

## RECURRENCE OF HYPERPROLACTINEMIA AND CONTINUATION OF OVARIAN ACYCLICITY IN CAPTIVE AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*) TREATED WITH CABERGOLINE

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**Abstract:** Hyperprolactinemia is associated with reproductive acyclicity in zoo African elephants (*Loxodonta africana*) and may contribute to the non-self-sustainability of the captive population in North America. It is a common cause of infertility in women and other mammals and can be treated with the dopamine agonist cabergoline. The objectives of this study were to assess prolactin responses to cabergoline treatment in hyperprolactinemic, acyclic African elephants and to determine the subsequent impact on ovarian cyclic activity. Five elephants, diagnosed as hyperprolactinemic ( $>11$  ng/ml prolactin) and acyclic (maintenance of baseline progestagens for at least 1 yr), were treated with 1–2 mg cabergoline orally twice weekly for 16–82 wk. Cabergoline reduced ( $P < 0.05$ ) serum prolactin concentrations during the treatment period compared to pretreatment levels in four of five elephants ( $11.5 \pm 3.2$  vs.  $9.1 \pm 3.4$  ng/ml;  $20.3 \pm 16.7$  vs.  $7.9 \pm 9.8$  ng/ml;  $26.4 \pm 15.0$  vs.  $6.8 \pm 1.5$  ng/ml;  $42.2 \pm 22.6$  vs.  $18.6 \pm 8.9$  ng/ml). However, none of the females resumed ovarian cyclic activity based on serum progestagen analyses up to 1 yr posttreatment. In addition, within 1 to 6 wk after cessation of oral cabergoline, serum prolactin concentrations returned to concentrations that were as high as or higher than before treatment ( $P < 0.05$ ). One elephant that exhibited the highest pretreatment prolactin concentration ( $75.2 \pm 10.5$  ng/ml) did not respond to cabergoline and maintained elevated levels throughout the study. Thus, oral cabergoline administration reduced prolactin concentrations in elephants with hyperprolactinemia, but there was no resumption of ovarian cyclic activity, and a significant prolactin rebound effect was observed. It is possible that higher doses or longer treatment intervals may be required for cabergoline treatment to result in permanent suppression of prolactin secretion and to mitigate associated ovarian cycle problems.

**Key words:** Acyclicity, cabergoline, elephant, hyperprolactinemia, prolactin.

### INTRODUCTION

Reproductive acyclicity is a serious problem for African elephants in North America, and efforts to breed African elephants in zoos have met with limited success.<sup>35</sup> As fewer than 10% of African elephants have produced offspring in the United States,<sup>30,31</sup> ‘captive extinction’ is a possibility without continued importations.<sup>35</sup> In a recent study, over one-third of captive North American African elephant females displayed some form of ovarian cycle problems.<sup>32</sup> Another report found two-thirds of these noncycling females (22 of 30) had significantly higher prolactin concentrations compared to normal, cycling females.<sup>13</sup> Secretion of prolactin above the normal range at times other than during gestation or lactation is termed ‘hyperprolactinemia’ and is a common cause of infertility in women and other mammals, includ-

ing gorilla, dogs, and rats, often resulting in ovarian acyclicity.<sup>3,9,10,16,18,19,21,33,34,36,37</sup>

Prolactin is produced in the anterior pituitary gland and increases during pregnancy and after birth as a result of estrogen-induced hyperplasia of the lactotrophs (prolactin-secreting cells) in the pituitary gland to promote milk production. Other states, such as anorexia, hypoglycemia, stress, and trauma, also can increase prolactin, but these are considered pathologic.<sup>8,37</sup> Chronic hyperprolactinemia is often associated with prolactin-secreting pituitary adenomas (i.e., prolactinomas) in humans, gorilla, and rats,<sup>4,8,9,15,23,36</sup> although the cause(s) of tumor formation are not well understood. Long-term hyperprolactinemia-induced infertility is generally associated with pituitary prolactinomas, which account for 40% of functioning pituitary tumors.<sup>15</sup> Prolactin secretion is regulated through a dopamine negative feedback mechanism, so any condition that interferes with its synthesis, release, or activity can affect prolactin secretion.<sup>26,27</sup> Excessive prolactin secretion impairs reproductive function by causing cycle disturbances or anovulatory subfertility.<sup>4</sup> Hyperprolactinemia can disrupt follicular maturation and steroidogenesis, ovulation, and corpus

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luteum development and function through effects at hypothalamic-pituitary or ovarian levels.<sup>17,22,37</sup> The cause of hyperprolactinemia in elephants has not yet been established, but its association with ovarian acyclicity makes it a concern for captive management.

Dopamine agonists, such as cabergoline or bromocriptine, are effective treatments for hyperprolactinemia and act by binding to lactotroph D2 receptors and reducing both the synthesis and secretion of prolactin.<sup>26,27</sup> Cabergoline is a well-tolerated dopamine agonist capable of rapidly normalizing prolactin as well as reducing tumor size in most cases in women.<sup>8,15</sup> It has also been used to routinely treat infertility and to control prolactin secretion in dogs.<sup>16,19,20</sup> Cabergoline has a long biological half-life (65 hr) and thus only needs to be administered once or twice per week, as compared to other dopamine agonists, such as bromocriptine or quinagolide, which require daily administration.<sup>3,10,34</sup>

Cabergoline was used in a preliminary trial to treat hyperprolactinemia in an acyclic captive Asian elephant (*Elephas maximus*).<sup>2</sup> Treatment consisted of 1 mg oral cabergoline administered twice weekly for 6 mo. Serum prolactin concentrations declined within days of treatment initiation, followed about 1 mo later by a normalization of progesterone concentrations and resumption of cyclic luteal phases within 7 mo. Given these promising preliminary results and the need to increase the reproductive rates of African elephants, the objectives of this study were to determine the efficacy of cabergoline therapy to treat acyclic African elephants with hyperprolactinemia and to determine if it could reinstate reproductive cyclicity.

## MATERIALS AND METHODS

### Animals and blood sample collection

Five female African elephants (22–36 yr of age) were used in this study. All animals were housed in indoor–outdoor enclosures. Elephants were identified as candidates for cabergoline treatment based on mean prolactin concentrations of >11 ng/ml for at least 1 yr prior to the study.<sup>6</sup> Each elephant also had been acyclic for at least 1 yr, based on serum progesterone analyses.<sup>7</sup> Because prolactin is known to be an inflammatory marker, all candidates were required to have a nonreactive lateral flow immunochromatography (Rapid Test) and multiple antigen immunoassay (MAPIA) for *Mycobacterium tuberculosis*<sup>25,29</sup> and were found to be healthy based on MAPIA results and veteri-

nary assessments for overall health at participating zoos. All elephants were accustomed to the blood sampling procedure, which was part of their husbandry routine. Blood was collected weekly from an ear vein without sedation, allowed to clot for at least 1 hr, and then centrifuged for recovery of serum. Serum was stored at –20°C or colder until analysis.

### Cabergoline treatment protocol

Treatment consisted of 1–2 mg cabergoline (Dostinex®, Pfizer, Inc., Kalamazoo, Michigan 49001, USA) given orally twice weekly for periods ranging from 16 to 48 wk. Table 1 summarizes the dose and treatment regimens used for individual elephants. All elephants received cabergoline at a starting dose of 1 mg twice weekly. Based on serum prolactin concentrations, the dose of cabergoline was increased to 2 mg twice weekly in two of the five elephants.

### Hormone assays

Serum progesterone were analyzed using a solid-phase progesterone radioimmunoassay (RIA) validated for elephants.<sup>7</sup> Serum prolactin was measured using a double-antibody <sup>125</sup>I RIA, also validated for elephants.<sup>5</sup> Progesterone and prolactin concentrations were measured in samples collected weekly for at least 1 yr before cabergoline treatment, throughout the treatment period, and then for a 1-yr posttreatment period.

### Statistical analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute, USA). Effectiveness of cabergoline treatment was evaluated by comparing means of prolactin and progesterone concentrations before, during, and after treatment dose for each elephant; thus, each female served as its own control. The *F*-test was used to test for differences in sample variation. Treatment-associated changes in prolactin and progesterone concentrations were analyzed using the one-way analysis of variance procedure and the Duncan option for multiple comparisons. Progesterone concentrations ≤0.10 ng/ml were considered baseline. Values are reported as mean ± standard deviation (SD). A *P*-value of <0.05 was considered statistically significant.

## RESULTS

Mean duration of cabergoline treatment was 44 wk (range, 16–82 wk). Cabergoline was well

**Table 1.** Cabergoline dose and treatment duration for individual elephants.

Elephant	Age (yr)	Dose (mg, 2×/wk)	Duration of treatment (wk)
1	22	1	46
2	27	1	34
		2	48
3	31	1	19
		2	16
4	28	1	16
5	36	1	39

tolerated by all elephants; none of the elephants required a dose reduction or discontinuation as a result of side effects.

Mean ( $\pm$ SD) serum prolactin concentrations before, during, and after cabergoline treatment are presented in Table 2, and longitudinal hormone profiles are shown in Figures 1–5. Within 7–8 wk of treatment, serum prolactin concentrations decreased in two of five elephants (Table 2; Figs. 1, 4;  $P < 0.05$ ). Based on a minimal serum prolactin response in two elephants, the dose of cabergoline was increased from 1 mg twice weekly to 2 mg twice weekly after 34 wk in elephant 2 and after 19 wk in elephant 3. After the dosage increase, prolactin concentrations decreased to normal levels in both females (Figs. 2, 3, respectively). Elephant 5 did not respond to cabergoline therapy. This female exhibited the highest prolactin concentrations throughout the study period, being slightly higher during than before treatment (Table 2; Fig. 5;  $P < 0.05$ ). Within 6 wk after cabergoline withdrawal, serum prolactin increased to concentrations that were as high (Table 2; Fig. 3;  $P < 0.05$ ) or higher (Table 2; Figs. 1, 2, 4;  $P < 0.05$ ) than pretreatment levels. Serum progesterone throughout the study period remained at baseline concentrations ( $\leq 0.10$  ng/ml) in all elephants, and there was no evidence of ovarian cyclic activity (Figs. 1–5).

**Table 2.** Mean ( $\pm$ standard deviation [SD]) serum prolactin concentrations (ng/ml) in elephants with hyperprolactinemia before, during, and after oral cabergoline treatment.

	Elephant 1	Elephant 2	Elephant 3	Elephant 4	Elephant 5
Before treatment	11.5 $\pm$ 3.2 <sup>a</sup>	20.3 $\pm$ 16.7 <sup>a</sup>	26.4 $\pm$ 15.0 <sup>a</sup>	42.2 $\pm$ 22.6 <sup>a</sup>	75.2 $\pm$ 10.5 <sup>a</sup>
During treatment					
1 mg	9.1 $\pm$ 3.4 <sup>b</sup>	10.2 $\pm$ 4.6 <sup>b</sup>	19.0 $\pm$ 7.6 <sup>b</sup>	18.6 $\pm$ 8.9 <sup>b</sup>	80.0 $\pm$ 0.0 <sup>bA</sup>
2 mg		7.9 $\pm$ 9.8 <sup>b</sup>	6.8 $\pm$ 1.5 <sup>c</sup>		
After treatment	43.6 $\pm$ 21.2 <sup>c</sup>	59.5 $\pm$ 27.4 <sup>c</sup>	24.5 $\pm$ 21.0 <sup>a</sup>	69.3 $\pm$ 21.7 <sup>c</sup>	78.7 $\pm$ 5.8 <sup>ab</sup>

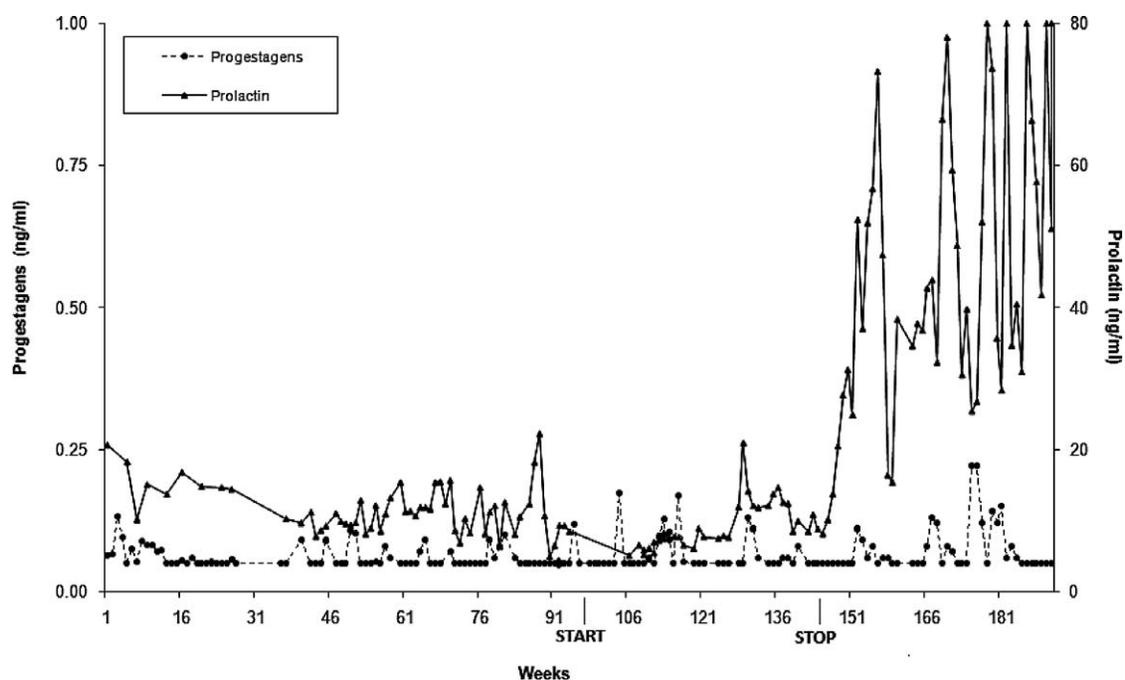
<sup>abc</sup> Means in the same column with different superscripts are significantly different ( $P < 0.05$ ).

<sup>A</sup> Prolactin concentrations for all samples during the treatment period exceeded the upper assay limit and so were assigned a value of 80 ng/ml.

## DISCUSSION

The present results indicate that oral cabergoline treatment is effective at reducing serum prolactin concentrations in elephants with hyperprolactinemia; however, it was not 100% effective, as only four of the five elephants responded with a significant reduction. Furthermore, although in an earlier study normalization of prolactin concentrations facilitated the return of normal ovarian cycles in an Asian elephant,<sup>2</sup> none of the African elephants in this study resumed a normal cyclic progesterone pattern. Thus, while the use of cabergoline shows promise in reducing elevated prolactin concentrations in both Asian and African elephants, it is possible that a longer treatment course or higher doses may be required for successful reinitiation of ovarian activity.

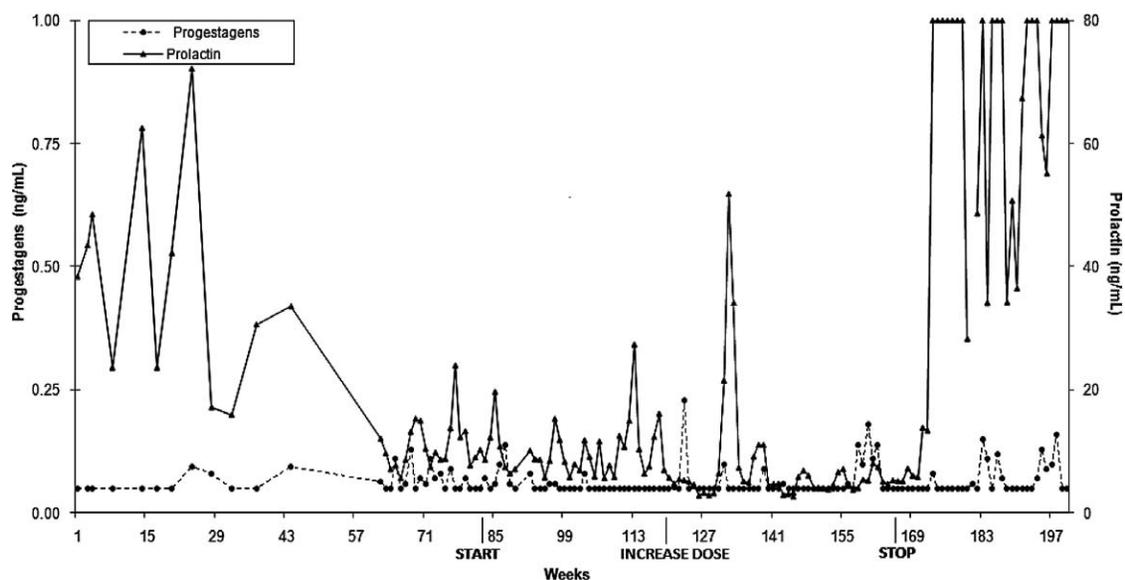
In women, the recommended dosage of cabergoline for treatment of hyperprolactinemia and associated infertility is 0.25 mg administered twice weekly.<sup>8</sup> The dose can be increased by 0.25 mg weekly on the basis of suppression of serum prolactin, up to a maximal twice-weekly dose of 1 mg to ensure prolactin secretion normalizes. A minimum duration of 1 yr for cabergoline therapy is recommended.<sup>8</sup> In contrast, treatment duration in our study ranged from 16 to 48 wk. The wide range of treatment duration was due to the individual prolactin responses to cabergoline treatment and this study's conservative approach to the use of cabergoline for the first time in African elephants. A large controlled study involving 459 hyperprolactinemic women investigated the effect of cabergoline on ovarian acyclicity and found that serum prolactin concentrations returned to normal in 83.4% of treated patients and that ovulatory cycles or pregnancy occurred in 72% and 52% of patients, respectively.<sup>35</sup> By contrast, in this study, although serum prolactin returned to normal concentrations with cabergoline treatment, none of the elephants exhibited a progesterone pattern indicative of



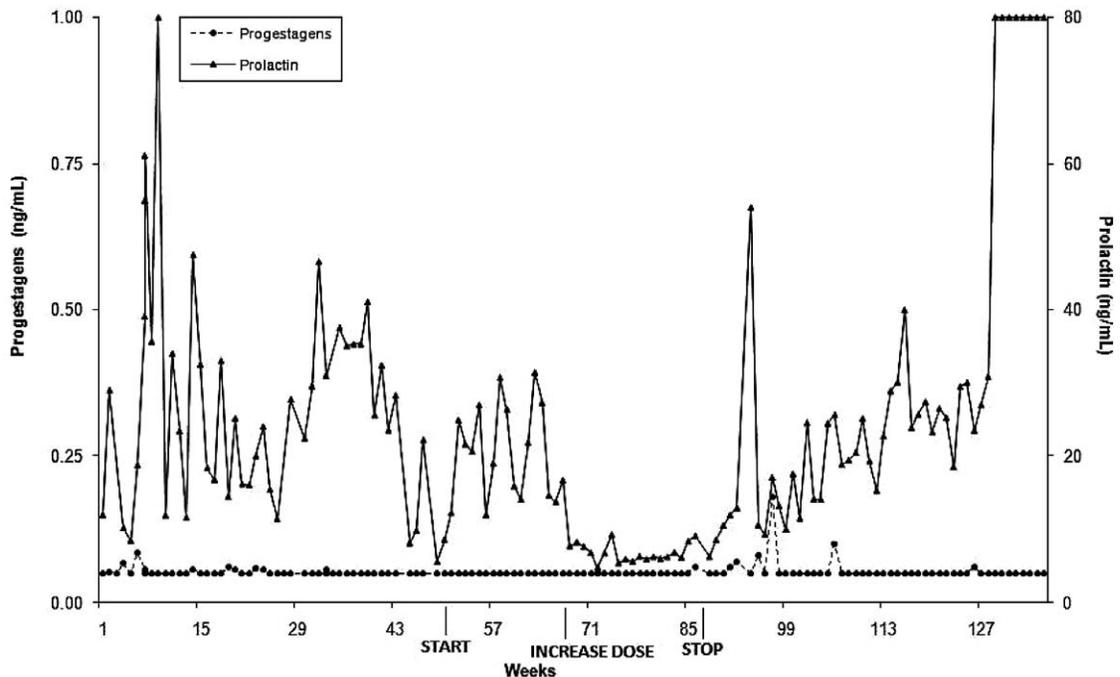
**Figure 1.** Serum progesterone and prolactin concentrations before, during, and after cabergoline treatment in a female African elephant 1. Cabergoline dose was 1 mg twice weekly for 46 wk.

normal ovarian activity or ovulation. The treatment was based conservatively on the highest human dosage and was increased after a lack of response in two elephants, but clearly was not sufficient.

In other species, cabergoline treatment regimens vary considerably. In a limited trial of one 17-yr-old hyperprolactinemic, acyclic gorilla with a confirmed pituitary mass consistent with prolactinoma, initial treatment with 0.25 mg (0.002



**Figure 2.** Serum progesterone and prolactin concentrations before, during, and after cabergoline treatment in female African elephant 2. Cabergoline dose started at 1 mg twice weekly for 34 wk and then was increased to 2 mg twice weekly for 48 wk.



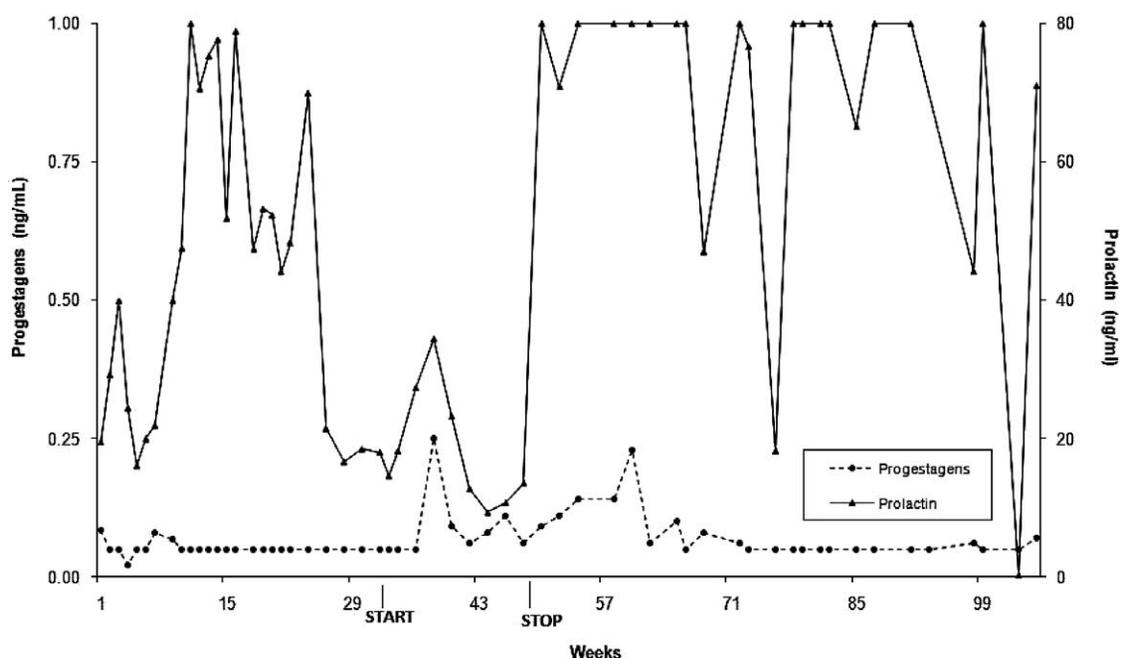
**Figure 3.** Serum progesterone and prolactin concentrations before, during, and after cabergoline treatment in female African elephant 3. Cabergoline dose started at 1 mg twice weekly for 19 wk and then was increased to 2 mg twice weekly for 16 wk.

mg/kg) cabergoline twice weekly was ineffective.<sup>9</sup> Prolactin concentrations remained elevated (68.7 ng/ml); thus, the dose was increased to 0.5 mg (0.004 mg/kg) twice weekly 3 mo later. After 6 mo, prolactin concentrations were within normal limits (10.4 ng/ml), and a magnetic resonance imaging (MRI) study confirmed that the mass was notably smaller, so treatment was discontinued. Subsequently, the gorilla was observed copulating regularly, and within 6 mo it conceived. In dogs, effective cabergoline treatment for estrus induction is typically 0.005 mg/kg once daily orally for 40 consecutive days.<sup>24</sup> In this present study, the higher cabergoline dose was only about 0.0005 mg/kg. Given the recommendations on cabergoline treatment for women and the successful outcome of cabergoline usage in a gorilla and dogs, elephants may need to be treated longer and at a higher dose to reinstate ovarian activity.

Cabergoline treatment normalizes serum prolactin levels and reduces tumor size in most human patients with prolactinomas. Eighty percent of prolactinomas treated with dopamine agonists shrink by more than 25% of the original volume, and in almost all patients, therapy is associated with a 50% reduction in serum prolac-

tin.<sup>8</sup> Tumor shrinkage and prolactin reduction are often observed within a week or two after starting therapy but in some cases may not commence for several months. In nontumoral hyperprolactinemia, prolactin concentrations normalize within 48 hr after starting therapy.<sup>1</sup> In this current study, a decline in prolactin concentrations occurred within 1 wk in two elephants but was not observed until 1–3 mo after cabergoline therapy initiation in the other two responders. This variation in the time required to initiate a prolactin reduction could indicate different etiologies of hyperprolactinemia of both tumoral and nontumoral types. If consistent with hyperprolactinemia in women, two of the study elephants may have had tumoral hyperprolactinemia. However, confirming the presence of prolactinomas requires MRI technology, which is not practical for elephants at the present time.

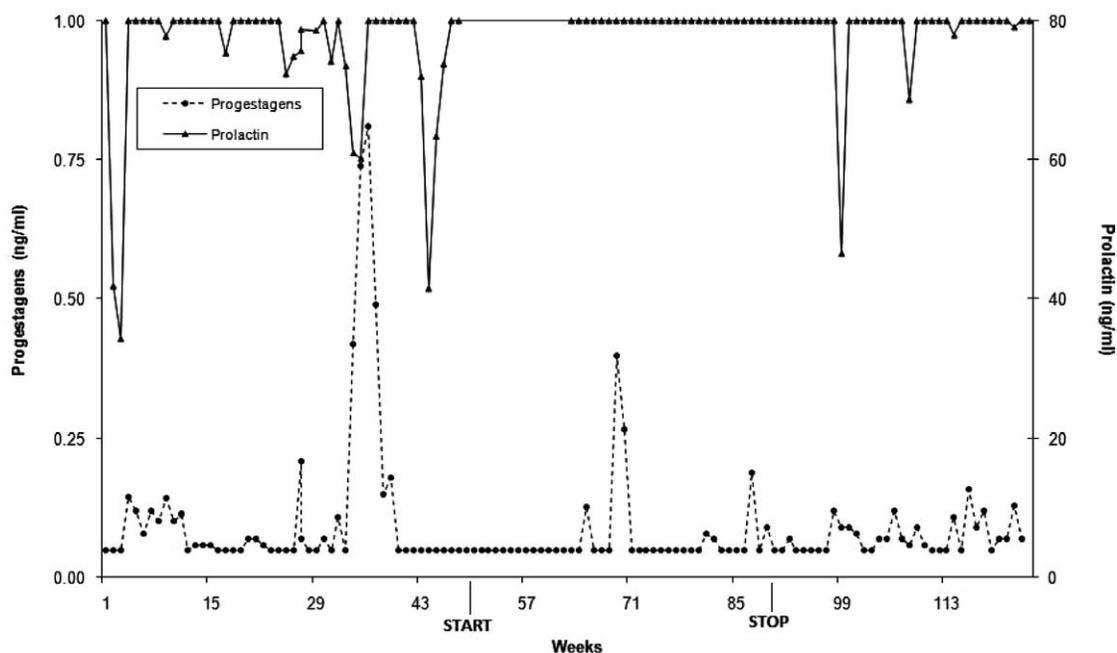
A meta-analysis of 19 studies, including a total of 743 patients, was conducted to assess the effect of dopamine agonist withdrawal on hyperprolactinemia recurrence in women with nontumoral hyperprolactinemia and prolactinomas.<sup>12</sup> Results indicated that hyperprolactinemia can recur after cabergoline withdrawal in a considerable proportion of patients. There was a higher proportion of



**Figure 4.** Serum progesterone and prolactin concentrations before, during, and after cabergoline treatment in female African elephant 4. Cabergoline dose was 1 mg twice weekly for 16 wk.

treatment success in idiopathic (nontumoral) hyperprolactinemia (32%; 95% confidence interval [CI], 5–80%), compared to both microprolactinomas (21%; 95% CI, 10–37%) and macroprolacti-

nomas (16%; 95% CI, 6–36%). Furthermore, the probability of treatment success was highest when cabergoline was used for at least 2 yr. In this study, a significant rebound effect was observed within 6



**Figure 5.** Serum progesterone and prolactin concentrations before, during, and after cabergoline treatment in female African elephant 5. Cabergoline dose was 1 mg twice weekly for 39 wk.

wk after cabergoline withdrawal, with serum prolactin increasing to concentrations that were as high as or higher than pretreatment levels in the four responding elephants. The elephant that did not respond to cabergoline had the highest pretreatment prolactin concentration of all study elephants. This finding may suggest that treatment duration may need to be longer to cause a decrease in prolactin levels in elephants with very high pretreatment prolactin levels. Taken together, these findings further suggest that cabergoline treatment of a longer duration in elephants might be more efficacious in normalizing prolactin secretion and mitigating reproductive problems.

Other than tumors, impairment of hypothalamic production of dopamine or a compression of the pituitary stalk that impairs dopamine transport to the pituitary can cause hyperprolactinemia. Head trauma and large pituitary adenomas other than prolactinomas can result in hyperprolactinemia, as can renal or hepatic failure because of decreased prolactin clearance.<sup>8,37</sup> Based on health records, none of these conditions are likely causes of hyperprolactinemia in the elephants in this study. Similarly, polycystic ovarian syndrome is often associated with elevated prolactin in women,<sup>11</sup> but it is not common in elephants.<sup>32</sup> Finally, pharmacologic agents that reduce dopamine secretion or action, such as neuroleptics, antihypertensives, psychotropic drugs, and anti-ulcer medication,<sup>8,11,28</sup> can elevate prolactin but were not part of any treatment regimens in the elephants of this study. Thus, the etiology of hyperprolactinemia in elephants remains to be established.

In conclusion, maintaining a sustainable African elephant population and identifying causes of ovarian acyclicity are currently high priorities of the Association of Zoos and Aquariums Elephant Species Survival Plan. There is a legitimate concern within the zoo elephant community that high rates of reproductive problems may lead to the eventual extinction of this captive population. The proportion of females with hyperprolactinemia has increased over the past 7 yr,<sup>13</sup> from 37% of noncycling females in 2004<sup>7</sup> to 71% in 2011.<sup>14</sup> Perhaps related, rates of ovarian cycle problems have also increased over this 7-yr period, from 22% in 2004 to 41% in 2011.<sup>14</sup> The concomitant increase in ovarian problems and rates of hyperprolactinemia suggests a physiologic link between the two. Based on the results of this study, cabergoline treatment effectively reduced serum prolactin concentrations in hyperprolactinemic African elephants but did not reinstate cyclicity, perhaps because the treatment dose or duration

was inadequate. Thus, further research is warranted to elucidate the physiologic mechanism(s) and pathogenesis of hyperprolactinemia and to identify effective therapies for treatment of this condition and associated ovarian cycle problems in elephants.

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