Hormone Secretion in the Asian Elephant (Elephas maximus): Characterization of Ovulatory and Anovulatory Luteinizing Hormone Surges

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ABSTRACT

In the elephant, two distinct LH surges occur 3 wk apart during the nonluteal phase of the estrous cycle, but only the second surge (ovLH) induces ovulation. The function of the first, anovulatory surge (anLH) is unknown, nor is it clear what regulates the timing of these two surges. To further study this observation in the Asian elephant, serum concentrations of LH, FSH, progesterone, inhibin, estradiol, and prolactin were quantified throughout the estrous cycle to establish temporal hormonal relationships. To examine long-term dynamics of hormone secretion, analyses were conducted in weekly blood samples collected from 3 Asian elephants for up to 3 yr. To determine whether differences existed in secretory patterns between the anLH and ovLH surges, daily blood samples were analyzed from 21 nonluteal-phase periods from 7 Asian elephants. During the nonluteal phase, serum LH was elevated for 1–2 days during anLH and ovLH surges with no differences in peak concentration between the two surges. The anLH surge occurred 19.9 ± 1.2 days after the end of the luteal phase and was followed by the ovLH surge 20.8 ± 0.5 days later. Serum FSH concentrations were highest at the beginning of the nonluteal phase and gradually declined to nadir concentrations within 4 days of the ovLH surge. FSH remained low until after the ovLH surge and then increased during the luteal phase. Serum inhibin concentrations were negatively correlated with FSH during the nonluteal phase (r = −0.53). Concentrations of estradiol and prolactin fluctuated throughout the estrous cycle with no discernible patterns evident. In sum, there were no clear differences in associated hormone secretory patterns between the anLH and ovLH surge. However, elevated FSH at the beginning of the nonluteal phase may be important for follicle recruitment, with the first anLH surge acting to complete the follicle selection process before ovulation.

INTRODUCTION

Early reports profiling LH secretion in female elephants noted the presence of multiple surges during the nonluteal phase of the cycle in both the Asian (Elephas maximus) and African (Loxodonta africana) species [1–5]. However, it was not until later that a consistency in the timing of these surges became apparent. In 1995, daily blood samples were collected from an Asian elephant at the National Zoological Park (Front Royal, VA) and analyzed for progesterone to estimate the time for artificial insemination. All serum samples were subsequently analyzed for LH, and after three consecutive cycles, it was clear that two distinct surges occurred approximately 3 weeks apart during the nonluteal phase of the cycle (unpublished results). In 1996, Kapustin et al. [6] described a similar pattern of LH secretion in African elephant females. In both species, the first and second LH surges are quantitatively and qualitatively indistinguishable, yet only the second surge results in an ovulatory increase in serum progesterone concentration. Hence, the terms anovulatory LH (anLH) and ovulatory LH (ovLH) surges are used to define these two events [6]. Detection of this double LH surge requires the collection of daily blood samples because concentrations generally are elevated above baseline for only 1 day. No doubt this explains why the presence of these two precisely timed surges was not recognized in earlier studies.

Questions now remain as to what controls the timing of these two surges and what is the function of the first, anovulatory surge. This study evaluated secretory patterns of LH, FSH, progesterone, inhibin, estradiol, and prolactin throughout the estrous cycle in the Asian elephant to determine whether the endocrine milieu surrounding the anLH and ovLH surges differs in a manner that might suggest a reason for this functional difference.

MATERIALS AND METHODS

Animals and Blood Sample Collection

Nine Asian elephant females (18–48 yr of age) were evaluated for periods of 6 mo to 3 yr. Three females were housed at the National Zoological Park, and the other six were residents of the Dickerson Park Zoo (Springfield, MO). All animals were housed in indoor-outdoor enclosures and exposed to natural photoperiod. The National Zoological Park does not have an elephant bull, whereas Dickerson Park Zoo elephants had olfactory and some visual contact with a mature Asian bull. None of the elephants in this study were bred during sample collection periods. At both institutions, weekly blood samples were collected routinely for progesterone analysis to monitor the estrous cycle. Additional blood samples were collected daily throughout the nonluteal phase in one National Zoological Park and all six Dickerson Park Zoo elephants for a total of 21 interluteal periods.

All elephants at both institutions were handled in a free-contact management system and were accustomed to the blood sampling procedure. Blood was collected from an ear vein without sedation, allowed to clot for at least 1 h, and then centrifuged for recovery of serum. Serum was stored at −20°C or colder until analyzed for LH, FSH, progesterone, prolactin, estradiol, and inhibin.

Hormone Iodinations

LH (LER-1374A), FSH (LER-1976A), prolactin (NIDDK-oPRL-I-2), and inhibin (porcine inhibin-α peptide) were iodinated using chloramine-T. For each iodin-
The assay containing 1 ml PBS with 0.5% BSA (pH 7.4). To determine hormone differences between the anLH and ovLH surges, means were calculated for each day beginning 10 days before and continuing for 5 days after each surge, and within-day comparisons were made using t-tests. The relationship between FSH and inhibin secretion during the nonluteal phase was determined by regression analysis. Baseline values were those remaining after all high values had been excluded. For presentation, hormone data were aligned according to the ovLH surge (designated Day 0). To determine hormone differences between the anLH and ovLH surges, means were calculated for each day beginning 10 days before and continuing for 5 days after each surge, and within-day comparisons were made using t-tests. The relationship between FSH and inhibin secretion during the nonluteal phase was determined by regression analysis.

RESULTS

Figure 1 shows mean secretory concentrations of LH, FSH, and progesterone throughout the estrous cycle in an Asian elephant monitored for a 3-yr period (n = 10 cycles).
Asian elephant monitored for a 3-yr period (n = 10 complete estrous cycles). In general, the estrous cycle was ~16 wk in duration with an 11-wk luteal phase. Baseline serum concentrations of LH throughout the cycle averaged 1.5 ± 0.8 ng/ml and rarely exceeded 2.5 ng/ml. For all elephants combined, mean baseline LH concentrations were 1.4 ± 0.1 ng/ml. Mean concentrations for the anLH surge (peak, 14.7 ± 1.3 ng/ml; range, 6.9–27.6 ng/ml) and ovLH surge (peak, 16.5 ± 1.0 ng/ml; range, 7.8–28.4 ng/ml) during the nonluteal phase were not different (P > 0.05). On average, the anLH surge occurred 19.9 ± 1.2 days after the decline of progesterone to nonluteal phase concentrations (range, 16–23 days), with the ovLH surge occurring 20.8 ± 0.9 days later (range, 18–23 days). In 70% of the surges, LH was significantly elevated above baseline for only 1 day. When LH was elevated for 2 days, the other value was always markedly lower than the peak (mean, 3.9 ± 1.2 ng/ml; range, 2.3–9.6 ng/ml). The timing of the ovLH surge relative to the luteal phase increase in progesterone varied among and within individuals. The percentage of ovLH surges occurring 1–4 days before the increase in progesterone was 19.2%, compared to 80.8% of surges that occurred after progesterone had begun to rise. In 70% of the cycles, a single, transient 1- to 2-day decrease in progesterone concentration was observed during the first week of the luteal phase. No LH surges were observed after the fourth day of the luteal phase or after the transient progesterone drop regardless of the day on which it occurred. At no time was a sustained increase in serum progesterone concentration observed after an anLH surge.

A distinct cyclic pattern of FSH secretion was observed that followed the 16-wk estrous cycle, with the rise and fall of concentrations trailing those of progesterone by several weeks (Figs. 1 and 2). Concentrations were lowest during the last week of the nonluteal period, then increased steadily during the luteal phase. Serum FSH peaked about 8–9 wk into the cycle just before progesterone began to decline. Thereafter, FSH concentrations decreased gradually throughout the nonluteal period, declining during the anLH surge and reaching nadir concentrations just before the ovLH surge (Fig. 3). In contrast, serum concentrations of inhibin were negatively correlated with FSH (r = −0.53; P < 0.05) during the nonluteal phase, being lower at the time of the anLH surge and higher during the ovLH surge (Fig. 3). Peaks of FSH coincident with anLH and ovLH surges were observed only occasionally, ~50% of the time.

Serum prolactin concentrations fluctuated throughout the estrous cycle with no clear pattern evident (Fig. 4). Concentrations averaged 4.8 ± 0.6 ng/ml, with occasional spikes exceeding 15 ng/ml. During the nonluteal phase, slightly elevated prolactin concentrations occasionally occurred coincident with anLH (66%) and ovLH (50%) surges, but this effect was not consistent. Similarly, serum estradiol concentrations varied throughout the estrous cycle. Estradiol concentrations averaged 12.8 ± 4.6 pg/ml, with some peaks observed up to 50 pg/ml during the nonluteal phase, but again the pattern was inconsistent (Fig. 5).

**DISCUSSION**

The finding of two distinct LH surges, 3 wk apart, during the nonluteal phase of the cycle in the Asian elephant confirms that the double LH surge phenomenon occurs in both Asian and African elephants. The timing of 18–23 days between the anLH and ovLH surges in this study was nearly identical to the 19–22 days reported by Kapustin et al.
[6] for the African species, as was the finding that the anLH and ovLH surges were qualitatively indistinguishable. The only notable differences between the two studies were that 1) the anLH surge occurred earlier after the return of progesterone to baseline during the nonluteal phase in the African elephant (12 days versus 20 days) and 2) the LH surge magnitude was lower in African than in Asian elephants (~3 ng/ml versus ~16 ng/ml). Although different LH antibodies were used in the two studies (GDN#15 in Kapustin et al. [6], 518-B7 in the present study), this species difference has been confirmed in our laboratory using the 518-B7 antisera (African elephant mean LH surge amplitude, 5.1 ± 1.2 ng/ml, n = 20).

Blood was collected only once daily, so estimation of LH surge duration was not possible. For the majority of surges, however, serum LH was elevated above baseline for only one day, which explains why previous studies using weekly blood sampling failed to recognize this pattern. In other mammalian species, the LH surge typically is less than 30 h in duration (rabbit, 4 h [8]; cat, 8 h [9]; sheep, 10 h [10]; ferret, 12 h [11]; llama, 12 h [12]; cow, 12 h [13]; rat, 12 h [10]; pig, 30 h [10]), although in a few species, like the horse [14] and dog [15], a prolonged rise and fall in LH can occur over several days. Thus, the 28-h surge duration estimated for the African elephant [6], and presumably also for the Asian elephant, is slightly longer than that in many other species, but not markedly so. What is unusual is the occurrence of two, precisely timed surges during the follicular phase of the cycle. It is clear that the second LH surge is ovulatory. What remains to be determined is the functional significance of the first surge, and why it does not induce ovulation.

The behavioral significance of the anLH surge also is not well understood. Most reports indicate that natural breeding in captivity is confined primarily to the late follicular, early luteal phase [7, 16, 17]. However, there are anecdotal tales of females displaying "false estrus," including eliciting bull interest, about 3 wk before "true estrus" (or conception), in both captive and wild elephants. It would be interesting to determine the relative proportion of females that actually exhibit sexual receptivity at the time of the anLH surge. In comparisons of behavior with endocrine changes, a significant 1- to 2-day transient decrease in progesterone secretion has been reported in Asian elephants during the first week of the luteal phase, with no breeding occurring after that drop [16]. Similar decreases in serum progesterone also were observed in this study, and coincidentally no LH surges were observed thereafter. It has been suggested that the initial increase in progesterone secretion may be of follicular origin, with the secondary rise resulting from ovulation [16]. The finding that both the ovLH surge (this study) and male interest [16] are observed before, but not after, the progesterone drop (both studies) infers that mating is somehow timed to ensure that semen deposition occurs immediately before ovulation. The mechanism by which bull elephants recognize this periovulatory period has been shown to be regulated by pheromonal signals from urine [18]. We have noted in our elephant at the National Zoological Park that temporal gland drainage occurs more frequently around the time (±2 days) of both LH surges. The production of temporal gland fluid in elephants usually is associated with stress or excitement [19], and significant changes in volume and composition occur in bulls during musth [18, 20, 21]. Temporal gland drainage occurs less frequently in females, especially Asians [18], and so its significance has received little attention. But if markers such as temporal gland drainage or urinary pheromone excretion accompany the first LH surge, they may serve as an early advertisement of impending fertility to attract bulls and ensure that they are available when ovulation occurs. The anLH surge may be more a consequence, rather than a cause, of these other physiological events.

As for the timing of the LH surges (i.e., between the drop in luteal progesterone and the anLH surge, and between the anLH and ovLH surges), the observed 3-wk intervals may not be coincidental. In fact, it was originally believed that the elephant estrous cycle was 3 wk in duration on the basis of presumed estrogen-related events like bull fleming responses [20, 22], urinary estrogen excretion [23], and changes in vaginal cytology [24]. It is conceivable that waves of follicular development occurring at 3-wk intervals are responsible for the apparent short estrous cycles, with only one wave culminating in ovulation and formation of a functional corpus luteum. Thereafter, elevated progesterone concentrations achieved during the luteal phase...
would inhibit further LH surges [5]. Removal of the progesterone block during the nonluteal phase would allow reinitiating follicular activity that culminates in LH surges, but the conditions at the first surge as compared to the second are fundamentally different so as not to permit ovulation to occur.

One objective of this study was to determine whether the hormonal milieu differs between the two surges. The most obvious approach is to examine follicular steroidogenic activity, since it is the positive feedback of estradiol on the hypothalamo-pituitary axis that induces an LH surge. Unfortunately, attempts to describe follicular dynamics through hormone analyses have met with limited success in the elephant. Estradiol concentrations are low throughout the estrous cycle and fluctuate without showing a clear, interpretable pattern [1, 3–5]. The results of this study were no exception. Kapustin et al. [6] did report that estradiol concentrations tended to be maximal the day before each LH surge and then declined significantly from that maximum by Day + 1 in the African elephant. We also observed that the highest concentration of estradiol tended to occur the day before each LH surge, on average. However, in both studies, estradiol concentrations varied considerably, and the profiles were not consistent among individuals. Thus, most studies, including data from our laboratory, have failed to reveal any obvious, repeatable pattern in estrogen secretion that would support or refute the existence of follicular waves. Perhaps because estrogen production is comparatively low in the elephant, current assay systems lack the sensitivity to detect subtle physiological changes. Alternatively, it is possible that the elephant ovary secretes estrogen metabolites other than estradiol, as has been shown for progestogens, for which the most abundant circulating forms are 5α-reduced compounds, not progestrone [25–27]. There is evidence that circulating estradiol may predominate in a conjugated form, rather than a free steroid form, in both Asian [28] and African [29] elephants. However, incubation of Asian elephant serum with β-glucuronidase/aryl sulfatase to hydrolyze estrogen conjugates before analysis did not provide any clearer evidence of cyclic follicular activity (unpublished results). So the question of the nature of follicular activity in the elephant remains largely unresolved.

Measurements of other serum hormones (FSH and prolactin) also failed to identify any obvious hormonal differences that might explain why one LH surge is ovulatory and the other is not. Comparatively, the pattern of FSH secretion in the Asian elephant differs somewhat from that in other mammals, in which concentrations typically are elevated coincident with the preovulatory LH surge, with a secondary FSH surge sometimes occurring after ovulation. Instead, FSH secretion in the elephant is more like that in the horse, in which concentrations are highest at the end of the luteal phase and decrease progressively during the follicular phase [30]. Although only one surge of LH occurs in the mare, it is present for up to a week before ovulation. It is possible that the protracted secretion of gonadotropins observed in the elephant serves to recruit functional follicles before ovulation, as has been suggested for the horse. By contrast, inhibin concentrations in the elephant are inversely related to FSH during the nonluteal phase, similar to what has been observed in the mare [30]. It may be significant that the horse often is used as a model for the elephant in developing biomedical procedures and therapies; thus, similarities in hormone profiles between these two species could have a fundamental basis.

In summary, the occurrence of two, precisely timed LH surges during the nonluteal phase of the estrous cycle has now been documented in both the Asian and African elephant. This finding is of interest for several reasons. Comparatively, this pattern of LH secretion has not been described for other species to our knowledge. From a practical standpoint, the phenomenon has proven to be an invaluable tool for timing breeding, especially for artificial insemination. With identification of the first LH surge, breeding or insemination can be scheduled for 3 wk later. In fact, two African elephants at the Indianapolis Zoo conceived after using LH to time the artificial insemination procedure (personal communication with D. Olson, Indianapolis Zoo, Indianapolis, IN; and R. Hermes and T. Hildebrandt, both of the Institute for Zoo and Wildlife Research, Berlin, Germany). However, further studies are needed to identify the functional significance of the nonovulatory LH surge, the results of which could potentially identify a novel mechanism associated with the control of ovarian function. The next step should be to use longitudinal ultrasound evaluations of the reproductive tract, such as that developed by Hildebrandt et al. [31] in conjunction with endocrine analyses, to establish whether multiple waves of follicular development occur during the inter-luteal phase and, if so, what the ovarian response is to each of the two LH surges. Continued studies, such as those conducted by Rasmussen and Schulte [18], of how pheromonal signals are affected by the hormonal milieu also could lead to a clearer understanding of the relationship between physiology and behavior in the elephant. The captive populations of Asian and African elephants are not self-sustaining. Understanding the relationships between endocrine status, behavior, and fertility is critical to helping zoos develop reliable breeding programs to maximize reproductive efficiency.

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HORMONE SECRETION IN ASIAN ELEPHANTS

1299