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Investigation of individual and group variability in estrous cycle characteristics in female Asian elephants (*Elephas maximus*) at the Oregon Zoo

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Abstract

Evaluating ovarian cycle activity through longitudinal progestagen monitoring is important for optimizing breeding management of captive elephants and understanding impact of life events (births, deaths, and transfers) on reproductive function. This study summarized serum progestagen profiles for eight Asian mainland elephants (*Elephas maximus indicus*) and one Bornean elephant (*E. maximus borneensis*) at the Oregon Zoo over a 20-yr interval, and represents the longest longitudinal dataset evaluated to date. Estrous cycle characteristics were more varied than previously reported for this species, with an overall duration of 12 to 19 wk, luteal phase duration of 4 to 15 wk, and follicular phase duration of 2 to 12 wk. In general, there was more cycle variability across than within individual elephants. Compared with other elephants in the group, the Borneo female exhibited consistently longer cycle lengths, higher progestagen concentrations, and greater cycle variability; however, it is not known if this represents a subspecies or an individual difference. Cycle durations did not appear to change over time or with age, and the first pubertal cycle was similar to subsequent cycles. Variability in duration of the follicular phase was greater than that of the luteal phase. In addition, there was a significant negative relationship between luteal and follicular phase durations, suggesting a possible regulatory role of the follicular phase in maintaining a relatively consistent cycle duration within individuals. Overall, we found these elephants to be highly resilient in that major life events (births, deaths, and changes in herd structure) had minimal effect on cycle dynamics over time. In conclusion, the higher range in cycle phase characteristics is likely because of the larger number of elephants studied and longer duration of longitudinal monitoring, and may be more representative of the captive population as a whole. Furthermore, identification of significant interanimal variability suggests that understanding the complexities of herd reproductive characteristics could facilitate development of more effective institution-specific breeding management strategies.

Keywords: Elephant; Reproduction; Estrous cycle; Follicular phase; Luteal phase; Progesterone

1. Introduction

The Asian elephant (*Elephas maximus*) is listed as endangered, with estimates of only 25 000 to 50 000 remaining in the wild, and approximately 16 000 managed under human care [1–4]. In North America, there are 269 (53 male, 216 female) individuals in the Asian Elephant Regional Studbook [5]. Currently, this population is not self-sustaining and historically has relied on supplemental importations from range countries to sustain numbers, an increasingly unrealistic option as

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import restrictions increase. Weise [6] reported that to maintain a captive population of approximately 250 Asian elephant females in North America, seven to nine female calves must be born annually. The actual birth rate has been only 2.6 female calves per year over the past 10 yrs [5], which is considerably lower than the rate needed to maintain the population. Thus, institutions must rely on all available tools to maximize reproductive efficiency, including a combination of assisted reproductive techniques and managed natural breeding introductions. To accomplish this goal, it is critical to understand the range of normal variation in Asian elephant reproductive cycles, as well as how management and social factors might impact those cycles.

Based on behavioral observations, the Asian elephant estrous cycle was originally believed to be 18 to 27 days in length [7,8]. Elephant ovarian cycles were initially difficult to monitor because the CL produces primarily 5α-dihydroprogesterone (5α-DHP) and little actual progesterone [9–13]. Through improved progestagen monitoring with assays that detected 5α-DHP, the elephant estrous cycle was found to be the longest of any mammal published to date, with a 13 to 17 wk duration, consisting of an 8 to 10 wk luteal phase and a 4 to 6 wk follicular phase [14–16]. Another unique aspect of the elephant estrous cycle is the occurrence of two LH surges, approximately 20 days apart during a normal follicular phase, with only the second surge inducing ovulation [16–18].

Because of the long ovarian cycle, elephant females are fertile only three to four times per year, and for only 2 to 3 days per cycle [19]. With so few opportunities to impregnate elephant females, breeding managers must have accurate data on individual elephants to understand subtle variations in estrous cycle patterns. Published estimates of the Asian elephant estrous cycle duration are derived from a surprisingly limited number of individuals studied for variable, and often short, intervals [7,8,13–15,19,20]. With such a long-lived species, it can take decades to compile a dataset large enough to identify subtle variations in estrous cycle patterns. Thus, most studies to date have been of insufficient duration to evaluate cycle characteristics within or among females with respect to age, management, and social (e.g., herdmate introductions and deaths) factors.

The Oregon Zoo has the longest-running dataset of routine weekly blood samples for Asian elephants, including data from males and females, breeding and non-breeding. This study summarizes serum progestagen data for eight Asian mainland elephants (*Elephas maximus indicus*) and one Bornean elephant (*E. maximus borneensis*) housed at the Oregon Zoo, several of which have been monitored for over two decades. Longitudinal serum progestagen data were analyzed to determine: 1) cycle variability across and within individuals; 2) role of luteal and follicular phase durations in maintaining duration of cycle; 3) effect of age on cycle parameters; 4) characteristics of the first pubertal cycle compared to subsequent cycles; 5) impact of management and social changes (transfers in, transfers out, births, deaths) on long-term cycle dynamics; and 6) timing of progestagen peaks relative to the luteal midpoint (as a potential method of predicting the end of the luteal phase). Results of evaluations of more than 3000 weekly data points and over 180 estrous cycles, encompassing puberty through senescence, are presented. This study represents the most thorough investigation published to date on estrous cycle characteristics of Asian elephants, on both a group and an individual basis.

2. Materials and methods

2.1. Animals and sample collection

This study utilized serum progestagen data collected between 1988 and 2008 for nine female Asian elephants (n = 8 *E. m indicus*; n = 1 *E. m borneensis*) housed at the Oregon Zoo (Table 1). All elephants were conditioned to blood sampling procedures (without anesthesia), as part of the normal management routine. Blood samples (3–9 mL) were collected approximately weekly (interval range 3–11 days) from either an ear or leg vein, generally in the morning. Blood was maintained at ~4 °C, allowed to clot at room temperature, then centrifuged (~1500*g*) and frozen. Serum was stored at −20 °C or colder until analysis.

During the 20-yrs study period, females were housed as a single herd or as two separate herds comprising two to four individuals each. Two to four adult bulls were present at any given time and were housed separately, except when placed with females for breeding or for male/female socialization. Over this time, elephants considered dominant members of the herd(s), based on keeper observations of social interactions, were OZ-F1 (highest ranking in all groups), OZ-F4 (secondary to OZ-F1), and OZ-F7 (after OZ-F1 and OZ-F4 had died). These three females were euthanized during the study. Elephant OZ-F1 was euthanized at age 44, after treatment for osteomyelitis, distal metacarpus of digit four on the left front foot, and tenosyn-

Table 1
Estrous cycle data of all females housed at the Oregon Zoo during the study period.

Elephant	Origin†	Age range during study (y)	No. cycles	Cycle characteristics‡						
				Estrous cycle (wk)	Rank§	Luteal phase (wk)	Rank§	Follicular phase (wk)	Rank§	Peak progestagen (pg/mL)
OZ-F1	Wild (Thailand)	36–44	26	13.0 ± 1.5 13.0 ± 0.3 (M = 13) [11–16] **OZ-F3,OZ-F5,OZ-F6, OZ-F7,OZ-F8,OZ-F9*	1	9.1 ± 1.2 9.1 ± 0.2 (M = 9) [7–12] **OZ-F7*	4	3.9 ± 1.3 3.9 ± 0.3 (M = 4) [2–6] **OZ-F3,OZ-F5, OZ-F6,OZ-F9*	1	511 ± 159 511 ± 30 (M = 468) [235–908]
OZ-F2	Wild (Thailand)	37–39	7	14.0 ± 1.3 14.0 ± 0.5 (M = 13) [13–16] **OZ-F9*	2	7.9 ± 2.3[‖] 7.9 ± 0.9 (M = 9) [5–11] **OZ-F7*	2	6.1 ± 1.7 6.1 ± 0.6 (M = 6) [4–8]	5	453 ± 129 453 ± 49 (M = 461) [277–615]
OZ-F3	Captive	4–14	22	15.2 ± 1.6 15.2 ± 0.3 (M = 15) [12–19] **OZ-F1,OZ-F9*	3	8.7 ± 2.0 8.7 ± 0.4 (M = 9) [5–13] **OZ-F7*	3	6.5 ± 2.6[‖] 6.5 ± 0.6 (M = 6) [2–12] **OZ-F1,OZ-F7*	6	512 ± 131 512 ± 27 (M = 499) [276–857]
OZ-F4	Captive	26–44	12	15.2 ± 1.2 15.2 ± 0.3 (M = 15) [13–17]	4	9.4 ± 1.5 9.4 ± 0.4 (M = 9) [7–13] **OZ-F7*	6	5.6 ± 1.6 5.6 ± 0.5 (M = 6) [3–9]	4	330 ± 78 330 ± 22 (M = 344) [220–476]
OZ-F5	Captive	25–44	15	15.5 ± 1.2 15.5 ± 0.3 (M = 15) [14–19] **OZ-F1*	5	7.9 ± 1.6 7.9 ± 0.4 (M = 8) [4–11] **OZ-F7,OZ-F8*	1	7.5 ± 1.7 7.5 ± 0.4 (M = 8) [4–10] **OZ-F1,OZ-F7*	8	279 ± 73 279 ± 17 (M = 265) [183–459]
OZ-F6	Captive	6–26	54	15.7 ± 1.1 15.7 ± 0.2 (M = 16) [13–19] **OZ-F1*	6	9.2 ± 1.3 9.2 ± 0.2 (M = 9) [6–13] **OZ-F7*	5	6.5 ± 1.4 6.5 ± 0.2 (M = 7) [3–10] **OZ-F1,OZ-F7*	7	677 ± 307 677 ± 41 (M = 588) [285–1828]
OZ-F7	Wild (Thailand)	34–51	37	16.3 ± 1.9 16.3 ± 0.3 (M = 16) [13–22] **OZ-F1*	7	11.5 ± 1.8 11.5 ± 0.3 (M = 11) [8–16] **OZ-F1,OZ-F2,OZ-F3, OZ-F4,OZ-F5,OZ-F6*	9	4.9 ± 1.1 4.9 ± 0.2 (M = 5) [3–8] **OZ-F3,OZ-F5, OZ-F6,OZ-F9*	2	906 ± 335 906 ± 53 (M = 856) [298–1808]

(continued on next page)

Table 1
(continued)

Elephant	Origin†	Age range during study (y)	No. cycles	Cycle characteristics‡ Estrous cycle (wk)	Rank§	Luteal phase (wk)	Rank§	Follicular phase (wk)	Rank§	Peak progestagen (pg/mL)
OZ-F8	Wild (Thailand)	18–20	6	16.7 ± 1.2 16.7 ± 0.5 (M = 16) [16–19] **OZ-F1*	8	11.3 ± 2.0 11.3 ± 0.8 (M = 11) [9–15] **OZ-F5*	8	5.3 ± 1.4 5.3 ± 0.6 (M = 5) [4–8]	3	910 ± 170 910 ± 69 (M = 986) [630–1060]
OZ-F9	Wild (Borneo)	7–15	9	19.0 ± 2.9‖ 19.0 ± 1.0 (M = 19) [13–22] **0Z-F1,OZ-F2,OZ-F3*	9	10.4 ± 2.5‖ 10.4 ± 0.8 (M = 10) [7–15]	7	8.0 ± 2.4‖ 8.0 ± 0.8 (M = 7) [6–13] **OZ-F1,OZ-F7*	9	1488 ± 433 1488 ± 137 (M = 1352) [1093–2645]
All females¶		4–51	188	15.4 ± 2.0 15.4 ± 0.1 (M = 15) [11–22]		9.5 ± 2.0 9.5 ± 0.1 (M = 9) [4–16]		5.9 ± 1.9 5.9 ± 0.1 (M = 6) [2–13]		664 ± 374 664 ± 26 (M = 549) [183–2645]
All females** (excl. Outliers)		4–51	179	15.4 ± 1.6 15.4 ± 0.L (M = 15) [12–19]		9.4 ± 1.9 9.4 ± 0.1 (M = 9) [4–15]		5.9 ± 1.8 5.9 ± 0.1 (M = 6) [2–12]		655 ± 369 655 ± 26 (M = 548) [183–2645]
All females†† (*E. m* *indicus*)		4–51	179	15.3 ± 1.8 15.3 ± 0.1 (M = 15) [11–22]		9.5 ± 1.9 9.5 ± 0.2 (M = 9) [4–16]		5.8 ± 1.9 5.8 ± 0.1 (M = 6) [2–12]		622 ± 318 622 ± 23 (M = 539) [183–1828]

* Median duration differs significantly ($P < 0.05$) from other elephants.
† All captive-born individuals were born at the Oregon Zoo.
‡ Data shown as mean ± SD, mean ± SEM, (M = median), (min-max).
§ Kruskal-Wallis rank of median duration for estrous cycle, luteal phase, and follicular phase (shortest = 1, longest = 9).
‖ SD of individual is greater than SD of all females combined with no outliers or individuals removed.
¶ Data for all females and all complete estrous cycles.
** Data for all females with outliers for estrous cycle duration among the group removed.
†† Data for all females with OZ-F9 (*E. maximus borneensis*) removed.

ovitis of the digital flexor tendons of the left front foot. Her medications included ibuprofen, acetaminophen, butorphanol, and ceftiofur. Elephant OZ-F4 was also euthanized at age 44, after treatment for severe pododermatitis of both front feet. Her medications included ibuprofen and acetaminophen. Both of these females exhibited a dramatic decline in behavior-assessed quality of life over a period of weeks, which precipitated euthanasia. Elephant OZ-F7 was euthanized at age 51 after a more gradual decline in health that included moderate pododermatitis of both front feet, and severe degenerative joint disease of carpi and multiple metacarpophalangeal joints in both front feet. Her medications included ibuprofen and butorphanol.

2.2. Radioimmunoassays

Unextracted serum was analyzed using a solid-phase progesterone RIA (Coat-a-Count progesterone; Siemens Diagnostic Solutions Corporation, Los Angeles, CA, USA). The antibody has a 9% cross-reactivity to 5α-DHP and has been used successfully for tracking progestagen profiles in elephants [13,16,21]. The manufacturer's protocol was used, but with the highest standard not included and with one lower standard added, produced by 2-fold dilution of the lowest standard with assay buffer. From 2006 forward, all volumes of the assay protocol were halved, after testing to verify that this caused no difference in assay results. The assay was validated for elephants at the Oregon Zoo by demonstrating parallelism between serial dilutions of high progestagen serum pools and the standard curve. Analyses of progesterone standard added to low hormone serum pools resulted in recoveries between 95 and 110%. Assay sensitivity was 50 pg/mL, and intra- and interassay coefficients of variation were <15%. Throughout the study period, assays were conducted within 1 mo after sample collection.

2.3. Data analysis

Baseline progestagen values were calculated for each individual using an iterative process developed for Asian elephants [16]. For each animal, all data points with values above the mean plus 1.5 times the 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 two consecutive weeks, with a duration of at least 4 wk; 2) the follicular phase was defined as progestagen concentrations below the baseline for at least two consecutive weeks; 3) single point fluctuations above or below baseline were assigned to 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. For each luteal phase, a midpoint date was calculated based on the beginning and end of the progestagen rise. If the midpoint was between two data points, the earlier date was used as the midpoint. Progestagen peaks were designated as the highest point during each luteal phase.

Only complete estrous cycles (n = 188) were used in most analyses; cycles for which there were incomplete luteal or follicular phases were excluded. The exception was the analysis of the temporal relationship of the luteal peak to the luteal midpoint, in which complete luteal phases (n = 203) were analyzed. Gestation periods (n = 2) were excluded from all analyses. Elephant OZ-F7 gave birth to a calf that died 1 day later and she started cycling normally after 3 mo. Elephant OZ-F4 gave birth to a calf in 1994, but that gestation period and subsequent lactational anestrus occurred within a time gap in the dataset, so these data were automatically excluded.

Distribution measurements were calculated using Excel (Microsoft® Office Excel 2003 for Windows XP; Microsoft, Corp., Redmond, WA, USA). All other statistics were run using InStat (InStat 3.0 b for Mac OSX, GraphPad Software, Inc., La Jolla, CA, USA). Based on Kolmogorov and Smirnov normality tests, many data distributions were non-normal. Thus, data were analyzed either with non-parametric tests or were log-transformed. Although the data had non-normal distributions (Fig. 1), mean, SD, and SEM are reported, in addition to medians, to allow comparison to other published studies. In addition, the coefficient of variation (CV) is reported for characterizing relative variability.

Estrous cycles within the group were considered outliers if the duration exceeded the group mean ± 2*SD. Outliers within the group were removed only for the purpose of reporting range in cycle duration. Estrous cycles within an individual were considered outliers if the duration exceeded the individual's mean ± 2*SD. All statistical tests were run without removing outliers.

Cycle durations were compared among individuals with a Kruskal-Wallis test, with post-hoc pairwise com-

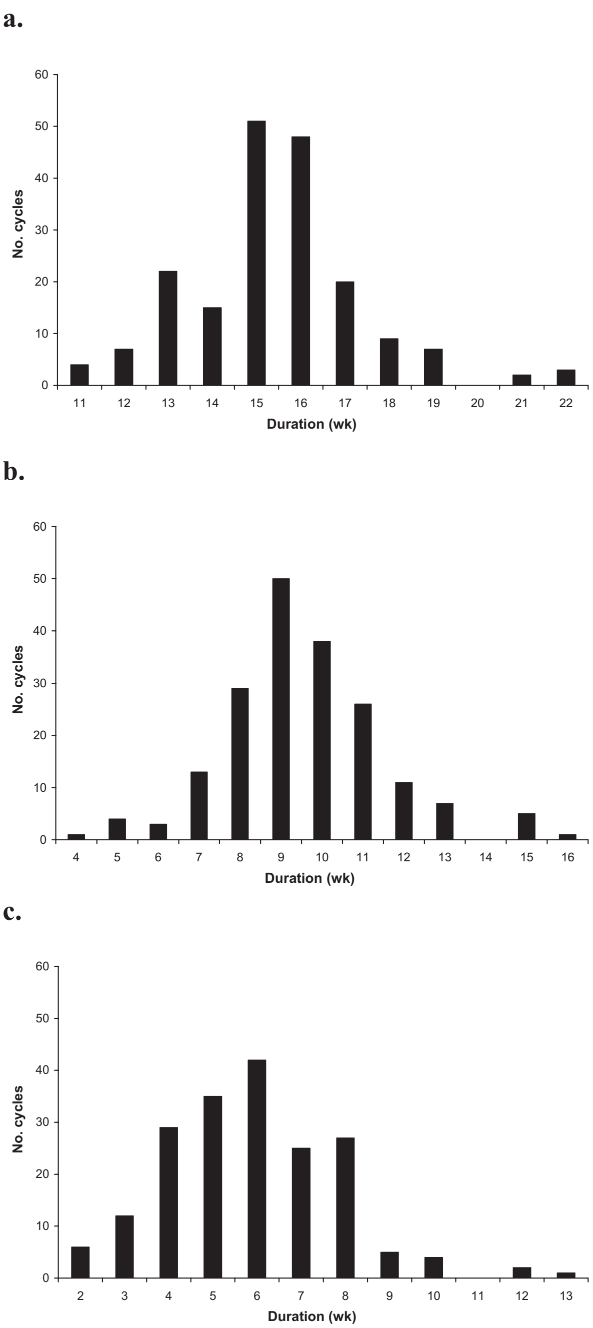

Fig. 1. Distribution of estrous cycle (a), luteal phase (b), and follicular phase (c) durations for all elephants at the Oregon Zoo during the study period (n = 188 complete cycles).

parisons using Dunn's Multiple Comparisons. Variability in cycle duration was assessed by comparing the coefficient of variation across females for the full cycle, the follicular phase, and the luteal phase. This analysis was performed on raw data to characterize relative variability. The relationship between luteal and follicular phase durations was assessed with linear regression analysis and Pearson correlations on log-transformed data, for all females combined and for each female individually.

To determine if cycle duration changed with age, each elephant's data were divided into consecutive time bins. Only elephants with a sufficient number of cycles to divide into time bins of at least 2 yrs in duration (n = 7) were included in the analysis. For elephants with 25 or more cycles (n = 3), data were divided into 3-yrs time bins and compared with Kruskal-Wallis rank tests. For elephants with fewer than 25 cycles (n = 4), data were divided into two equal-sized time bins (generally 2–3 yrs in duration) and compared with *t* tests on log transformed data. For females that began cycling during the study period (n = 3), the mean of the first pubertal cycle was compared with the mean of subsequent cycles. The first pubertal cycle was considered similar if the duration was within 2*SD of the mean of subsequent cycles. A Bonferroni correction was used to correct for effects of multiple tests.

Outlier cycles within individuals were assessed for temporal relationships to major life events (births, deaths, and transfers) listed in Asian Elephant (*Elephas maximus*) North American Regional Studbook [5]. A major event was considered associated with an outlier cycle if that event occurred either during the outlier cycle or within the cycle immediately before or after the outlier.

The separation (in weeks) of peak progestagen and luteal midpoint was calculated for every luteal phase (n = 203) to determine if the timing of the peak could be used to predict the end of the luteal phase. The timing of the peak in relation to the midpoint was also recorded to determine if there was a temporal pattern in these two markers.

3. Results

3.1. Estrous cycle characteristics

A total of 188 cycles was analyzed from the nine elephants in this study. The estrous cycle duration ranged from 11 to 22 wk, with the majority being 15 to 16 wk (Table 1, Fig. 1). The luteal phase ranged from 4 to 16 wk, with the majority being 8 to 11 wk. The follicular phase ranged from 2 to 13 wk, with the majority being 4 to 8 wk. The percentage of outlier cycles within the group was low. Three individuals (OZ-F1, OZ-F9, OZ-F7) exhibited a total of nine outlier cycles. With outliers removed (n = 179 cycles), the range for estrous cycle duration was 12 to

19 wk, luteal phase 4 to 15 wk, and follicular phase 2 to 12 wk (Table 1).

Individuals differed in median duration of the estrous cycle (H [8] =67.7, $P < 0.001$, n = 188), luteal phase (H [8] =67.3, $P < 0.001$, n = 203) and follicular phase (H [8] =74.7, $P < 0.001$, n = 193; Kruskal-Wallis tests). Post-hoc pairwise comparisons yielded significant differences between elephants. Two individuals had pronounced and significant differences in median estrous cycle duration compared to other elephants (Table 1). Elephant OZ-F1 had a comparatively short cycle, whereas elephant OZ-F9 (*E. m borneensis*) had an unusually long cycle. Both of these elephants had significant differences in follicular phase durations as well, but their luteal phases were similar to those of the other elephants. Another elephant, OZ-F7, had the longest luteal phase, but the second shortest follicular phase, which resulted in an overall cycle duration that was not different from the other elephants (significantly different only from OZ-F1).

Variability in estrous cycle duration (Table 2) was considerably less than the variability in luteal and follicular durations, both for all females combined (estrous CV = 13%, luteal CV = 21%, follicular CV = 33%) and also within each individual. Variability was generally greater across than within individuals. Only one individual, the Borneo elephant (OZ-F9), exhibited variability in estrous cycle duration that was greater than that of all females combined, and her variability in luteal duration was also high (CV = 24%). Three individuals exhibited variability in luteal duration that was greater than that for all females combined, and two individuals exhibited variability in follicular duration that was greater than that for all females combined. There was no apparent relationship in this group of elephants between socially dominant status and estrous cycle dynamics.

The Borneo elephant (OZ-F9) consistently had the highest peak progestagen concentrations of all the elephants (Table 1), which were greater than 1.5 times higher than the second highest peak concentrations observed in elephant OZ-F8. The lowest peak concentrations were observed in elephants OZ-F5 and OZ-F4.

Table 2
Variability in estrous cycle and phase durations (CV) of all females housed at the Oregon Zoo during the study period (n = 188 complete cycles).

Elephant	Estrous cycle (CV)	Luteal phase (CV)	Follicular phase (CV)
OZ-F1	0.11	0.13	0.34*
OZ-F2	0.09	0.29*	0.27
OZ-F3	0.10	0.23*	0.40*
OZ-F4	0.08	0.15	0.28
OZ-F5	0.08	0.20	0.22
OZ-F6	0.07	0.14	0.21
OZ-F7	0.11	0.16	0.22
OZ-F8	0.07	0.17	0.26
OZ-F9†	0.16*	0.24*	0.29
All females	0.13	0.21	0.33

* CV of individual is greater than CV of all females combined with no outliers or individuals removed.
† OZ-F9 is *E. m borneensis*.

3.2. Relationship of luteal and follicular phases

Across all individuals, luteal and follicular phase durations were negatively correlated ($r = -0.47$, $P < 0.0001$). Within individuals, luteal and follicular phase durations were negatively correlated in six individuals (OZ-F2, OZ-F3, OZ-F4, OZ-F5, OZ-F6, and OZ-F8; overall $r = -0.73 \pm 0.06$; range of individual r values = -0.60 to -0.90, $P < 0.05$).

3.3. Age category analysis

Estrous cycle characteristics did not change with age ($P > 0.05$), with the exception of one elephant, OZ-F6, who exhibited a shortening of the luteal phase (H(5,50) = 18.5, P = 0.0023) in the last 3 yrs of the study (mean ± SEM, 8.0 ± 0.4 wk), compared to the preceding years (9.5 ± 0.2 wk). Three females began cycling during the study period: OZ-F3 at age five, OZ-F6 before age six, and OZ-F9 at age 12 (Fig. 4). The first pubertal cycle was similar to subsequent cycles for elephants OZ-F6 and OZ-F9 in estrous cycle and phase durations. The first pubertal cycle was different from subsequent cycles for elephant OZ-F3 for estrous cycle duration, but follicular and luteal phase durations were similar. The first cycle for OZ-F3 was her shortest cycle.

3.4. Impact of major life events on cycle dynamics

Major life events (births, deaths, transfers) had minimal effects on cycle dynamics of this group of elephants. Twenty major life events occurred during the study period, only four of which were associated with outlier cycles. The percentage of outlier cycles within individuals was low. Two individuals (OZ-F1 and OZ-F7) exhibited cycle durations that exceeded their individual mean ± 2*SD, for a total of five outlier cycles within all individuals. Elephant OZ-F1 (Fig. 2) experienced 12 major life events during the study. Two major life events (transfer of a herdmate and a birth) were associated temporally with two of her three outlier cycles (all of which were longer than normal), but the remaining 10 major life events had no detectable effect

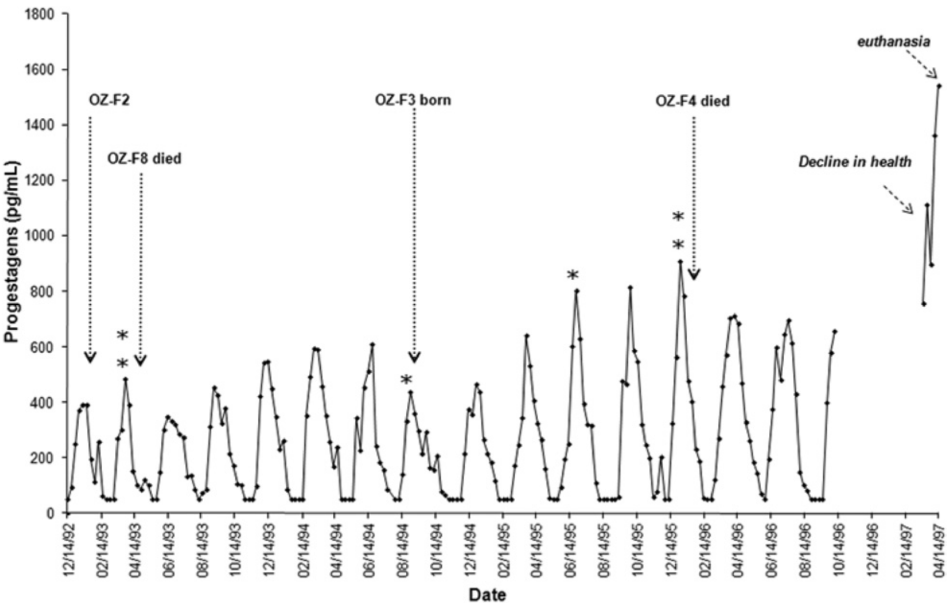

Fig. 2. Serum progestagen profile for elephant OZ-F1 showing major events and estrous cycle outliers.
* = Outlier within individual for cycle duration.
** = Outlier among group for cycle duration.

on her estrous cycle parameters. Elephant OZ-F7 (Fig. 3) experienced 16 major life events. Two major life events (transfer of a herdmate and her own death by euthanasia) were associated temporally with both of her outlier cycles (which were longer than normal), but the remaining 14 major life events had no detectable effect. In two of the three females that were euthanized (OZ-F1 and OZ-F4), progestagen concentrations in the final luteal phase were over three times higher than the preceding mean peak levels for that individual (e.g., Fig. 2).

3.5. Timing of the luteal peak relative to the luteal midpoint

Peak progestagen concentration and the luteal midpoint occurred in the same week in 30% of all luteal phases (n = 203), and within 1 wk of each other in the remaining 70% (Table 3). When peak progestagens and the luteal midpoint did not occur in the same week, the peak occurred before the midpoint in 71% of the cases. The peak occurred consistently before the midpoint in all individuals except for elephants OZ-F3 and OZ-F4, in which the order was mixed.

4. Discussion

In the present study, there was a wider range of estrous cycle and phase durations in Asian elephants than previously reported [7,8,13–15,19,20], likely because of the larger number of elephants studied and longer duration of longitudinal monitoring. As such, these data may be more representative of the captive population as a whole. Despite the high variability across individuals in the Oregon Zoo group, variation within individuals was relatively low. Each elephant's estrous cycle characteristics remained generally consistent over time, despite changes in social structure. A few outlier cycles were associated with significant life events, but in general the Asian elephants at the Oregon Zoo appeared to be quite resilient with respect to ovarian cyclicity in response to changes in management and social environment. Studies are now needed to assess whether the resilience observed in this study group is representative of Asian elephants at other captive facilities, or of captive African elephants (*Loxodonta africana*). African elephants exhibit much higher rates of ovarian acyclicity in captivity compared to Asian elephants [22], which might indicate a greater susceptibility to social or management disruption. In Freeman, et al. [22], the majority of noncycling African elephant females were ranked as dominant within a group, whereas no such effect of dominance status on ovarian cyclicity was observed in the Asian elephants of this study.

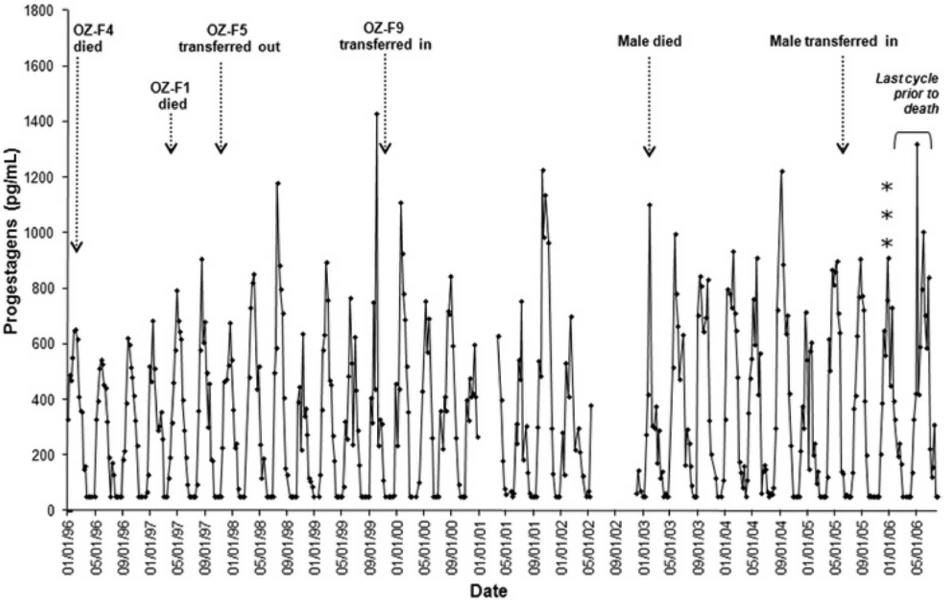

Fig. 3. Serum progestagen profile for elephant OZ-F7 showing major events and estrous cycle outliers. *** = Outlier within individual and among group for cycle duration.

Of potential relevance was the finding of pronounced elevations in progestagen concentrations in two (OZ-F1 and OZ-F4) of three elephants in the last luteal phase before death, which appeared to be related to the severity in health decline. In all three cases, individuals were euthanized because of ongoing foot pathologies associated with pain and discomfort that could no longer be managed to maintain quality of life. However, the declining health of OZ-F1 and OZ-F4 before euthanasia was more acute compared to a more gradual decline in well-being observed in OZ-F7. The cause of increased progestagen secretion is unknown, but some could be of adrenal origin, and thus may serve as an indicator of distress associated with declining health.

The overall duration of the estrous cycle appeared to be maintained by an inverse relationship between follicular and luteal phase durations. A relatively short follicular phase tended to be followed by a longer luteal phase, and vice versa, so the total estrous cycle duration remained constant. A similar negative correlation between luteal and follicular phase durations also was observed among groups of semicaptive Asian elephants in Thailand [20]. We further showed that the follicular phase was more variable than the luteal phase. Because previous studies have shown that the timing between the two LH surges was highly consistent [16–18], the variability in follicular duration in Asian elephants appeared to be driven by variation between the start of the follicular phase and the first LH surge. In the Thai elephant study, Thitaram, et al. [20] found that the follicular phase duration was indeed related to the interval between the end of the luteal phase and the first LH surge, which ranged from 7 to 41 days. It is not clear how or why phase durations are adjusted to maintain a more consistent cycle duration, but one possible mechanism could be through estrous synchrony induced by other members of the group. Although the elephants in this study were not obviously synchronous in their cycles, subtle changes in cycle characteristics may still be occurring. African elephants have been shown to synchronize estrous cycles with herdmates, the degree of which was dependent on changes in social interactions [23].

Age had little detectable effect on estrous cycle parameters. Eight of the nine elephants showed no changes with age in estrous cycle, luteal, or follicular phase durations. However, for some elephants the data were limited to only a few years. In the elephant for which we have the longest dataset (OZ-F6, age 6–26 yrs), the luteal phase shortened in the last time bin (age 23–26 yrs). This time period coincided with the death of her mother, OZ-F7, who had been the matriarch of the group. However, no significant differences were observed in her overall cycle or follicular phase durations. The oldest female in this study, OZ-F7, cycled normally until she died at age 51. There is little

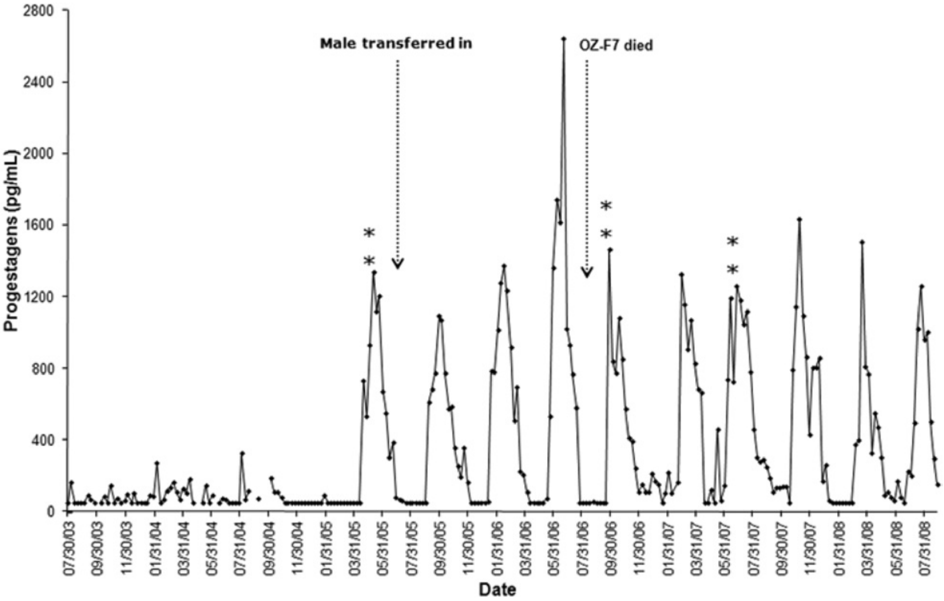

Fig. 4. Serum progestagen profile for Borneo elephant OZ-F9 showing major events and estrous cycle outliers. This female began cycling at 12 y of age.

** = Outlier among group for cycle duration.

hormonal data on geriatric elephants, and menopause in female elephants has not yet been demonstrated. Overall, based on our results, we inferred that management decisions can be made with the expectation that a cycling female Asian elephant will continue cycling as she ages, with a similar timing to that seen when she was younger.

Similarly, in the three cases for which we have pubertal cycles, the first cycle appeared similar to subsequent cycles. Quality of the first pubertal ovarian cycle varies among mammals (see review in [24]). For example, in the gilt and filly, the pubertal CL appears to have a normal lifespan, but may or may not be associated with behavioral estrus. In the ewe and heifer, a transient (1–4 days, ewe; 3–10 days, heifer) rise and fall in progesterone is detected in peripheral blood (80% of ewes and 50% of heifers) before the first "normal" CL. In general, the first cycle is not accompanied by estrus in the ewe, and may or may not be accompanied by estrus in the cow. The first estrus in the cow can also be anovulatory. Without ultrasound data, it is not possible to know if the first progestagen cycle in the pubertal elephant is associated with ovulation, but at present that is our assumption.

Of potential practical significance was the finding that the progestagen peak and luteal midpoint data may be useful in planning AI. The progestagen peak occurred at or near the luteal midpoint for most cycles, so its identification could be used to estimate the end of the luteal phase. This would help managers estimate when to begin the daily blood collections that are needed to identify the first LH surge; thereafter, AI could be scheduled to coincide with the second LH surge, which occurs ~ 18 to 20 days later [16–18,20].

An unexpected finding was how different the Borneo elephant was in cycle characteristics compared to the group. Borneo elephants have phenotypic traits (small size, small ears, long tail) that differ from those of Sumatran and mainland Asian elephants; however, there is debate as to whether it is a separate subspecies [4,25]. The Borneo elephant in this study had significantly longer estrous cycles, higher peak progestagen concentrations, and began cycling at a much later age than other elephants in the group (all of which were mainland Asian elephants). Onset of puberty is controlled by many factors, including nutrition and body weight, and tends to be related more to weight than age [26]. This female has always been substantially smaller than herdmates of similar age, which could be due to subspecific size differences in Borneo vs. mainland elephants, but we cannot rule out individual differences. The Borneo elephant in this study was wild born and imported at ~6 yrs of age [5], whereas the other two elephants for which we have puberty data were both captive-born. Captive-born Asian elephants have been known to conceive at just <4 yrs of age [19], whereas wild counterparts reach sexual maturity at 10 to 14 yrs of age [27]. There are only two other Borneo

Table 3
Timing of the progestagen peak versus the luteal midpoint for all females housed at the Oregon Zoo during the study period (n = 203 luteal phases).

Elephant	Separation between progestagen peak and luteal midpoint									Total*
	0 wks	1 wk		2 wks		3 wks		4 wks		
		Peak before	Peak after	Peak before	Peak after	Peak before	Peak after	Peak before	Peak after	
OZ-F1	6	11	2	5	1	3	0	0	0	28
OZ-F2	3	1	1	1	0	1	0	0	0	7
OZ-F3	10	3	5	1	2	1	0	1	0	23
OZ-F4	4	3	4	0	1	1	0	0	0	13
OZ-F5	2	7	2	2	2	4	0	0	0	19
OZ-F6	21	14	8	7	4	2	0	0	1	57
OZ-F7	11	10	4	7	1	3	2	1	1	40
OZ-F8	2	3	0	1	0	0	0	0	0	6
OZ-F9	1	3	1	1	0	4	0	0	0	10
All females (total)†	60	55	27	25	11	19	2	2	2	203
All females (% peak before/after)‡	NA	67%	33%	70%	31%	90%	10%	50%	50%	
All females (# for time span)§	60	82		36		21		4		203
All females (% for time span)‖	30%	40%		18%		10%		2%		

* Total equals the number of luteal phases for each elephant.
† Total number of luteal phases for which the peak occurs before the midpoint or after the midpoint for each category of separation.
‡ Percentage of luteal phases for which the peak occurs before the midpoint or after the midpoint for each category of separation.
§ Total number of luteal phases for each category of separation.
‖ Percentage of luteal phases for each category of separation.

elephants in captivity for which hormonal data are available, and they appear to exhibit progestagen cycle characteristics similar to other Asian elephants (Hildebrandt, personal communication). Thus, the Borneo elephant in this study may simply exhibit unusual cycle characteristics.

In conclusion, our analysis of longitudinal progestagen data has further clarified many aspects of Asian elephant reproduction: the range of estrous cycle duration is broader than previously realized; cycles within an individual are quite consistent over time; follicular and luteal phase durations are strongly and inversely related; and the progestagen peak can potentially be used to predict the luteal midpoint for the purposes of timing AI. Our next goal is to use this study as a model for similar analyses of hormonal data of other elephants managed by the Elephant Species Survival Plan (SSP; with ~112 Asian and ~127 African females) to evaluate hormonal relationships on an individual, herd, and population basis. Currently, blood is collected from about half of the captive elephant females in North America at regular weekly or biweekly intervals to monitor estrous cycles [28]. In addition, the SSP now requires longitudinal progestagen evaluations of all females between 8 and 35 yrs of age to ensure females are reproductively sound before making breeding recommendations [13]. As a result, there is a growing database of serum hormonal profiles for elephants in North America, yet little has been done with these data. We hope that our analysis might spur similar, larger-scale comparative analyses using the entire dataset across multiple North American zoos, for both Asian and African elephants. Such analyses could help identify subtle, yet important differences between species and among individuals in reproductive functioning, and further highlight factors that are most important for improving the breeding management of elephants.

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