Secretory Patterns of Serum Prolactin in Asian (Elephas maximus) and African (Loxodonta africana) Elephants During Different Reproductive States: Comparison With Concentrations in a Noncycling African Elephant

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Serum prolactin was quantified in adult female Asian (Elephas maximus) and African (Loxodonta africana) elephants during various reproductive states and the profiles compared to that in a noncycling African elephant. In reproductively normal elephants, there was no effect of season, estrous cycle stage, or lactational status on quantitative or qualitative prolactin secretion ($P > 0.05$), nor were there any differences ($P > 0.05$) in overall prolactin concentrations between species. In pregnant elephants, prolactin concentrations remained at baseline for the first 4–6 months of gestation. Thereafter, concentrations during early pregnancy averaged ~four-fold higher than those during the estrous cycle, increasing to ~100-fold over baseline during mid- to late gestation in both species. In contrast to cycling elephants, prolactin concentrations in an African elephant exhibiting chronic anovulation (on the basis of an acyclic serum progesterone profile) and mild galactorrhea were consistently about five-fold higher ($P < 0.05$), suggesting she is hyperprolactinemic. Other endocrinological assessments confirmed the hypogonadal state of this female. Serum estradiol concentrations were consistently at or below detectable levels. Additionally, no preovulatory luteinizing hormone (LH) surges occurred in daily serum samples analyzed over a 12-month period. The pituitary was not totally refractory, however, and responded with a several-fold increase in serum LH concentration (peak, 3.07 ng/ml) over baseline (0.75 ng/ml) after i.v. injection of gonadotropin-releasing hormone. This study describes normal baseline serum prolactin values for Asian and African elephants and is the first to identify hyperprolactinemia as a possible cause of reproductive acyclicity and galactorrhea in an African elephant. Zoo Biol 16:149–159, 1997. © 1997 Wiley-Liss, Inc.
INTRODUCTION

Prolactin, a polypeptide secreted by the lactotrophs of the anterior pituitary gland, plays an important role in the process of lactation in most mammalian species. Increased prolactin secretion during late gestation is believed to be at least partly responsible for the final stages of mammary gland development and initiation of milk secretion [Tucker, 1988]. After parturition, surges of prolactin occur in response to suckling and appear to maintain galactopoiesis [Davis et al., 1971; Concannon et al., 1978]. In both Asian (Elephas maximus) and African (Loxodonta africana) elephants, prolactin concentrations increase after the sixth month of gestation and remain elevated throughout pregnancy [McNeilly et al., 1983; Hodges et al., 1987; Brown and Lehnhardt, 1995], suggesting a similar role for prolactin in these species. However, other than these few reports, little is known about the physiological function of prolactin in elephants.

Secretion of prolactin above the normal range at times other than during gestation or lactation is termed “hyperprolactinemia” and is a common endocrine cause of infertility in mammals. This condition is often associated with altered hypothalamic dopaminergic, opiateergic and catecholaminergic activity, reduced gonadotropin-releasing hormone (GnRH) and gonadotropin secretion, and impaired ovarian function [Kann et al., 1978; Woolf, 1986; McNeilly, 1987; Jones, 1989]. The problem is especially pervasive in women where hyperprolactinemia accounts for a significant proportion of amenorrhea and anovulation in patients seeking treatment at infertility clinics [Jones, 1989]. In many of these cases, the women also exhibit mild to moderate galactorrhea.

In 1989, a nulliparous African elephant female was observed secreting a milk-like fluid from her mammary glands. Although of reproductive age (~35 years old), this female subsequently was diagnosed as anovulatory on the basis of an acyclic serum progesterone profile. Further evaluations of serum estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), inhibin, and cortisol failed to reveal the cause of ovarian inactivity. Thus the objectives of this study were to: (1) establish normal prolactin baseline values for cycling, pregnant, and lactating elephants (Asian and African), and (2) then determine if the galactorrhea and reproductive acyclicity observed in this African female were associated with abnormally high prolactin levels.

MATERIALS AND METHODS

Animals and Blood Sample Collection

The noncycling African elephant (Nancy) was housed at the National Zoological Park (NZP) in Washington, DC, in a herd with three adult Asian elephant females. She was acquired by the NZP as a 2-year-old in 1956, reportedly out of East Africa. She is nulliparous and has never been involved in a breeding program. Elephants at the NZP are fed herbivore pellets, a vitamin E supplement, and fresh fruits and vegetables three times daily, with mixed grass hay (timothy and/or orchard grass) and water available at all times. Because of a history of colic (often 3 or 4 episodes per year), alfalfa was removed from Nancy’s diet beginning in 1988. She
also receives steamed rolled oats and bran daily. All NZP elephants are extremely tractable and managed under a free-contact system with extensive keeper interaction.

In April 1989, a lump was discovered on Nancy’s left nipple and in May 1989 milk-like secretions from the mammary glands were observed for the first time. Lactation has continued uninterrupted from that time on. Between June and September 1989, the mammary glands were treated with warm compresses daily, with no alleviation of secretory activity. In 1992, vaginal vestibular polyps were identified by endoscopy, and in 1993 a large polyp was removed surgically. On 16–17 October and 20–24 November 1994, blood of unknown cause was shed profusely from the vulva. Beginning in January 1989 and continuing through the present, blood samples (10 ml) have been collected 1–3 times weekly. Additional daily samples also were collected between December 1994 and November 1995. In 1990, a GnRH challenge was administered to assess pituitary function. A pretreatment blood sample (Time 0) was collected immediately by an i.v. injection of 500 µg GnRH (Gonadorelin, Abbott Laboratories, Chicago, IL) and continued blood sampling at 15, 30, 45, 60, 75, 90, 105, and 120 min post-injection.

For comparative purposes, prolactin was measured in serum collected from Asian and African elephants during different reproductive states. Weekly serum prolactin was analyzed from normal cycling Asian elephants at the NZP (n = 3) over a 3-year period from 1989–1991 and African elephants at the Indianapolis (Indiana) Zoo (n = 4) over a 1-year period in 1990. In Asian elephants, weekly (NZP) or 1–4 times monthly (Burnet Park Zoo, Syracuse, NY; Elephant Breeding Center, Chitwan, Nepal) samples also were collected throughout two complete and three partial pregnancies and four ensuing lactational periods (2–6 months). In African elephants, single blood samples were collected from seven pregnant and three lactating females during routine culling at the Kruger National Park (Republic of South Africa). Stage of pregnancy was estimated in the field from fetal weights [Craig, 1984].

In general, blood samples were collected from a vein on the caudal aspect of the ear usually while the cow was in lateral recumbency. Elephants at the NZP, Burnet Park Zoo, and Indianapolis Zoo were well conditioned to the blood sampling procedure, which is part of their weekly routine. None of these elephants exhibited signs of stress or discomfort during sample collection. Blood usually was maintained at ~4°C and centrifuged (~1,500× g) within a few hours of collection. Serum was stored at ~20°C or colder until analysis.

Hormone Iodinations

Prolactin (NIDDK-oPRL-I-2), LH (LER-1374-A), FSH (LER-1976-A2), and inhibin (inhibin-α) were iodinated using chloramine-T. For each iodination, 5 µg of hormone (in 20 µl 0.05 M NaPO₄, pH 7.6) was incubated with 1 mCi carrier-free Na₁²⁵I (10 µl) for 1 min in the presence of 2.5 µg chloramine-T (10 µl in 0.05 M NaPO₄, pH 7.6). The reaction was stopped with sodium metabisulfite (10 µg in 10 µl 0.05 M NaPO₄, pH 7.6) and labelled hormone separated from free ¹²⁵I using anion-exchange chromatography (AG 2-X8, 100–200 mesh, chloride form; BioRad Laboratories, Melville, NY). Columns (1 × 5 cm) were prepared by equilibrating resin in 0.05 M NaPO₄, (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 NaPO₄, (pH 7.6). The iodination reaction mixture was layered onto the column and labelled hormone eluted with 2 ml 0.05 M NaPO₄ into a tube containing 1 ml phosphate-buffered saline (PBS) with 0.1% BSA (pH 7.4).
Radioimmunoassays

Serum prolactin was measured by a heterologous $^{125}$I double-antibody radioimmunoassay [Brown and Lehnhardt, 1995]. The assay employed an antihuman prolactin antiserum (NIDDK-anti-hPRL-3) and ovine prolactin (NIDDK-oPRL-I-2) label and standards. All assay dilutions were made in PBS-BSA buffer (0.01 M PO$_4$, 0.5% BSA, 2 mM EDTA, 0.9% NaCl, 0.01% thimerosal, pH 7.4), with the exception of second antibody where BSA was omitted. For samples collected during pregnancy, the assay was incubated at room temperature for 2 days in a total volume of 500 µl. Standards (100 µl; 0.039–20 ng/tube) and/or sample (10–100 µl) were brought up to 300 µl in buffer and incubated with 100 µl first antibody (diluted 1:15,000) for 3 hr followed by the addition of 100 µl $^{125}$I-prolactin (20,000 cpm). On day 2, antibody-bound complexes were precipitated by incubation for 1 hr with 1 ml of sheep anti-rabbit gamma globulin (diluted 1:200 plus 5% polyethylene glycol; PEG) and centrifugation for 30 min at 2,500× g. To increase sensitivity for analyzing prolactin concentrations during all other reproductive states, the assay was modified by increasing the incubation time and using a higher dilution of first antibody. Standards (100 µl; 0.02–2.5 ng/tube) and/or sample (10–100 µl) were incubated with antibody (diluted 1:60,000) for 24 hr followed by the addition of $^{125}$I-prolactin. On day 3, antibody-bound complexes were precipitated with second antibody/PEG and centrifugation as described above. In both assay systems, the antibody bound 20–35% of the $^{125}$I-prolactin with ~3% nonspecific binding. Assay sensitivities were 0.05 and 0.01 ng/tube, respectively.

To further characterize the endocrine status of the noncycling African female, other hormone analyses were performed periodically (usually at 4–6-month intervals annually or bi-annually) and the values compared to published data [Brown et al., 1991; Brown and Lehnhardt, 1995]. Serum steroids (progesterone, estradiol, and cortisol) were measured using solid-phase $^{125}$I radioimmunoassays (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). Assay sensitivities were 30 pg/ml, 5 pg/ml, and 5 ng/ml, respectively. Serum protein hormones (LH, FSH, and inhibin) were quantified using $^{125}$I double-antibody radioimmunoassays developed in our laboratory [Brown et al., 1991]. Standard preparations were NIH-LH-S18, NIDDK-FSH-S16, and synthetic porcine inhibin-α fragment, respectively. Assay sensitivities were 0.07, 0.5, and 0.01 ng/tube, respectively. For all assays, intra- and interassay coefficients of variation were < 10%.

Milk secretions (n = 6 samples collected throughout June of 1991) were analyzed using standardized procedures for percentage dry matter, fat, ash, sugar, and crude protein [Oftedal et al., 1988] and gross energy [Oftedal et al., 1993].

Statistical Analyses

Data are presented as means ± SEM. Seasons were defined as winter (January–March), spring (April–June), summer (July–September), and autumn (October–December). For each female, prolactin concentrations were averaged across each season. Data for Nancy also were averaged across season and year of study. Increases in serum progesterone were considered to be indicative of a luteal phase if concentrations exceeded 0.05 ng/ml for > 2 consecutive weeks. For each individual, average prolactin concentrations during the luteal and follicular phases were calculated, as well as an overall mean for each female throughout the entire estrous cycle. Those
values were then averaged to produce a single value per female for each reproductive state. For comparing differences in prolactin concentrations across seasons or reproductive states or between species, individual means were averaged and compared using Duncan’s New Multiple Range tests or Student’s t-tests.

RESULTS

There were no differences ($P > 0.05$) between Asian and African elephants in prolactin concentrations throughout the estrous cycle (Table 1). There also was no effect of season or estrous cycle stage ($P > 0.05$) on prolactin secretion in either species (Table 1), although random fluctuations occurred throughout the year (Fig. 1). During gestation, prolactin concentrations generally averaged about four-fold higher during early pregnancy than during the estrous cycle (Table 1), increasing to ~100-fold over baseline during mid- and late gestation ($P < 0.05$) (Fig. 2, Table 1). No species differences in circulating prolactin concentrations throughout gestation were observed ($P > 0.05$) (Table 1). Prolactin concentrations during lactation also were similar ($P > 0.05$) to normal cycling, nonlactating females. However, secretion was more variable as indicated by the broader range in mean concentrations (Table 1).

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>n</th>
<th>Mean ± SEM (ng/ml)$^a$</th>
<th>Range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncycling</td>
<td>1</td>
<td>22.0 ± 2.9$^c$</td>
<td>4.4–54.9</td>
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<tr>
<td>Normal cycling</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asian</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td></td>
<td>4.5 ± 0.9$^d$</td>
<td>0.5–11.6</td>
</tr>
<tr>
<td>Follicular</td>
<td></td>
<td>4.4 ± 0.8$^d$</td>
<td>0.7–14.7</td>
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<tr>
<td>Overall</td>
<td></td>
<td>4.4 ± 0.6$^d$</td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td></td>
<td>4.2 ± 0.7$^d$</td>
<td>1.8–9.9</td>
</tr>
<tr>
<td>Follicular</td>
<td></td>
<td>4.8 ± 0.6$^d$</td>
<td>1.5–10.3</td>
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<tr>
<td>Overall</td>
<td></td>
<td>4.7 ± 0.5$^d$</td>
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<tr>
<td>Pregnant$^b$</td>
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<tr>
<td>Asian$^c$</td>
<td>4</td>
<td>21.4 ± 4.6$^d$</td>
<td>1.2–50.2</td>
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<tr>
<td>Early</td>
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<td></td>
<td></td>
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<tr>
<td>Mid</td>
<td>4</td>
<td>468.2 ± 121.8$^d$</td>
<td>50.1–1,064.2</td>
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<tr>
<td>Late</td>
<td>3</td>
<td>525.1 ± 80.3$^d$</td>
<td>152.3–1,038.3</td>
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<td>African$^d$</td>
<td>3</td>
<td>17.9 ± 8.4$^d$</td>
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<tr>
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<td></td>
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<tr>
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<td>358.1 ± 188.9$^d$</td>
<td>195.3–653.7</td>
</tr>
<tr>
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<td>1</td>
<td>397.3</td>
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<td>Lactation</td>
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<td></td>
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<tr>
<td>Asian$^e$</td>
<td>4</td>
<td>10.6 ± 4.9$^d$</td>
<td>0.4–60.1</td>
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<tr>
<td>African$^e$</td>
<td>3</td>
<td>11.4 ± 7.1$^{d,1}$</td>
<td>3.6–25.1</td>
</tr>
</tbody>
</table>

$^a$Values with different superscripts differ ($P < 0.05$).
$^b$Stages of pregnancy are designated: Early (1–6 months), Mid (7–14 months), Late (15 months–birth)
$^c$Values represent means of weekly samples from complete (n = 2) and partial (n = 3; 20–30 samples/individual) pregnancies.
$^d$Values represent means of a single blood sample collected from culled females.
$^e$Values represent means of 1–3 times weekly blood samples collected for 2–6 months postcalving.
The relatively stable secretion of progesterone at basal levels in the African female, Nancy, indicated she was anovulatory, except for two isolated episodes in 1989 and 1995 where luteal phase increases in progesterone secretion lasting 8 and 11 weeks, respectively, were observed (Fig. 3A). Otherwise, < 1% of the progesterone values exceeded 0.05 ng/ml. The hypogonadal state of this female was further confirmed by estradiol analysis where concentrations consistently remained at baseline, rarely exceeding 10 pg/ml. Prolactin concentrations in this female fluctuated considerably and were unaffected ($P > 0.05$) by season. However, there was a marked quantitative difference with prolactin concentrations averaging five-fold higher ($P <

Fig. 1. Longitudinal profiles of weekly serum prolactin concentrations throughout the year in representative, normal-cycling Asian, and African elephants.

Fig. 2. Serum prolactin concentrations in an Asian elephant housed at the Burnet Park Zoo (Syracuse, NY) throughout gestation and after parturition. Day 0 represents the time of observed breeding.
0.05) than those observed in cycling elephants (Figs. 3B, 4). Rather, concentrations were comparable to those observed during early pregnancy (Table 1). All other hormone determinations in this female produced values that were within baseline ranges for cycling elephants. Overall means were: cortisol (15.6 ± 0.9 ng/ml; range, 5.0–45.8 ng/ml), LH (0.83 ± 0.05 ng/ml; range, 0.71–1.82 ng/ml), FSH (12.5 ± 1.6 ng/ml; range, 5.0–21.8 ng/ml) and inhibin (0.41 ± 0.03 ng/ml; range, 0.18–0.65 ng/ml). However, regular fluctuations in LH, FSH, and inhibin secretion, typical of patterns observed during the normal estrous cycle [Brown et al., 1991], were not observed. Further analysis of LH in daily serum samples over a 12-month period also revealed
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an absence of preovulatory-type surges, although concentrations after GnRH administration increased from 0.75 ng/ml (Time 0) to 2.38, 3.07, 2.95, 2.89, 3.06, 2.74, 2.51, and 2.34 ng/ml at 15, 30, 45, 60, 75, 90, 105, and 120 min postinjection, respectively.

Mean chemical composition of Nancy’s mammary secretions was as follows: dry matter (12.18 ± 0.28%; range, 11.50–13.44%); fat (4.35 ± 0.27%; range, 3.40–5.33%); ash (0.94 ± 0.15%; range, 0.71–1.70%); sugar (1.41 ± 0.03%; range, 1.30–1.50%); crude protein (4.41 ± 0.12%; range, 4.05–4.78%); and energy (0.73 ± 0.02 kcal/g; range, 0.67–0.80 kcal/g). There was no apparent relationship between the degree of galactorrhea (on the basis of subjective visual assessment) and fluctuations in serum prolactin concentrations.

DISCUSSION

Longitudinal analysis of serum prolactin in normal cycling Asian and African elephants revealed no effect of season on pituitary secretory activity. Although prolactin concentrations increase during long days in many seasonally breeding ungulates [Bubenik, 1990], neither reproduction nor lactotroph activity appears to be affected by photoperiod in the elephant. This finding is consistent with limited information on free-ranging African elephants where no seasonal differences in serum prolactin concentrations were observed in animals culled throughout the year [McNeilly et al., 1983]. Likewise, there was no apparent effect of estrous cycle or lactational stage on circulating prolactin concentrations, although the data were highly variable especially in lactating animals. The variability during lactation is not surprising because both suckling and time postpartum influence prolactin release [Tucker, 1988]. Consequently, comparatively higher prolactin concentrations during lactation in elephants have been observed in some studies [Hodges et al., 1987; Brown and Lehnhardt, 1995], but not others [McNeilly, 1983]. Furthermore, like other pituitary hormones, prolactin is secreted episodically, which likely explains the day-to-day variability observed in all females. Prolactin concentrations during pregnancy were markedly elevated in both Asian and African elephants, a finding noted previously by us [Brown and Lehnhardt, 1995] and others [McNeilly et al., 1983; Hodges et al., 1987]. In fact, the rise in serum prolactin observed after ~6 months of gestation is so dramatic that this measurement alone can be used to diagnose pregnancy based on a single sample collected after the first third of gestation [McNeilly et al., 1983; Hodges et al., 1987; Brown and Lehnhardt, 1995].

In comparison with normal cycling elephants, prolactin concentrations in Nancy were several-fold higher, although not as high as those observed throughout most of gestation. This female also was anovulatory on the basis of progesterone analyses, with the exception of two isolated and inexplicable periods of elevated secretion. She also exhibited varying degrees of galactorrhea throughout the 7.5-year study. Taken together, these findings suggest she is hyperprolactinemic. Although a direct causal relationship between elevated serum prolactin and anovulation in this elephant has not been established, in women even marginal elevations in circulating prolactin can affect reproduction, with deficient luteal activity often cited as the first evidence of compromised endocrine function [Jones, 1989]. The cause of hyperprolactinemia in this elephant is unknown, although in other species it often results from a prolactin-secreting pituitary adenoma (prolactinoma). Because hypothalamic dopamine regulates pro-
lactin release through an inhibitory mechanism, any lesion interfering with its synthesis, release, or activity can affect prolactin secretion [MacLeod et al., 1976; Yen, 1982; MacLeod and Lamberts, 1986]. For example, long-term absence of dopamine negative feedback can cause hypertrophy and/or hyperplasia of pituitary lactotrophs, eventually leading to prolactinoma development [Yen, 1978; Jones, 1989]. Estrogens can stimulate prolactin release; however, a direct relationship between estradiol secretion and prolactinoma formation has yet to be confirmed [Jones, 1989]. Furthermore, the most prominent hormonal consequence of hyperprolactinemia is hypogonadism, marked by low concentrations of circulating estradiol and progesterone [Gomez et al., 1977; Robbins, 1986], consistent with the endocrine profile presented by Nancy. Compared to cycling elephants [Brown et al., 1991], normal fluctuations in LH, FSH, and inhibin also were absent in Nancy. The pituitary was not completely refractory, however, because a significant amount of LH was released after GnRH administration. Although a lack of comparable data in female African elephants precludes determining if her pituitary response was “normal”, it was within the range of responses observed in anesthetized, free-ranging African elephant bulls given a similar GnRH challenge [Brown et al., 1993].

The question now remains how, or if, to treat this condition. The most common nonsurgical approach for reducing prolactin secretion in women involves oral administration of ergot alkaloids, such as bromocriptine, which act as dopamine agonists. However, numerous side effects have been reported including gastrointestinal disturbances, anxiety, orthostatic hypotension, and dizziness [Robbins, 1986], which raises serious safety concerns about it use in elephants. The decision to treat the disease in women often is driven by a need to correct infertility. In Nancy’s case, re-establishing reproductive cyclicity for breeding purposes is not a consideration. The secretory activity of her mammary glands is mild and also not considered a major problem. Comparatively, the mammary fluid is somewhat lower in sugar than normal elephant milk [McCullagh and Widdowson, 1970; Oftedal, 1984; Mainka et al., 1994], suggesting that lactose synthesis is reduced [O. Oftedal, pers. comm.].

Prolactin concentrations in patients with hyperprolactinemia vary widely, ranging from mild elevations (2–5-fold above baseline) to levels several orders of magnitude greater [Woolf, 1986]. The modest elevation in prolactin concentrations observed in Nancy (only ~5-fold above normal) suggests that if a tumor is present, it is not large. Tumor growth also appears to be relatively slow in other species because prolactin secretion often remains stable for years, with no correlation between serum prolactin concentrations and duration of the disease [Gomez et al., 1977; Kleinberg et al., 1977; Robbins, 1986]. The finding that Nancy’s prolactin concentrations have remained unchanged for the past 7.5 years also suggests it is not a rapidly progressive disorder and not in need of immediate treatment.

Thus, although the NZP African elephant has been diagnosed with a mild case of hyperprolactinemia, no specific treatment will be rendered at this time. Instead, plans are to continue evaluating her endocrine status by weekly progesterone analyses in conjunction with yearly prolactin determinations to monitor the progress of the disease. Because hyperprolactinemia in women also can be caused by hypothyroidism [Honbo et al., 1978; Woolf, 1986], plans are to include measurements of thyroid hormones and pituitary thyroid stimulating hormone in future analyses.
CONCLUSIONS

1. Prolactin secretion does not appear to be affected by stage of the estrous cycle or season in Asian or African elephants.

2. There were no differences in prolactin concentrations between Asian and African elephants.

3. Although a causative relationship has not been established, elevated prolactin concentrations were associated with chronic anovulation and galactorrhea in an African elephant.

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REFERENCES


Oftedal, O.T.; Bowen, W.D.; Boness, D.J. Energy transfer by lactating hooded seals and nutrient deposition in their pups during the four days from birth to weaning. PHYSIOLOGICAL ZOOLOGY 66:412–436, 1993.


