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Endocrine correlates of puberty in female Asian elephants (*Elephas maximus*) at the Pinnawala elephant orphanage, Sri Lanka

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Abstract

Background: Previous studies have established ovarian cycle characteristics of adult Asian elephants using progestagen analyses, but little work has been done on young elephants to determine age at puberty. Demographic studies of wild Asian elephants suggest females give birth at about 12–18 years of age (conceiving at 10–16 years of age based on a 2-year gestation). However, there are a few examples of zoo elephants giving birth at only 5–6 years of age, so they would have started cycling much earlier. This study was carried out at the Pinnawela Elephant Orphanage (PEO) in Sri Lanka, where a herd of >80 captive elephants breeds successfully, resulting in a unique opportunity to monitor hormones and document initiation of ovarian cyclicity in young females, thus contributing to the normative reproductive database for this species.

Results: We measured serum progestagens in samples collected every 10 days for 18 – 24 months from 11 females (3.5–15 years of age), and found six (5.5 – 12 years of age) already were cycling at study onset. Four females started cycling during the study at 4.5, 5.5, 7.5 and 15 years of age. There were no quantitative or qualitative differences between the first pubertal luteal phase and those of subsequent cycles. Of the 46 ovarian cycles observed, 78% were associated with clear behavioral signs of estrus (heightened bull attentiveness, and willingness of females to be mounted) during the late non-luteal period when progestagens were low. The average body weight at puberty was ~48% of that of adult female elephants at PEO.

Conclusions: Asian elephants under human care, including under semi-captive conditions, may reach puberty earlier than those in the wild, perhaps due to better nutrition and reaching a body weight capable of supporting reproductive activity at a younger age. Thus, facilities with bulls need to carefully manage elephants to avoid accidental pregnancies in young females that may be too small to safely carry a pregnancy to term.

Keywords: Elephant, Estrus, Ovarian cycle, Progestagens, Puberty

Background

Ovarian cycle activity in elephants is easily determined by weekly or bi-weekly progestagen analyses of serum, urine or feces [1–3]. A properly maintained record of progestagen data is key to determining the reproductive capacity of individual animals, as well as allowing proper planning and timing of breeding, and accurate pregnancy diagnosis [4]. A substantial endocrine database related to ovarian cycle characteristics now exists for Asian elephants, particularly those in western zoos [3]. Elephants have the longest estrous cycle of any land mammal, ranging between 13 and 17 weeks, with a 3 to 6-week follicular phase and 7 to 12-week luteal phase [4, 5]. By contrast, substantially less is known about aspects of reproductive function in 'domesticated' Asian elephants in range countries. Despite their being managed under human care for thousands of years, few captive populations are self-sustaining [6]. Today, with the exception of Myanmar, it is illegal to capture wild Asian elephants for any purpose; thus, to sustain this important cultural heritage, successful captive breeding programs are essential, and could benefit by a thorough understanding of basic biological functioning, including age at puberty onset [4].

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From observational data, Asian elephants begin reproducing at around 12–18 years of age in the wild [7, 8]. In North American zoos, the average age at first conception based on studbook data is 16.9 years [9]. Logging elephants of Myanmar show low fecundity up to about 13 years of age, followed by a rapid increase to a peak at 19 years of age [10]. There are examples of captive births occurring at much younger ages, however, raising questions about when females actually reach puberty and are capable of reproduction. For example, the Asian elephant studbook in the U.S. lists two zoo births in females less than 7 years of age [11]. We also have preliminary hormone data in Asian elephants showing initiation of ovarian cyclicity at 6, 7 and 8 years of age [4, 5]. Unfortunately, few facilities collect samples for hormone monitoring before 8 years of age, so it is unclear how common it is for females to start cycling that young.

Of the ~4000 Asian elephants in Sri Lanka, about 300 are maintained in captivity, mostly at temples or held by private owners [12]. The largest captive population in Sri Lanka is at the Pinnawala Elephant Orphanage (PEO), managed by the Department of National Zoological Gardens, which is a rare exception in having not only a sustaining, but also a growing population [13, 14]. The PEO began as an elephant orphanage in 1975 with five baby elephants. Today it is a successful captive breeding center with a current population of over 80 elephants, including 45 that were born at the orphanage; there now are three generations of elephants at PEO [14]. Thus, this facility provided a unique opportunity to study hormonal changes in young elephant females, at or near the time of puberty. The aim of this study was to analyze serum progestagens in young Asian elephants at the PEO to determine pubertal status. Changes in body weight also were examined in relation to age and ovarian cycle activity. Such information could help improve the on going breeding program at PEO, and also provide information to captive facilities globally on how to best manage young elephants for maximal reproductive success.

Methods

Animals and sample collection

The PEO is open to the public and located in the wet-zone, mid-country region of Sri Lanka (92 m elevation). It consists of 11 hectares with sheds for night accommodation, a grassland area with a large water hole, and access to the adjacent Maha Oya river. Elephants are not tamed and are managed under semi-captive conditions as a group comprising adult females and male/female calves, which are allowed full social interactions throughout the day. Adult females are chained overnight from approximately 1600 – 0800 h; calves under 4 years of age are untethered and stay in close proximity. Females and young bulls (<8–10 years of age) are walked as a group to the river (<1 km) for bathing twice a day (at 1000 and 1400 h) for 2 h each, and fed a mixture of browse, supplemented with local fruits. A full time veterinarian is onsite, and individual records of health and reproductive events are maintained on all elephants, starting at birth or arrival at PEO. This study was approved by the Animal Care and Use Committee at the Peradeniya University, Kandy, Sri Lanka, and Conservation & Research Center/National Zoological Park Animal Care and Use Committee (#06-20).

Eleven female elephants at PEO were evaluated and are described in Table 1. All calves of wild origin were brought

Table 1 Summary of the age and origin of female elephants utilized in this study, and mean (± SEM) estrous cycle characteristics and serum progestagen concentrations

Female	Date of Birth	Age at Onset and End of Study (years)	Captive or Wild Born	Number of Cycles	Luteal Phase Length (days)	Follicular Phase Length (days)	Total Cycle Length (days)	Baseline Progestagens (ng/ml)	Luteal Peak Progestagens (ng/ml)
Tikiri	~Sep 2002	3.0 – 4.5	Wild	0				0.63	
Mali*	~ Nov 2001	3.2 – 5.0	Wild	4	55.1 ± 6.8^{a}	24.2 ± 6.5	90.0 ± 1.7^{a}	0.73	2.25 ± 0.12^{c}
Maya*	~Jan 2000	4.0 – 5.8	Wild	4	$79.0 \pm 4.2^{b,c}$	25.3 ± 9.5	104.3 ± 13.5^{b}	0.55	3.26 ± 0.50^{d}
Uthpala	Aug 1999	5.5 – 7.5	Captive	7	$78.3 \pm 7.1^{b,c}$	33.4 ± 2.8	114.6 ± 6.0^{c}	0.34	0.94 ± 0.10^{a}
Ashokamala*	Aug 1999	6.5 – 8.3	Captive	4	$78.0 \pm 3.4^{b,c}$	28.3 ± 0.8	109.3 ± 2.8^{c}	0.20	0.89 ± 0.15^{a}
Surangi	~Dec 1997	7.0 – 9.2	Wild	7	$76.9 \pm 2.8^{b,c}$	29.0 ± 3.2	$104.8 \pm 4.5^{b,c}$	0.76	2.06 ± 0.20^{c}
Supamali**	~Jun 1997	7.3 – 9.1	Wild	2	$69.5 \pm 3.3^{a,b}$	28.5 ± 3.3	$98.0 \pm 1.0^{a,b}$	0.50	2.23 ± 0.04^{c}
Meena**	~Nov 1997	8.3 – 10.3	Wild	3	$77.7 \pm 2.8^{b,c}$	35.0 ± 8.3	112.7 ± 5.6^{c}	0.52	2.01 ± 0.27^{c}
Sandali	~Jul 1993	11.6 – 13.6	Wild	7	$65.7 \pm 5.9^{a,b}$	27.0 ± 5.2	88.5 ± 5.7^{a}	0.43	1.28 ± 0.16^{b}
Kanthi	~Aug 1992	12.8 – 14.8	Wild	6	82.3 ± 6.3^{c}	31.0 ± 2.0	113.3 ± 6.2^{c}	1.70	4.71 ± 0.17^{e}
Manika*	~1990	14.3 – 16.2	Wild	3	83.7 ± 10.9^{c}	33.7 ± 2.1	117.3 ± 12.6^{c}	1.13	3.43 ± 0.25^{e}
Overall Mean					74.6 ± 2.8	30.6 ± 1.1	105.3 ± 3.2	0.68 ± 0.13	2.31 ± 0.38

*Exhibited first estrous cycle during the study period
**Females conceived during study period
[a,b,c,d,e]Values within column differ ($P < 0.05$)

to the PEO before 3 years of age, except Manika who was rescued from an army camp in Northern Sri Lanka and arrived at 14 years of age. Age was estimated by height and weight measurements in comparison with known ages of elephants born at PEO. During the first 6 months of the study, elephants were trained for blood collection using positive reinforcement with bananas as rewards. Blood samples were then collected every 10 days for 18 to 24 months from an ear vein without sedation while in a standing position. Samples were centrifuged at 1500 g for 10 min within an hour of collection and the serum stored at -20 °C until analysis. Serum progestagen concentrations were analyzed using a single-antibody enzyme immunoassay validated for elephant serum [15] that relied on a monoclonal progesterone antibody (1:10,000, CL425; Coralie Munro, University of California, Davis), horseradish peroxidase conjugated progesterone label (1:40,000) and progesterone standards (Catalog #P0130, Sigma Chemical Co., St. Louis, MO). Assay sensitivity was 0.016 ng/ml. Inter- and intra-assay coefficients of variation of high and low control samples run in each assay ($n = 42$) were 8.8 and 13.7%, respectively.

Behavioral observations were carried out daily for 2 h while elephants were in the field (0800 – 1000 h) and for 2 h at the river (1400 – 1600 h). Behaviors included those directed from female to male (standing closer, touching, smelling genitalia, urine or dung) and from male to female (genital inspection, urine testing, flehmen and mounting). Signs of estrus (present/not present) were recorded daily; estrous females were allowed access to one or more bulls over a period of about a week in an isolated section of the grassland area, or occasionally in the river, for natural mating.

Body weight (to the nearest 5 kg) was measured monthly using an electronic weigh bridge (Weightronics Levli Ltd., Sri Lanka). An additional five non-pregnant, cycling females (32.3 ± 6.5 years of age; 20 – 40 years) were weighed monthly to compare with young elephants.

Statistical analysis

Mean data are presented as ± standard error (SEM). Estrous cycle characteristics were determined based on serum progestagen patterns [5]. For each animal, progestagen values above the mean plus 1.5 times the standard deviation (SD) were removed and the process repeated until no values exceeding the mean + 1.5*SD remained. The remaining data points defined the baseline for that individual. The first and last week of the luteal phase were based on the following criteria: 1) the luteal phase was defined as progestagen concentrations greater than baseline for at least 2 consecutive weeks, with a duration of at least 4 weeks; 2) the follicular phase was defined as progestagen concentrations below the baseline for at least 2 consecutive weeks; 3) single point fluctuations above or below baseline were considered within the same phase as the surrounding points; 4) data points on the baseline were included in the previous phase; 5) when data were not available for a given week, and that week appeared to coincide with the start or end of a luteal phase, it was added to the luteal phase. Estrous cycle duration was calculated as the number of weeks from the first increase in serum progestagens until the next increase. Cycle durations were compared among individuals with a Kruskal-Wallis test, with post-hoc pairwise comparisons using Dunn's Multiple Comparisons [5]. Differences between the first cycle luteal phase and average luteal phase lengths for the individuals that started cycling during the study were determined by paired t-tests.

Results

A summary of ovarian cycle characteristics is shown in Table 1. Ten of 11 females exhibited progestagen patterns indicative of normal ovarian activity during the study period. Of these, six were already cycling, at ages 5.5, 7.0, 7.3, 8.3, 11.6 and 12.8 years; two of these conceived for the first time within 2–3 cycles of study onset at ~8 years of age. Four females exhibited their first cycle during the study at 4.5, 5.0, 7.5 and 15.0 years of age. One female that was sampled from 3.0 – 4.5 years of age was acyclic throughout. Total estrous cycle lengths varied across individuals, ranging from 90.0 to 117.3 days, a result of significant differences in luteal phase length. There were no differences in luteal phase characteristics at puberty onset (length, 69.3 ± 11.1 days; peak progestagens, 2.33 ± 0.56 ng/ml) compared to subsequent cycles (83.7 ± 10.9 days; 2.31 ± 0.38 ng/ml), respectively ($P > 0.05$). Progestagen profiles in Fig. 1 represent peri-pubertal (a,b) and pubertal (c) elephants, and illustrate the similarity and consistency of cycles within individuals, and between pubertal and subsequent cycles. Pregnancy was easily diagnosed in the females that conceived, as post-breeding progestagens were several-fold higher than preceding luteal phase concentrations (e.g., Fig. 1d).

Of 46 documented cycles in 10 females, 36 were associated with behavioral signs of estrus in the last 2 weeks of the follicular phase; no estrous behaviors were observed during luteal phases. All 10 showed signs of behavioral estrus during some cycles. In five of the females (Mali, Maya, Uthpala, Ashokamala, Sapumali), 100% of the cycles were associated with behaviors that corresponded with changes in hormonal status; i.e., just before the rise in progestagens. By contrast, clear signs of behavioral estrus were detected in only 50% of the cycles in one of the older females (Kanthi), who had been mounted with observed intromission multiple times but never conceived. Interestingly, mean baseline and peak progestagen concentrations in that female were the highest compared to the other elephants. Estrous behaviors

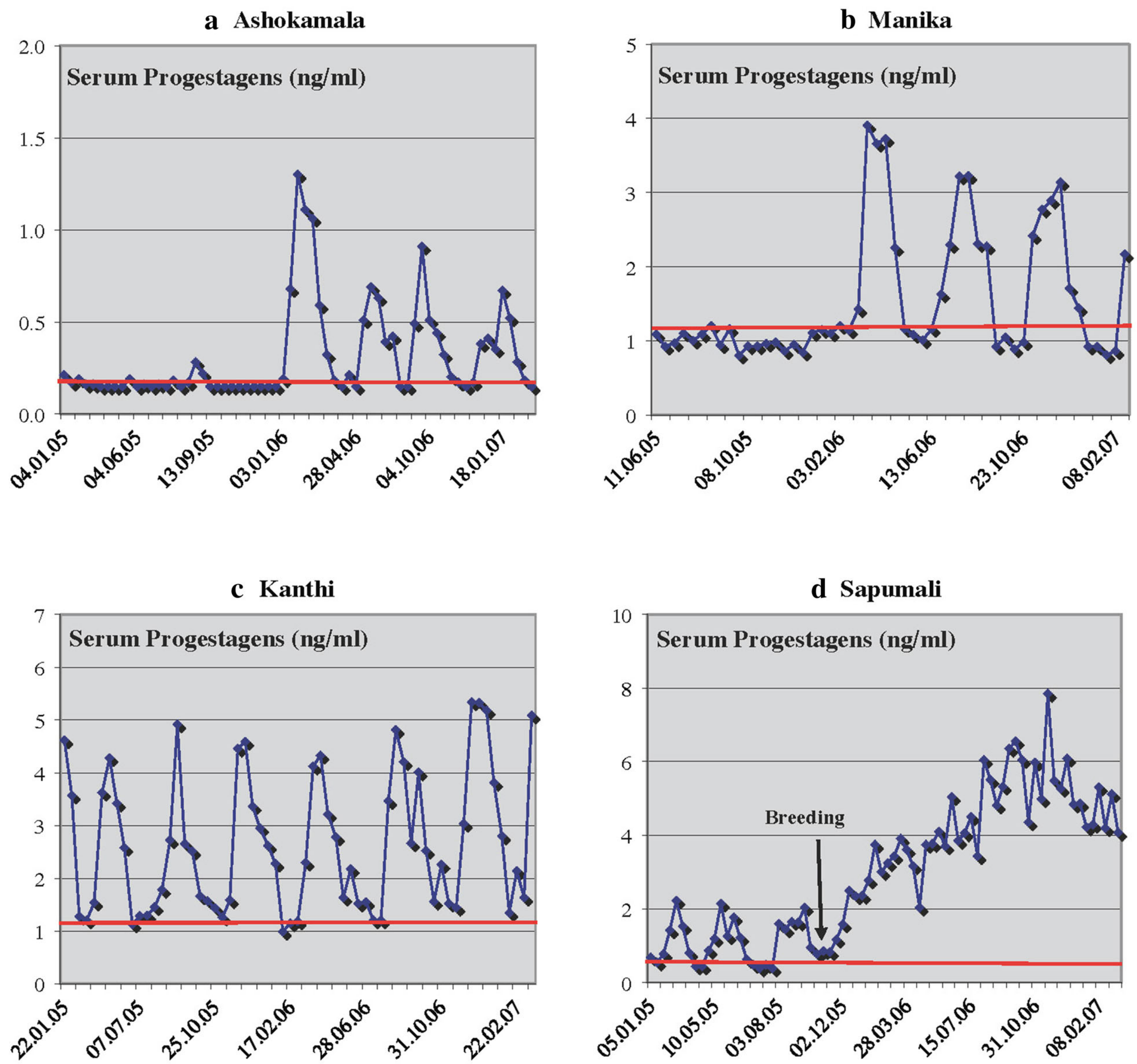

Fig. 1 Representative serum progestagen profiles of elephants that reached puberty during the study (**a**, **b**), were cycling at study onset (**c**) and conceived after study onset (**d**). *Red* line indicates baseline concentration

associated with the first estrous cycle were observed in all four females that started cycling during the study period. No estrous behavior was observed in the youngest female (Tikiri), who showed no cyclic ovarian activity during the study period.

Body weights at puberty were 950 kg (4.5 years of age), 1020 kg (5.5 years), 1265 kg (7.5 years) and 1645 kg (15.0 years), which corresponded to 38, 40, 50 and 65% of the adults' weight (2520 ± 101 kg), respectively. The weights of the two females at first conception were 1950 kg at 8.1 years of age (77% of adult weight) and 2275 kg at 8.7 years of age (90%). Weights (and ages) of the six females that were cycling at study onset were 1590 kg (5.5 years), 1700 kg (7.0 years), 2180 kg (7.3 years), 1800 kg (8.3 years), 1695 kg (11.6 years) and 2285 (12.8 years), all of which were over 60% of the averaged adult weight. The one female that never cycled weighed only 690 kg (27% of adult weight) at the end of the study period.

Discussion

Based on PEO records, age at first calving is about 14 years (range, 10 – 25 years); thus, average conception is ~12 years of age given a 20 to 22-month gestation [4]. In our study, three females exhibited their first pubertal cycles between 4.5 and 7.5 years of age, younger than we had anticipated. Several other elephants under 9 years of age also were cycling at the start of the study, and two conceived at ~8 years old. Only one female did not cycle, but she was very young, only 4.5 years of age at study conclusion. Altogether, these data suggest that female Asian elephants may attain pubertal status at an earlier age than originally believed, which could have significant management implications if females conceive too young [4]. However, without hormonal data it is impossible to know when wild elephants begin cycling relative to first conception. In zoos, controlled breeding generally limits this. The fact that wild Asian elephants often do not give birth before 12–18 years of age [7, 8] suggests they either do not begin cycling until right before conception or there are other behavioral or physiological factors that prevent pregnancy in younger animals.

Overall mean values for estrous cycle, luteal phase and follicular phase lengths of PEO elephants were within the ranges reported for other captive populations [2–5]. In the four cases for which hormones documented pubertal cycles, the first was comparable to subsequent cycles, similar to the finding of Glaeser for two Asian females in the U.S. [5]. Quality of the first pubertal ovarian cycle varies among mammals [16]. For example, in the gilt and filly, the pubertal corpus luteum appears to have a normal lifespan, whereas in the ewe and heifer, a transient (ewe, 1 – 4 day; heifer, 3 – 10 day) rise and fall in circulating progesterone is detected before the first "normal" luteal phase. Without ultrasound data we cannot know for sure if the first progestagen cycle in the pubertal elephant is associated with ovulation, but at present that is the assumption [5]. In general, the first cycle is not accompanied by behavioral estrus in the ewe, and may or may not be in the heifer, gilt and filly [16]. By contrast, all of the pubertal cycles in the PEO elephants were associated with clear estrous behaviors, which were facilitated by the routine practice of allowing access to bulls.

In other ungulates, the onset of puberty can be influenced by age, weight, health and environmental factors [17–19], although often it is body weight that is most strongly correlated with initiation of ovarian cyclicity. In cattle, Wiltbank [20] refers to a critical age-to-weight ratio that must be reached before heifers attain puberty, whereas Meirelles et al. [19] suggested most begin cycling at about 65% of the mature body weight. In this study, pubertal female body weights were highly variable: 38 – 65% of the adults. Other females that were cycling at study onset were at 63 – 90% of the adult weight, whereas the young female that never cycled was only at 27%. A Borneo elephant that did not cycle until 12 years of age was always substantially smaller than her similar aged herdmates [5]. Based on behavior and PEO records, all of the females had been exposed to or were mated by adult bulls prior to and during the study, but only two conceived, at 77 and 97% of the average adult body weight. Thus, while female elephants can begin cycling at a lower relative body weight, it does not necessarily mean they can or will conceive. One female at PEO (Manika) did not cycle until 15 years of age. She was an orphan that was brought to the PEO from a Northern army camp. Her general body condition was good, and she weighed in excess of 2400 kg, more than many others at PEO that had begun cycling at younger ages. Thus, it is not known if the delay in exhibiting estrous cyclicity was due to unfavorable conditions, or if cycles were initially suppressed due to the stress of a new environment and social group.

Conclusion

In conclusion, daily behavioral observations aid in the detection of behavioral estrus, as bulls are attracted to estrous females through pheromonal signals in urine [21]. However, this strategy only works well if animals are managed in a herd setting with access to bulls, like at PEO. With this study, we add to the limited hormonal database on young Asian elephants, and support findings that females can initiate ovarian cyclicity several years earlier than reports of wild elephant first conception ages indicate. The reason for this seemingly accelerated rate of sexual development is unclear, but could be related to higher levels of nutrition in captivity compared to diets in the wild [3]. In this regard, it may be similar to early puberty onset associated with increased body fat in girls [22]. It is not known if early puberty poses health problems for elephants, but it could present management problems for facilities with bulls if females breed before they are physically (i.e., adequate body size) or psychologically ready. There also may be trade-offs between reproduction and survival for elephants. Evaluation of an extensive longitudinal dataset of semi-captive timber elephants in Myanmar ($n = 8006$) found an association between reproduction and adult survival, being positive in early life, but negative later on [23]. Reproduction and survival trade-offs were greater after peak reproduction was achieved, and investing in offspring after the age of 30 years decreased survival probabilities. That study was conducted on logging elephants however, which are subjected to intense workloads not experienced by elephants at PEO. Whether similar reproductive-lifespan trade offs occur in other elephant populations remains to be determined.

Abbreviation

PEO: Pinnawala elephant orphanage

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Availability of data and materials
There are no additional files; data are available upon request. The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions
All authors contributed to the study design, which served as the Masters project for SM, SM and RCR were responsible for obtaining and analyzing breeding records at PEO. SM conducted behavioral observations, weighed the animals and collected and processed the blood samples, under the mentorship of NKJ and JLB. JLB and SM participated in laboratory analyses, analysis and interpretation of hormone data. All authors read, provided input on and approved the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Animal Care and Use Committee at the Peradeniya University, Kandy, Sri Lanka, and Conservation & Research Center/ National Zoological Park Animal Care and Use Committee (#06-20).

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