)	(2.30–6.78)	(2.50–6.23)	(4.32–8.84)	(3.33–60.41)	
TSH	0.75 ± 0.42	0.97 ± 0.36	0.66 ± 0.15	0.56 ± 0.14	0.69 ± 0.15
(ng/ml)	(0.61–1.08)	(0.41–2.74)	(0.42–1.27)	(0.37–0.86)	
Estradiol	14.74 ± 2.28^{a}	44.88 ± 2.67^{b}	24.86 ± 6.09^{a}	36.04 ± 3.09^{b}	
(pg/ml)	(13.89–15.54)	(18.11–45.33)	(14.31–42.34)	(21.24–62.71)	
Free T3	1.93 ± 0.26	1.39 ± 0.24	1.61 ± 0.27	1.41 ± 0.21	1.56 ± 0.12
(pg/ml)	(1.06–2.98)	(0.73–2.86)	(0.70–3.49)	(0.41–3.68)	
Free T4	1.01 ± 0.06	0.87 ± 0.05	0.91 ± 0.03	0.93 ± 0.04	0.94 ± 0.02
(ng/dl)	(0.74–1.44)	(0.63–0.97)	(0.72–1.10)	(0.72–1.46)	
Total T3	123.95 ± 6.25	126.72 ± 6.13	124.03 ± 4.30	123.27 ± 4.38	123.97 ± 2.62
(ng/dl)	(91.37–158.35)	(110.65–153.95)	(99.34–148.07)	(89.49–177.49)	
Total T4	11.20 ± 0.57	11.12 ± 0.46	10.06 ± 0.36	10.76 ± 0.41	10.73 ± 0.24
(μg/dl)	(8.62–14.54)	(9.53–12.52)	(7.58–12.25)	(8.45–16.56)	
Cortisol	23.32 ± 4.21	20.04 ± 7.83	20.37 ± 4.86	27.53 ± 5.92	24.15 ± 3.27
(ng/ml)	(11.13–51.55)	(7.59–73.54)	(5.74–59.63)	(4.05–110.91)	

[a,b,c] Mean values with different superscripts are significantly different across all groups ($P < 0.001$).

[A] Combined data from both species for hormones where there was no significant difference ($P < 0.001$) between species or reproductive status groups.

[B] Excludes LH surge data.

concentrations, with concentrations in non-cycling females being similar to baseline levels observed in cycling animals (Figs. 2 and 3). Significant differences ($P < 0.05$) also were observed in mean prolactin concentrations between African and Asian elephants, and between cycling and non-cycling African females. The species difference was due to the cyclic pattern of secretion observed in African, but not Asian elephants (Figs. 2 and 3). Prolactin concentrations in Asian elephants were similar to baseline levels observed in African elephants, and did not increase during the follicular phase as was observed for Africans. The difference related to cyclicity status was due to 11 of 30 African elephant females that had elevated ($\geqslant 15$ ng/ml) mean prolactin concentrations (overall mean of outliers, 31.97 ± 4.44 ng/ml; range, 15.5–60.41 ng/ml) (Fig. 4). Excluding these data, overall mean prolactin for non-cycling African elephants was 6.73 ± 0.53 ng/ml, which was similar to cycling African females ($P > 0.05$). Three of the females with elevated prolactin exhibited occasional bouts of mammary enlargement and fluid production, whereas the others were asymptomatic.

Estradiol secretion also was variable in non-cycling females, but was higher on average than that in cycling females ($P < 0.05$), with mean concentrations exceeding 30 pg/ml in all but 2 non-cycling Asian and 5 non-cycling African elephants. There was no correlation between mean estradiol and prolactin concentrations throughout the cycle ($P > 0.05$). Furthermore, mean estradiol values were similar between those with normal (47.7 ± 3.0 pg/ml) and elevated (38.0 ± 5.0 pg/ml) prolactin concentrations.

There were no differences ($P > 0.05$) related to cyclicity status, season, estrous cycle stage or species for overall mean concentrations of TSH, free T3, free T4, total T3 or total T4 (Table 2). Combining all groups, only TSH and free T3 approached significance for a seasonal effect ($P = 0.09$ and $P = 0.11$, respectively), with concentrations appearing to be lower during the summer months. Average correlations between thyroid hormones were significant ($P < 0.05$) for free T3 and free T4 ($r = 0.28$), free T3 and total T3 ($r = 0.41$), free T4 and total T3 ($r = 0.22$), free T4 and total T4 ($r = 0.49$), but not between thyroid hormones and TSH ($P > 0.05$; $r < 0.08$ for each comparison). Correlations between prolactin and thyroid hormones or TSH were not significant ($P > 0.05$; $r < 0.15$).

For cortisol, there also were no differences related to cyclicity status, season, estrous cycle stage or species ($P > 0.05$). However, five individuals exhibited overall mean concentrations that were higher on average than their cohorts (Fig. 5). Four of these were non-cycling females ($n = 1$ Asian, 3 African). None of these outlier females had elevated prolactin. An analysis of the variation in cortisol secretion based on measures of the coefficient of variation (CV), standard deviation (SD), and difference between minimum and maximum values (DIFF) revealed no differences ($P > 0.05$) among cycling Asian (CV, $62.3 \pm 10.2\%$; SD, 29.1 ± 9.2 ng/ml; DIFF, 90.5 ± 31.1 ng/ml), non-cycling Asian ($54.9 \pm 9.5\%$; 18.3 ± 5.1 ng/ml; 47.7 ± 18.3 ng/ml), cycling African ($49.3 \pm 6.6\%$; 16.4 ± 7.1 ng/ml; 87.7 ± 30.1 ng/ml) and non-cycling African ($48.4 \pm 5.4\%$; 15.9 ± 4.6 ng/ml; 66.8 ± 17.5 ng/ml) elephants, respectively. There were no correlations between mean cortisol and prolactin concentrations ($P > 0.05$) (see Fig. 5).

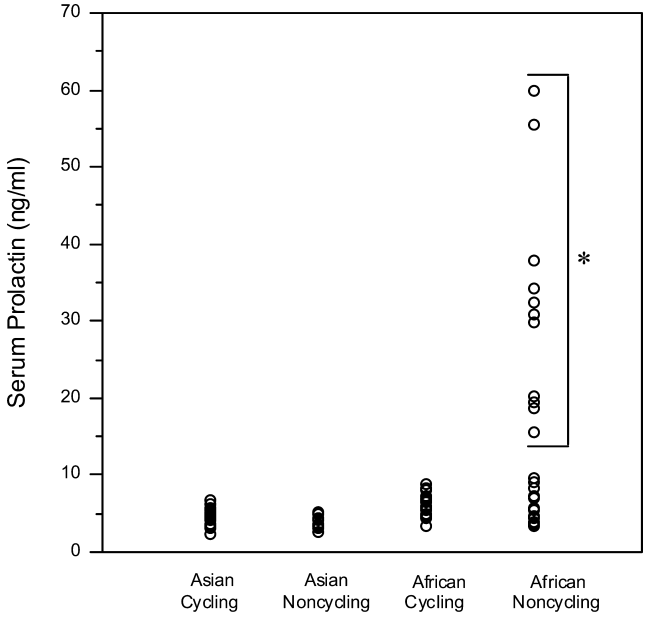

Fig. 4. Scatter plot of overall mean serum prolactin concentrations in cycling and non-cycling Asian and African elephant females. The bar with an asterisk identifies individual mean values that were determined to be outliers.

To evaluate if sample age affected hormone estimations, analysis of protein (LH and prolactin) and steroid (progesterone and cortisol) hormones in subsets of samples spanning 2–7 years between evaluations revealed no differences ($n = \sim 100$ samples per hormone; t test, $P > 0.05$).

4. Discussion

While the 'symptom' of ovarian inactivity in elephants is physiological (i.e., baseline progestin secretion), the 'etiology' likely involves both physiological and psychological mechanisms. In this study, a comprehensive analysis of serum hormones from non-cycling Asian and African elephants was conducted to determine if acyclicity was related to any specific endocrine dysfunctions. The females in this study represented 75 and 100% of the acyclic Asian and African elephants, respectively, identified in a recent survey (Brown et al.,

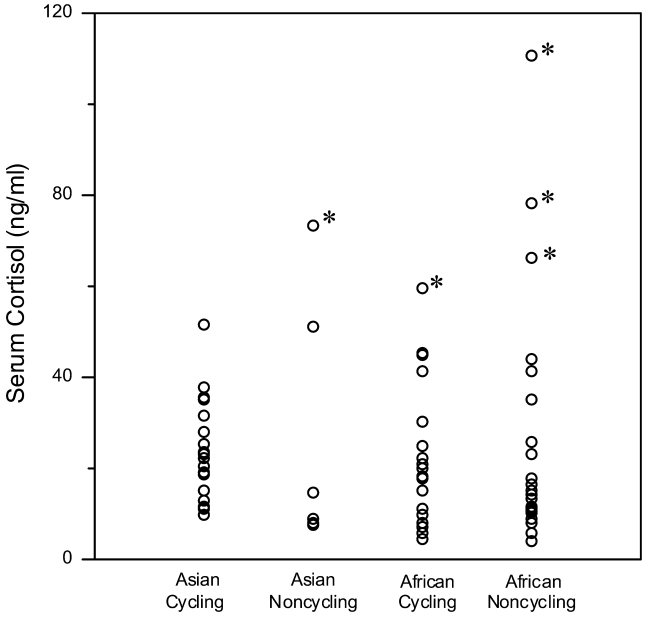

Fig. 5. Scatter plot of overall mean serum cortisol concentrations in cycling and non-cycling Asian and African elephant females. Asterisks represent individual mean values identified as outliers.

in press). To permit appropriate comparisons, however, it was first necessary to develop a normative species-specific endocrine database for each of the hormones examined. An earlier study had evaluated reproductive hormones (LH, FSH, progestin, estradiol, inhibin, and prolactin) in normal cycling Asian elephants (Brown et al., 1999b). In this study, a similar assessment was conducted in the African elephant. In addition, because reproductive dysfunction can be related to metabolic or other endocrine problems, thyroid (T3, T4, and TSH) and adrenal (cortisol) function also was examined in cycling and non-cycling females.

Hormone secretory patterns for the Asian elephants of this study were comparable to the data in previous reports (Brown et al., 1991, 1999b). Patterns also were comparable to those observed for African elephants, with the exception of prolactin. In both species, FSH exhibited a secretory profile that lagged behind progestin changes by about a week. Concentrations were highest at the end of the luteal phase and decreased progressively throughout the non-luteal phase. In other species, a decline in FSH during the follicular phase is considered an important component of dominant follicle selection (Fortune et al., 2001; Ginther et al., 2001; Zeleznik, 2001). FSH may well serve that purpose in elephants; however, the unusually protracted profile along with the long follicular phase of the elephant (up to 6 weeks) makes species comparisons difficult. The finding of two LH surges, the first anovulatory and the second ovulatory, occurring 3 weeks apart during the follicular phase of the cycle also was similar to earlier reports for both species (Brown et al., 1999b; Kapustin et al., 1996). The ability to conduct a direct species comparison for LH in this study revealed that while baseline concentrations did not differ, LH surges were of greater magnitude on average in Asian elephants. This finding has some practical implications in that natural or artificial breeding often is timed to coincide with the ovLH surge (i.e., 3 weeks after the anovulatory LH surge) (Brown, 2000). The lower surge concentrations in African elephants can make identifying the first, anLH surge more difficult (Brown, unpublished).

A notable species difference in prolactin secretion during the estrous cycle was confirmed, a finding inferred from previous reports (Bechert et al., 1999; Brown et al., 1999b). In the African elephant, prolactin may be involved in follicular development because concentrations are elevated during the non-luteal phase (Bechert et al., 1999). By contrast, in Asian elephants prolactin remains stable concentrations throughout the cycle (Brown and Lehnhardt, 1997; Brown et al., 1999a,b; Carden et al., 1998). Prolactin has been shown to regulate ovarian function in other species (Clarke et al., 1997; Gaytan et al., 1997; Murray et al., 1996), so it is possible that the follicular phase increase in African elephants is due to a positive feedback of estrogen, as has been demonstrated in other species (Lawson et al., 1993). An earlier study using transrectal ultrasound identified two waves of follicular activity in African elephants that culminated in LH surges (Hermes et al., 2000), and presumably these follicles produce estrogens. However, no relationship between prolactin and estradiol was found in this study. But then monitoring circulating estrogens has never been very informative (Brown, 2000). Recently, an evaluation of urinary estrogens suggested that two follicular waves occur during the non-luteal phase of the Asian elephant, because concentrations increased before each LH surge (Czekala et al., 2003). Perhaps more investigations into follicular dynamics and urinary estrogen patterns might help explain why there is a species difference in prolactin secretion during the estrous cycle in elephants.

As with the other protein hormones described above, the ability of our assay to measure bioactive TSH may be limited by the use of heterologous antigens. Given that caveat, TSH immunoactivity clearly was demonstrated. To our knowledge, this is the first study to characterize thyroid hormone (TSH, T3, and T4) function in Asian and African elephants, and so these data represent an important contribution to the biological database for these species. Thyroid hormones have been shown to regulate gonadal function, especially in seasonal breeders (Billings et al., 2002). Although elephants are not considered seasonal in captivity (Brown, 2000), there was a reduction, though non-significant, in TSH and free T3 during the summer months for all animals combined. In addition to seasonal influences, both

hyperthyroid and hypothyroid conditions have been linked to cessation of ovarian activity and anovulatory cycles in some species (Doufas and Mastorakos, 2000; Krassass, 2000). It can, however, be difficult to discriminate between direct and indirect effects given that many individuals with thyroid problems also have other disorders that can negatively impact reproduction. In this study, comparison of cycling and non-cycling elephants revealed no significant differences in thyroid hormone or TSH concentrations that might explain the observed ovarian acyclicity. Concentrations of thyroid hormones were generally similar to those reported for other mammalian species (Anderson et al., 1988). Only one outlier was identified and that was a female with elevated TSH (2.74 ng/ml). She was a non-cycling Asian that was overweight and had numerous other health problems, including arthritis, dry skin, and bouts of lethargy. These are all potential symptoms of hyperthyroidism, although other measures of thyroid function (T3 and T4) were not altered in this female, nor was cortisol or prolactin significantly different.

Cortisol concentrations in most elephants of this study were at the lower limit of assay detection. However, there were a few individuals that exhibited consistently higher cortisol concentrations. No obvious reasons for these elevated levels were apparent. Cortisol measures often are used as an index of stress, and chronic elevations have been related to poor reproduction (Moberg, 1985, 1990). Indeed, four of the five females with 'elevated' cortisol were not cycling; however, these individuals represent only a small proportion of the total acyclic group and overall there were no differences in cortisol concentrations between cycling and non-cycling elephants. Furthermore, evaluations of related parameters of cortisol secretory activity (i.e., CV, SD, and DIFF) also suggested there were no consistent differences in adrenal function between cycling and non-cycling elephants. This does not mean that altered adrenal function is not related to reproductive problems in elephants, but perhaps evaluating random corticoid activity may not be the most accurate assessment method. Rather, it might be more informative to evaluate adrenal activity in the context of changes in environmental or social factors, and in elephants where cyclicity status has changed. There now are several examples of cycling elephants that have become acyclic, and non-cycling elephants that reinitiated ovarian activity. These individuals are now the focus of continuing studies by our laboratory.

One potential problem identified in acyclic elephants was that of elevated prolactin. Although not as high as that observed during pregnancy (Brown and Lehnhardt, 1995, 1997), immunoactive prolactin concentrations were on average 6-fold higher in 11 of 30 non-cycling African elephants. Prolactin concentrations in human patients with hyperprolactinemia can vary widely, ranging from mild elevations (2- to 5-fold above baseline) to levels several orders of magnitude greater (Woolf, 1986). An association between high prolactin and ovarian acyclicity was documented earlier in a non-cycling African elephant at the NZP (Brown and Lehnhardt, 1997). In that female, hyperprolactinemia was associated with mammary swelling and the secretion of a watery milk-like fluid. In this study, only a few elephants with high prolactin exhibited these symptoms. Galactorrhea is a common clinical feature of hyperprolactinemia, but it is not obligatory (Jones, 1989). By far, the most prominent hormonal consequence of hyperprolactinemia is hypogonadism (Gomez et al., 1977; Robbins, 1986), with deficient luteal function cited as the first evidence of compromised endocrine function (Jones, 1989). That certainly was the case in this study where all elephants with elevated prolactin exhibited a lack of cyclic luteal activity. By contract, reduced pituitary gonadotropin secretion often is associated with hypogonadism in hyperprolactinemic patients (Jones, 1989; Woolf, 1986), but this was not the case in acyclic elephants.

The causes of hyperprolactinemia are diverse. Often it is associated with prolactin-secreting pituitary adenomas (prolactinomas) (Jones, 1989). Hypothalamic dopamine regulates prolactin release through an inhibitory mechanism, so any lesion interfering with its synthesis, release or activity can affect prolactin secretion (MacLeod and Lamberts, 1986; MacLeod et al., 1976; Woolf, 1986; Yen, 1982; Zacur, 1999). Transient increases in prolactin can be caused by sleep, protein meals, hypoglycemia, chest wall irritation, surgical stress, pregnancy, and renal failure (Jones, 1989; Woolf, 1986; Zacur, 1999). It is unlikely that any of these are causes of elevated prolactin in acyclic elephants because their condition is chronic, not transient. Estrogens can stimulate prolactin release (Jones, 1989; Zacur, 1999), but no relationship between estrogen and prolactin secretion was observed in these elephants. Hypothyroidism is another known cause of hyperprolactinemia (Jones, 1989; Woolf, 1986; Zacur, 1999). Decreased T4 results in increased TRH that subsequently stimulates TSH as well as prolactin release. However, altered thyroid function was not associated with hyperprolactinemia or ovarian acyclicity in this study.

Many types of stressors, both physiological and psychological, have been linked to hyperprolactinemia in addition to increased adrenocorticotropin hormone and cortisol secretion (Fink, 2000). An example of social stress-induced increases in prolactin secretion is illustrated by studies in groups of macaques (Fink, 2000). Subordinate individuals exhibited lower concentrations of sex steroids and higher prolactin levels compared to dominant monkeys. Placing the subordinate animal in a situation where it became dominant normalized prolactin and sex hormone secretion. A question of why

elevated prolactin was observed only in non-cycling African elephants is of considerable interest. It is tempting to speculate that prolactin may be more important in the control of ovarian function in African elephants because that is the only species where prolactin varies throughout the normal estrous cycle. Social stress could be a cause of reproductive problems as suggested by preliminary survey results where acyclicity was related to dominance status in captive African elephants (E. Freeman, E. Weiss, J. Brown, unpublished). In contrast to the monkey studies, it was the most dominant African female within a group that was reproductively suppressed. It remains to be determined whether these are cause or effect relationships, or if they are related to altered prolactin secretion. Given the differences in social and physical environments between captivity and the wild, it is important to understand how all aspects of management impact elephant health and, ultimately, reproduction. To that end, further evaluations of acyclicity, social stress, and behavior as they relate to hyperprolactinemia in elephants certainly would be warranted.

With the finding of hyperprolactinemia in some acyclic elephants, continued assessments are needed to ascertain the time course between the increase in prolactin secretion and cessation of ovarian activity, and if the condition worsens over time. Tumor growth is generally slow because prolactin concentrations often remain stable for years (Gomez et al., 1977; Kleinberg et al., 1977; Robbins, 1986). The earlier finding that prolactin in the NZP female remained unchanged for at least 7.5 years also suggests it is not a rapidly progressive disorder. There are treatments for hyperprolactinemia, the most common being oral administration of ergot alkaloids like bromocriptine or cabergoline which act as dopamine agonists (Zacur, 1999). Numerous side-effects have been reported for bromocriptine, however, including gastrointestinal disturbances, anxiety, orthostatic hypotension, and dizziness (Robbins, 1986). By contrast, cabergoline side-effects appear to be minimal (Jochle et al., 1989). The decision to treat hyperprolactinemia in women often is driven by the desire to correct infertility. Given the need to increase reproductive rates of African elephants to prevent captive extinction (Olson and Wiese, 2000), it might be efficacious to treat genetically valuable females with cabergoline in the hope it will reinitiate reproductive cyclicity.

Another cause of acyclicity in livestock and women is cystic ovarian disease (see reviews, Garverick, 1997; Hamilton et al., 1995; Meirow et al., 1993). Using transrectal ultrasound, Hildebrandt et al. (1997) found they were more prevalent in captive (21%) than in wild (1.4%) African elephants. It is not known if these cysts were associated with acyclicity though, because progestins were not monitored. At least one study reported an association between ovarian cysts and acyclicity in an African elephant (Brown et al., 1999a), and a recent reproductive survey of elephants in North America identified another two individuals, both African, where a lack of ovarian cyclicity was associated with follicular cysts (Brown et al., in press). Ovarian cysts are either estrogen active or estrogen inactive (Garverick, 1997; Kesler et al., 1981). In the three individuals identified with ovarian cysts, average estradiol was higher on average (50–70 pg/ml), but it is premature to conclude that elevated estrogens are caused by ovarian cysts in all acyclic elephants.

As for treating ovarian cysts, 80% of domestic cows respond to exogenous GnRH or human chorionic gonadotropin (hCG) with a resumption of normal cyclic activity (Kesler et al., 1981; Seguin et al., 1976). One African elephant with an ovarian cyst failed to respond to GnRH and hCG treatment (Brown et al., 1999a,b). Because the cyst was unresponsive to direct and indirect gonadotropic stimulation, it was suggested this female may have chronic cystic ovarian disease. This is a problem that occurs in ~20% of dairy cows diagnosed with follicular cysts (Seguin et al., 1976) and is related to a lack of follicular LH and FSH receptors (Brown et al., 1986). If so, the prognosis for recovery of normal ovarian cyclicity is poor. Still, the wide safety margin of these treatments, especially for GnRH, suggests that more females with ovarian cysts should be treated to establish efficacy. Until there is more concomitant ultrasound and hormonal data, it will be difficult to determine the relationship between reproductive tract pathologies and ovarian cyclicity status. However, more zoos now recognize the importance of reproductive monitoring, so understanding these relationships and developing effective treatments may be possible in the near future.

Ultimately, more physiological studies are needed to determine the cause of reproductive pathologies in Asian and African elephants. These should include analyses of the nutritional, disease, and health status of individual elephants, in addition to investigating other potential social or environmental factors as they relate to ultrasound and hormonal results. One encouraging finding was the baseline secretion of gonadotropins. In cases of hypogonadal infertility or after castration, gonadotropin secretion increases as a result of pituitary gonadotroph hypertrophy and lack of steroidal negative feedback (e.g., Wright et al., 1996). Thus, in elephants the lack of cyclic gonadotropin activity may due to inhibitory mechanisms that are not of organic origin, and therefore could be reversible. Information obtained from studies that integrate species- or individual-specific behavior with reproductive physiology could lead to the development of more appropriate management programs for successful captive breeding. Hopefully, continued studies like these will elucidate what factors are related to reproductive dysfunction in elephants in time for mitigating actions to be taken.

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