Reproductive Endocrine Monitoring of Elephants: An Essential Tool for Assisting Captive Management

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Considerable information now is available about the basic reproductive biology of elephants, especially females. However, as important as this knowledge is, it no longer is enough to simply compile it into a database. The potential exists for using endocrine monitoring techniques to solve real problems. This review summarizes our current knowledge of elephant endocrinology and offers suggestions on how to use the technology to maximize reproductive potential. The estrous cycle can be monitored through the analysis of serum progestogens, primarily 5α-reduced compounds, and consists of an 8- to 12-week luteal phase and a 4- to 6-week inter-luteal period. Proof of ovarian cyclicity currently is mandatory before Species Survival Plan breeding recommendations are approved. However, because many adult females are not cycling normally, the reproductive monitoring of all cows throughout their life span is now encouraged. Complete endocrine evaluations in conjunction with ultrasound examinations and behavioral assessments are needed to identify causes of reproductive failure and develop mitigating treatments. Progestogen analyses also are effective for monitoring pregnancy, but only if longitudinal samples are collected. Alternatively, pregnancy can be diagnosed in occasional samples using serum prolactin or possibly relaxin measurements after 20 weeks of gestation. Parturition can be predicted on the basis of the rapid decrease in progestogens that occurs about 2–5 days before birth. An updated model of ovarian dynamics during the estrous cycle suggests that two waves of follicular development occur 3 weeks apart during the non-luteal phase, possibly under the control of follicle-stimulating hormone. Each follicular wave culminates in a luteinizing hormone (LH) surge, with the second surge inducing ovulation and corpus luteum formation. The functional signifi-

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cance of the first, anovulatory LH surge is under investigation, but from a practical perspective it can be used to schedule breeding (by artificial insemination or natural mating) to coincide with the ovulatory LH surge. Less is known about the reproductive biology of bulls, aside from the fact that musth is associated with dramatic changes in androgen secretion. Studies are needed to determine whether poor libido and inadequate semen quality observed in some mature elephants are due to testicular steroidogenic dysfunction. When blood samples cannot be collected for routine hormone analysis, gonadal activity can be monitored non-invasively through the measurement of excreted steroid metabolites (males: androgens; females: estrogens, progestogens) in urine and feces. Lastly, suggestions for future research priorities are provided. Zoo Biol 19:347–367, 2000.
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Key words: hormones; progestogens; estrous cycle; pregnancy; non-invasive monitoring; testis

INTRODUCTION

In the past few years, several significant reviews on the fundamentals of reproductive biology/endocrinology of elephants have been published [Mikota et al., 1994; Niemuller et al., 1998; Hodges, 1998]. The purpose of this review is to add to that knowledge and show how hormone monitoring can be used to better manage elephants. Neither Asian (*Elephas maximus*) nor African (*Loxodonta africana*) elephant populations are self-sustaining in captivity, and pressure to cease importing replacement animals from the wild makes it essential that reproductive rates be improved [Wiese, 2000; Olson and Wiese, 2000]. The challenges facing elephant managers are daunting, consisting of both logistical and physiological problems. Major areas of concern are the following: 1) too few breeding bulls are available; 2) transporting animals for breeding is expensive and stressful; 3) elephants of both sexes often exhibit a lack of sexual interest; 4) females are aging and experiencing problems such as uterine fibroids and ovarian cysts that may prevent conception; 5) many adult bulls are not producing good-quality semen; and 6) increasing numbers of adult females are not cycling. The success or failure of breeding programs will depend, in part, on using available technology to assess reproductive activity. Routine endocrine monitoring, the focus of this article, is now viewed as a valuable tool for making informed decisions about the reproductive management of elephants. Recommendations, based on published literature, personal experience, and discussions with colleagues, are provided on how to apply endocrine techniques to elephant management. These are guidelines only; their use and application ultimately will depend on the needs, resources, and priorities of each facility.

CIRCULATING HORMONES DURING THE ESTROUS CYCLE
Progestogens

Early reports on Asian elephants suggested an estrous cycle length of about 3 weeks on the basis of estrogen-related events, such as changes in vaginal cytology [Watson and D'Souza, 1975], behavior [Eisenberg et al., 1971; Jainudeen et al., 1971], and measurement of urinary estrogens [Ramsay et al., 1981]. It was not until later, when progestogen assays were identified that could monitor luteal activity, that the true ovarian cycle of 13–17 weeks was recognized for the first time in both species [Asian: Hess et al., 1983; African: Plotka et al., 1988]. It is now accepted that the

elephant estrous cycle consists of an 8- to 12-week luteal phase and a 4- to 6-week non-luteal period (Fig. 1). This cyclicity can be characterized by measuring serum or plasma progestogens in weekly samples using standard immunoassays, including commercially available progesterone radioimmunoassay (RIA) kits [e.g., Brown et al., 1991; Olsen et al., 1994; Brown and Lehnhardt, 1995; Kapustin et al., 1996; Carden et al., 1998]. Although cycle length is variable among cows, it can be remarkably consistent within an individual. At the National Zoological Park, for example, three Asian elephants cycle consistently at 13-, 16-, and 17-week intervals. Estrous synchrony probably is not common, although it occasionally is observed among females at a facility [Turczynski et al., 1992; Bechert et al., 1999] or occurs in females for limited periods [J.L. Brown, unpublished data].

The elephant apparently is unique in that the major circulating progestogen is not progesterone, but 5α-reduced pregnanes [Heistermann et al., 1997; Hodges et al., 1997; Schwarzenberger et al., 1997; Hodges, 1998]. In both species, 5α-pregnane-3,20-dione (5α-DHP) and 5α-pregnane-3-ol-20 one (5α-P-3-OH) are present in high concentrations in circulation compared to progesterone, with 5α-DHP predominating [Hodges et al., 1997; Hodges, 1998]. The ability of "progesterone" assays to monitor luteal func-
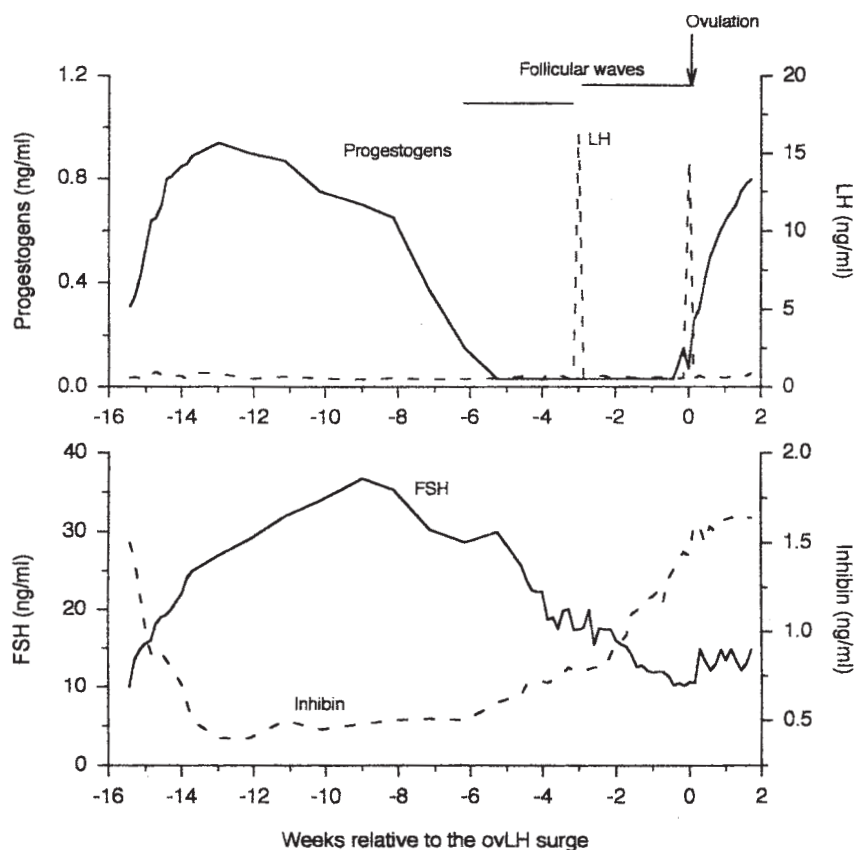

Fig. 1. Profiles of serum LH and progestogens (**top**), and FSH and inhibin (**bottom**) throughout the estrous cycle of the elephant. Horizontal lines (**top**) represent estimated time course of follicular waves during the nonluteal phase, with ovulation occurring only after the second ovulatory LH surge. (Adapted from Brown et al. [1991]; Kapustin et al. [1996]; Brown et al. [1999b]; and Hermes et al. [2000].)

tion in the elephant is due to varying antisera cross-reactivities with these pregnanes. The qualitative profiles generated by different assay systems are comparable; however, assays specific for 5α-DHP produce values that exceed those for "progesterone" by up to 20-fold [see review, Hodges, 1998]. In the Asian elephant, 17α-hydroxyprogesterone (17α-OHP) also is present in significant quantities in the bloodstream, although it is not as abundant as 5α-DHP [Hodges, 1988; Niemuller et al., 1993]. From a practical perspective, there is no reason to suggest that one assay system is better than another for tracking elephant luteal activity, at least qualitatively. Rather, it is more important to select a method (or laboratory) that meets cost and convenience needs, and stay with it for consistency. If testing procedures change, data are needed to allow interpreting variation in values resulting from differences in antibody specificities. Quality assurance typically is accomplished through analysis of control samples in each assay to assess inter-assay and inter-laboratory variability (serum controls can be obtained from Dr. Dennis Schmitt, Southwest Missouri State University, Springfield, MO).

Endocrine evaluations are essential for assessing cyclicity in adult females, and the Elephant Species Survival Plan (SSP) now requires it before making breeding recommendations. However, the database needs to be more inclusive of information gathered from puberty to senescence. Studbook records indicate that few cows produce a first calf after 30 years of age. Cows that have had calves at younger ages can reproduce into their 30s and 40s, but there appears to be a greater risk of calving problems and stillbirths in these older animals. In contrast, free-ranging females can produce calves into their 50s with apparently less difficulty. Whether fecundity differences between captive and free-ranging elephants is due to altered endocrine function, ovarian/uterine activity, or other factors will not be known until long-term reproductive monitoring is established. There is evidence, however, that older, nulliparous elephant cows develop reproductive tract pathologies, such as uterine and ovarian cysts [Hildebrandt et al., 2000a].

Based on the limited information to date, captive female elephants attain puberty several years earlier than those in the wild. In a study of five African and two Asian elephants, the first pubertal luteal phase increase in progestogens was observed between 7 and 8 years of age [J.L. Brown and A. Savage, unpublished data]. However, studbook records indicate that females can mature even earlier. Whenever possible, endocrine analyses should begin as soon as an animal will tolerate blood collection. Monthly sampling is adequate for initial evaluations, increasing to weekly as the female reaches an age of 4 or 5 years. This information is important as a means of evaluating developmental parameters of individuals in the population, to assess whether demographic changes in reproductive activity are occurring, and to guard against unexpected pregnancies in very young animals.

Estradiol

The period between successive luteal phases in the elephant generally is referred to as the non-luteal phase or inter-luteal period, rather than the follicular phase, as is common terminology for other mammals. The reason for this distinction is that, until recently, it was not known whether follicular activity was continuous throughout the non-luteal period or whether there was only a single wave of development that occurred just before ovulation. The original speculation that presumed changes in estrogen status reflect successive 3-week follicular waves [Plotka et al., 1988] has not been confirmed by actual measurements of circulating estradiol. In both species,

estradiol concentrations are low (generally <20 pg/mL) and fluctuate without any discernible pattern [Chappel and Schmidt, 1979; Hess et al., 1983; Brannian et al., 1988; Brown et al., 1999b]. However, a recent ultrasound study showed that two successive follicular waves, each about 3 weeks in duration, occur during the non-luteal period [African: Hermes et al., 2000], suggesting that "follicular phase" probably is proper terminology for the elephant. However, it is not known why estrogen measurements fail to clearly show the existence of these two 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. Finally, there is evidence that circulating estradiol predominates in a conjugated, rather than a free steroid form [African: Hodges et al., 1983; Asian: Czekala et al., 1992], although enzyme (β-glucuronidase/aryl sulfatase) hydrolysis of Asian elephant serum before analysis did not provide any clearer evidence of cyclic estrogen activity [J.L. Brown, unpublished data]. Continued studies are needed to clarify the nature of follicular steroidogenic activity in the elephant.

"Double LH Surge"

In other mammals, ovulation is induced by a single, pre-ovulatory LH surge at the end of the follicular phase of the estrous cycle [Knobil and Neill, 1998]. The elephant is unique, however, in that two precisely timed LH surges occur during the non-luteal phase [African: Kapustin et al., 1996; Asian: Brown et al., 1999b] The first surge is observed between 12 [Kapustin et al., 1996] and 21 [Brown et al., 1999b] days after progestogens decline to baseline, with the second surge occurring 3 weeks later (19–22 days) (Fig. 1). The surges are quantitatively and qualitatively similar, yet only the second induces ovulation. The terms anovulatory LH (anLH) and ovulatory LH (ovLH) surge are typically used to define these events. Detection of the surges requires the collection of daily blood samples because concentrations generally are elevated above baseline for only 1 day.

It is obvious that the ovLH surge induces ovulation and corpus luteum (CL) formation; however, the function of the anLH surge is less clear. It is possible that the "false estrus" occasionally observed in elephant females 3 weeks before "true estrus" (or conception) may be associated with the anLH surge [anecdotal observations]. Studies are needed to determine whether other physiological changes, such as temporal gland drainage or urinary pheromone excretion, accompany the anLH surge. If so, they may serve as an early advertisement of impending fertility to ensure that bulls are available when ovulation occurs. Additional endocrine analyses have been conducted to investigate why one LH surge is ovulatory and the other is not. In most cycles, progestogens increase a few days before the ovLH surge, followed by a 1- to 2-day transient decrease between days 2 and 9 of the luteal phase [Carden et al., 1998; Brown et al., 1999b]. A similar increase in progestogens is not observed before the anLH surge. Estradiol tends to be highest before each surge [Kapustin et al., 1996; Brown et al., 1999b], but concentrations are variable as discussed above. Prolactin secretion may [African: Bechert et al., 1999] or may not [Asian: Carden et al., 1998; Brown et al., 1999b] be elevated during the non-luteal phase, but there is no indication that concentrations are notably different between the early (i.e., associated with the anLH surge) or late (ovLH surge) follicular phase. One potentially significant finding is the pattern of follicle-stimulating hormone (FSH) secretion. In the

elephant, concentrations are highest at the end of the luteal phase and decrease progressively during the non-luteal phase (Fig. 1) [Asian: Brown et al., 1991; Brown et al., 1999b]. In other species, FSH concentrations typically are elevated coincident with the pre-ovulatory LH surge, with a secondary FSH rise occurring after ovulation [Knobil and Neill, 1998]. In humans and sheep, FSH declines slightly during the follicular phase, and the ability of a follicle to withstand this depression is suggested to be key in follicle selection and dominance [Baird, 1983; Campbell et al., 1999]. However, the "follicular phase" in other mammals is considerably shorter (generally <1 week) than the inter-luteal period in the elephant (up to 6 weeks); thus, these comparisons may not be relevant. Two possibilities as to the function of the protracted secretion of FSH in the elephant are that it may serve to: 1) recruit functional follicles during the last few weeks of the non-luteal phase leading up to ovulation, as observed in the horse [Ginther, 1992]; or 2) sustain waves of follicular development throughout the inter-luteal period as suggested by recent ultrasound findings [Hermes et al., 2000]. By contrast, inhibin concentrations are inversely related to FSH (Fig. 1), a relationship that does exist in other species [Knobil and Neill, 1998]. As for the timing of the LH surges (i.e., between the drop in luteal progestogens and the anLH surge, and between the anLH and ovLH surges), the observed 3-week intervals support earlier speculations that follicular waves occur at this frequency [Plotka et al., 1988], at least during the non-luteal phase.

Proposed Model for the Elephant Reproductive Cycle

This model updates previous versions [Plotka et al., 1988; Hodges, 1998] by incorporating information from recent endocrine and ultrasound studies. As shown in Figure 1, elevated progestogens during the luteal phase inhibit follicular development and LH release, whereas removal of the progestogen block reinitiates follicular activity. Elevated FSH at the beginning of the non-luteal phase recruits follicles and initiates two successive waves of follicular development that culminate in distinct, precisely timed LH surges. The first wave consists of multiple follicles that do not reach Graafian size or ovulate, but regress after the anLH surge. During the next 3 weeks, a second follicular wave results in the formation of one large dominant follicle that ovulates about 24 h after the ovLH surge. Elevated estrogens (which may or may not be measurable in circulation) during each wave trigger LH surges, but the follicles in the first wave are not functionally competent to ovulate, perhaps because of inadequate gonadotropin receptor numbers. Instead they luteinize in response to the anLH surge to form accessory CL that become steroidogenically active later in the cycle, a few days before the ovLH surge. It is possible that the Graafian follicle also secretes small amounts of progestogens in the days leading up to ovulation. A slight decrease in progestogens during the first few days of the luteal phase constitutes a shift in steroidogenic activity between the accessory CL and newly formed, postovulatory CL. Thereafter, progestogens increase in conjunction with continued CL maturation, followed about a week later by the gradual rise in FSH, which peaks at the end of the luteal phase. Throughout the cycle, inhibin of follicular origin is inversely related to FSH secretion, and may control its secretion.

Questions still remain, especially with regard to the functional significance of the two follicular waves during the non-luteal phase. The follicles in the first wave do not ovulate, but they may be obligatory to the process by forming accessory CL and producing the pre-ovulatory rise in progestogens that are necessary for ovulation

of the subsequent Graafian follicle. If so, this pattern of follicular growth would suggest it is appropriate to refer to the non-luteal phase as a true "follicular phase."

PREGNANCY AND PARTURITION
Pregnancy

Pregnancy in the elephant lasts 20–22 months and can be diagnosed on the basis of elevated progestogens beyond the normal luteal phase (after about week 12) (Fig. 2). On average, progestogen concentrations may [Hess et al., 1983; McNeilly et al., 1983; Olsen et al., 1994; Brown and Lehnhardt, 1995] or may not [Mainka and Lothrop, 1990] be higher during gestation, but overlap enough so that serial samples are required for accurate diagnosis. By contrast, pregnancy can be confirmed on the basis of a single blood sample by analyzing prolactin, which increases markedly after ~20 weeks of gestation (Fig. 2) [African: McNeilly et al., 1983; Hodges et al., 1987; Asian: Brown and Lehnhardt, 1995]. The immunoreactive "prolactin" observed during pregnancy probably is of placental origin, similar to lactogenic hormones identified in other species [Knobil and Neill, 1998]. Concentrations of relaxin also increase over non-pregnant levels by week 20 (Fig. 2) [Asian: Niemuller et al., 1998], so presumably this analysis could be used as a diagnostic tool. It is not known whether relaxin increases during pregnancy in African elephants. For a more rapid confirmation of pregnancy, a change in the 17α-hydroxyprogesterone (OHP): progesterone ratio has been shown to occur between weeks 2–7 after ovulation, with a ratio ≤0.7 indicating pregnancy [Asian: Niemuller et al., 1997]. Obviously, the practical benefit of diagnosing pregnancy at 3 weeks as compared to 4–5 months is considerable. Whether this hormone shift occurs in African elephants, or if this approach is valid using "progesterone" assays with differing antisera specificities remains to be determined. Concentrations of free estrogens are low and unchanging during gestation [Hess et al., 1983; Hodges et al., 1983], whereas conjugated estrogens increase significantly during the second half of gestation [African and Asian: Hodges et al., 1983, 1987].

At this time there is no evidence of a placental gonadotropin in the elephant. No immunoactivity has been found in pregnant Asian or African elephants using antibodies that detect equine chorionic gonadotropin [J.L. Brown, unpublished data] or Pregnancy Specific Protein B [J.L. Brown and R.G. Sasser, unpublished data]. Using a mouse oocyte retrieval bioassay, Allen et al. [1996] also did not detect gonadotropin bioactivity in placental extracts from early to mid-pregnancy in African elephants. Finally, immunoactive LH and FSH concentrations during gestation are not elevated above non-pregnant levels in either species [McNeilly et al., 1983; Brown and Lehnhardt, 1995; J.L. Brown, unpublished data]. Interestingly, fetal sex can be determined by measuring maternal testosterone through 60 weeks of gestation, at least in Asian elephants; concentrations are higher in the presence of a male calf [C. Duer, D.L. Schmitt, T. Tomasi, unpublished data].

Parturition

Considering the amount of time and energy invested in an elephant pregnancy, the ability to predict parturition is crucial. Fortunately, this can be done on the basis of a decrease in progestogens that occurs 2–5 days before birth (range, 1–10 days)
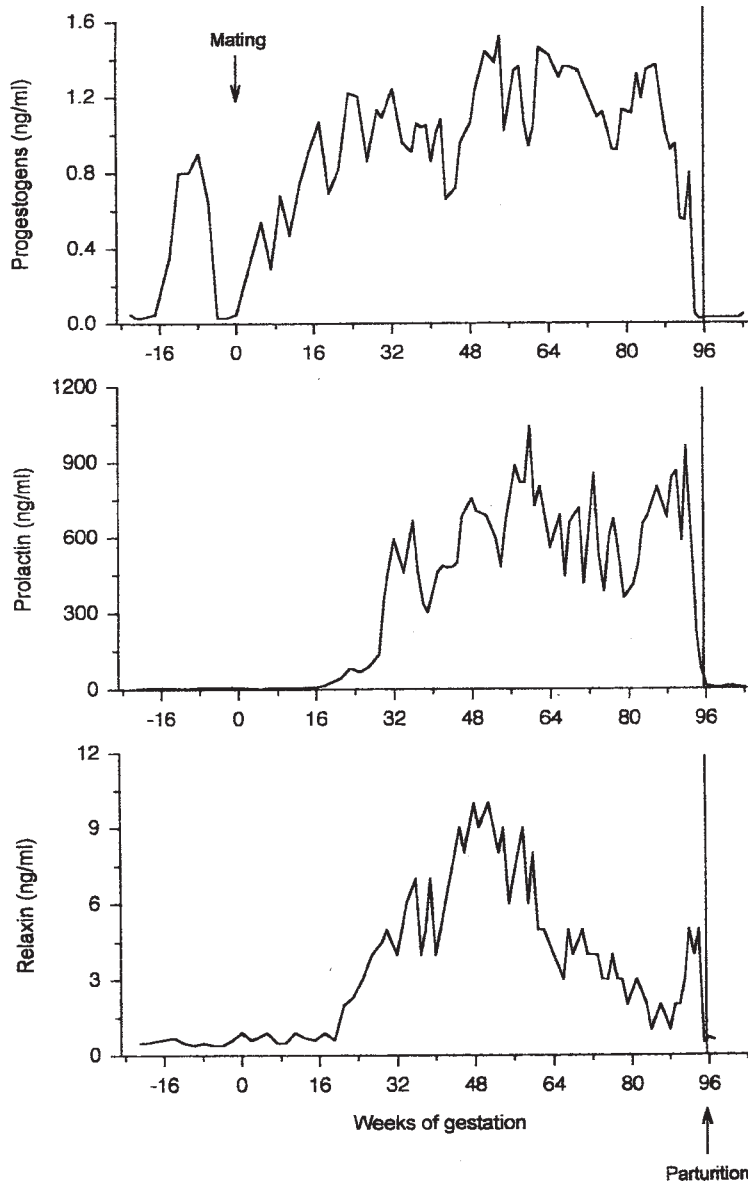
Fig. 2.   Profiles of serum progestogens (**top**), prolactin (**middle**), and relaxin (**bottom**) throughout gestation in the elephant. The vertical line indicates the time of parturition. (Adapted from Brown and Lehnhardt [1995] and Niemuller et al. [1998].)

[Brown and Lehnhardt, 1995; Carden et al., 1998; Doyle et al., 1999]. The prepartum decline in progestogens generally is rapid, occurring during a period of days (Fig. 3). Secretion is variable, however, making the selection of a low cutoff value difficult. These values also depend on which progestogen assay is being used. However, if concentrations fall to non-luteal levels (i.e., baseline), problems should be
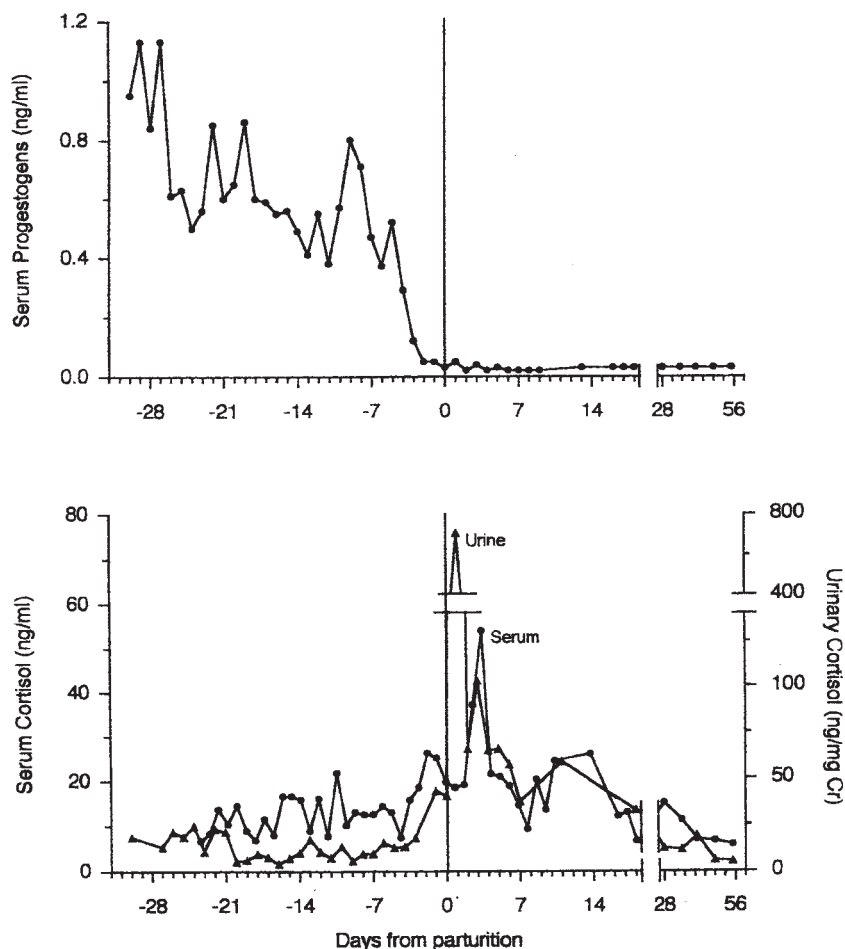
Fig. 3. Profiles of serum progestogens (**top**), serum cortisol (**bottom**), and urinary cortisol (**bottom**) during the peripartum period in an Asian elephant. The vertical line indicates time of parturition. (Adapted from Brown and Lehnhardt [1995].)

suspected if birth does not occur within a few days. If possible, the daily monitoring of progestogens during the last month of gestation is recommended. The 2- to 5-day "warning" that parturition is imminent ensures that staff are adequately prepared. It also is useful as a guide to more rapidly determine when a cow is in distress to reduce cow/calf mortality related to dystocia or other birthing problems.

In many species, the timing of parturition is initiated by the fetus through maturation of the hypothalamic–pituitary–adrenal axis and a corresponding increase in cortisol [Ginther, 1992; Knobil and Neill, 1998]. In one Asian cow, cortisol concentrations were low during gestation, but increased markedly at birth (Fig. 3) [Asian: Brown and Lehnhardt, 1995]. As a predictor of parturition, however, cortisol is not likely to be useful because adrenal activation can occur in response to a variety of stressors.

Postpartum Period

In captive elephants, the length of the postpartum anestrous period depends primarily on lactational status. In other mammals, lactation inhibits reproduction, with weaning reinitiating estrous cyclicity [Knobil and Neill, 1998]. On average, lactational anestrus in the elephant lasts about 46 weeks and is characterized by low progestogen concentrations [Asian: Olsen et al., 1994]. However, problems with retained placenta, reduced milk production, death of a calf, or premature weaning can reduce the postpartum period to as short as 8 weeks [Asian: Olsen et al., 1994; Brown and Lehnhardt, 1995]. Although prolactin probably is involved in initiating and/or maintaining milk production, concentrations are not consistently elevated above prepartum levels during the lactational period [African: McNeilly et al., 1983; Asian: Brown and Lehnhardt, 1995].

THE PROBLEM WITH "FLATLINERS"

It is now recognized that many reproductive-age females are not cycling [Brown, 1999; Brown and Lehnhardt, 1997; Brown et al., 1999a]. Termed "flatliners," serum progestogens in these individuals remain at baseline, indicating a lack of ovarian activity (Fig. 4). The cause of this acyclicity is unknown, nor have any reliable treatments been identified. It does not appear to be due to specific husbandry or management practices, because usually only one of a pair or group of elephants is affected. The possibility that social factors play a role in this disorder, however, cannot be excluded.
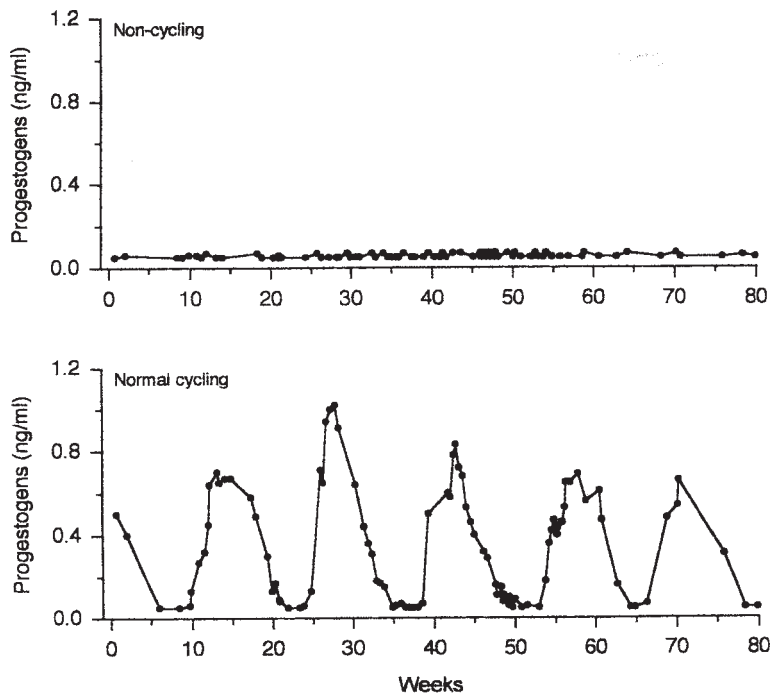

Fig. 4.   Representative profiles of serum progestogens in two elephants, one exhibiting normal estrous cycles (**bottom**) and the other showing an absence of estrous cyclicity (**top**).

To determine the magnitude of this problem, a survey was sent to institutions maintaining elephants to gather information about the reproductive status of the captive population (Table 1). Results are preliminary, but indicate that about half of elephant females are bled weekly, permitting assessment of reproductive cyclicity. Of these, 17% of Asian and 26% of African elephants are not cycling. Most of the flatliners in the survey are of reproductive age (average age, 24 ± 4 years), but because nulliparous females in captivity generally do not reproduce after about 30 years of age, there is limited time to address this problem before the population becomes too old. The survey also revealed that most elephants were acyclic at the time hormone monitoring was begun, or in a few cases were cycling, but then blood collection was stopped and when sampling was reinitiated, the female was no longer cycling. For this reason, it is recommended that all elephants be reproductively monitored continually throughout their reproductive life span to establish the time course and etiology of ovarian dysfunction, especially when animals are transferred to new facilities or are faced with altered social situations.

Two cases of ovarian inactivity have been evaluated in more detail, both involving African elephants. In one case, acyclicity was associated with mild hyperprolactinemia [Brown et al., 1997], a known cause of infertility in women. The female produced a watery, milklike fluid from her mammary glands; thus, a symptom of galactorrhea would indicate that endocrine problems exist. The other female had an ovarian follicular cyst [Brown et al., 1999a], also a cause of infertility in other species. Follicular cysts are abnormally large, anovulatory structures that persist in the absence of a CL. They apparently are not uncommon in African elephants, being more prevalent in captive (10–20%) than in wild (<2%) animals [Hildebrandt et al., 1997; Hildebrandt et al., 2000a]. Blood progestogens were not evaluated in those studies, so it is not possible to ascertain whether all ovarian cysts result in acyclicity. Obtaining that information obviously is important, because it appears from these limited studies that ovarian acyclicity in elephants has multiple etiologies.

As for treatment, no permanent resolution of reproductive inactivity in elephants has been accomplished to date. Administration of gonadotropin-releasing hormone (GnRH) and/or human chorionic gonadotropin, both common treatments for reproductive problems, including ovarian cysts, have not proven effective in the elephant [Brown et al., 1999a; D.L. Schmitt, personal communication]. In women, hyperprolactinemia generally is treated with ergot alkaloid drugs, such as bromocriptine. However, these drugs can cause hypotension and dizziness and so may be unsuitable for elephants. Newer prolactin-antagonist agents, such as cabergoline, have fewer side effects and may be an option. However, elevated prolactin has not been observed in other elephants, so it may not be a common disorder. Clearly, more work is needed to develop effective treatments. Studies are needed to examine the efficacy

TABLE 1. Survey results indicating the number of adult female elephants bled at a frequency to establish reproductive cyclicity status

Species	No. of females in captive population	No. surveyed	No. bled weekly (% of those surveyed)	No. of non-cycling females (% of those bled)
African	220	115	65 (56%)	17 (26%)
Asian	243	139	100 (72%)	17 (17%)

of one or multiple GnRH injections, different doses of chorionic or pituitary gona-
dotropins, or perhaps combinations of these treatments. Additionally, basic informa-
tion is lacking on many aspects of normal hormone function in cycling elephants,
especially the effects of GnRH on pituitary/gonadal function (Fig. 5). Without a sys-
tematic approach to diagnosing reproductive problems, and without these normative
data to compare treatment outcomes, devising adequate therapies will be difficult.

ENDOCRINE FUNCTION IN MALES

Less is known about the reproductive endocrinology of bull elephants, aside
from limited studies investigating musth. In both species, musth is a period of height-
ened aggressive and sexual behavior, characterized by increased temporal gland drain-
age, urine dribbling, and androgen secretion for periods of a few weeks to several
months [see reviews, Schmidt, 1993; Mikota et al., 1994; Niemuller et al., 1998].
The factors determining when a bull exhibits musth are related to age and nutrition,
and perhaps social status [Jainudeen et al., 1972; Cooper et al., 1990; Lincoln and
Ratnasooriya, 1996]. Musth can begin as early as 10–15 years of age in captivity, but
seldom is observed before age 25 in the wild. On average, concentrations of circulat-
ing androgens, including testosterone, dihydrotestosterone, and androstenedione, are
low during non-musth (<2 ng/mL), with occasional spikes up to 10 ng/mL [Jainudeen
et al., 1972; Rasmussen et al., 1984, 1990; Hall-Martin and van der Walt, 1984;
Cooper et al., 1990; Niemuller and Liptrap, 1991; Brown et al., 1993]. Concentra-
tions of testosterone increase dramatically during musth, averaging 10–20 ng/mL in
pre- and post-musth and sometimes exceeding 50 ng/mL in peak musth. The ratio of
androstenedione:testosterone also changes in favor of testosterone during musth
[Niemuller and Liptrap, 1991]. Frequent sampling of Asian bulls demonstrated that
androgen secretion is under LH control, with pulses of testosterone closely follow-
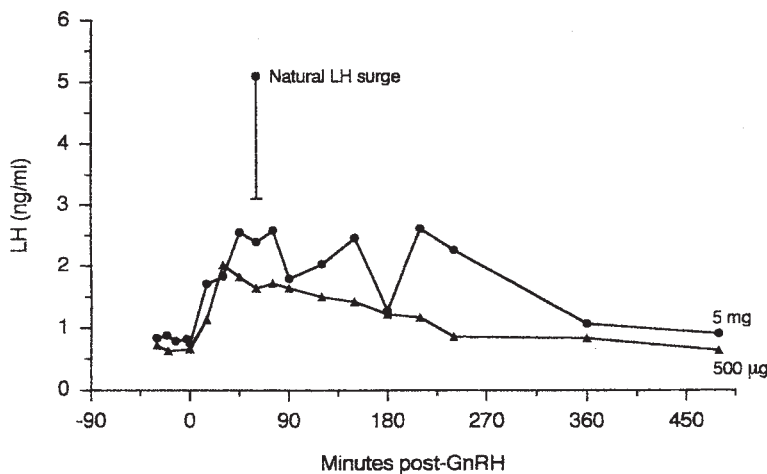ing those of LH (~1 pulse/3 hours), and little difference in hormone pulsatility be-

Fig. 5. Pituitary responses to two doses of GnRH (500 μg and 5 mg, i.v.) in an African elephant
diagnosed with an ovarian follicular cyst. Data are compared with the mean (– SEM) LH surge concen-
tration that occurs during the normal estrous cycle of the African elephant. Peak LH values post GnRH
appear to be lower than the natural LH surge.

tween musth and non-musth [Niemuller and Liptrap, 1991]. However, mean concentrations of LH increase about 4 weeks before musth begins, declining rapidly to baseline soon thereafter [Turczynski, 1993]. As in other mammals, LH secretion in elephants is stimulated by exogenous GnRH, with the testes of musth bulls exhibiting a hyperresponsiveness to LH [Brown et al., 1993; Lincoln and Ratnasooriya, 1996].

Musth has been compared to rut in other ungulates; however, this analogy is incorrect. Unlike rut, musth is not seasonal, although a mature bull may exhibit musth annually at the same time each year [Poole, 1994]. Musth also is not a prerequisite for breeding. In fact, the overaggressiveness often accompanying musth can reduce breeding interest, at least in captive bulls [Schmidt, 1993]. It is affected by nutritional status, because a common method of suppressing musth is water and food deprivation [Cooper et al., 1990; Schmidt, 1993]. Alternative therapies that decrease androgen secretion or activity are being sought to find a more ethical approach to controlling this problem. Compounds like the GnRH agonist luprolide acetate (Lupron; Tekada-Abbot, Abbott Park, IL) [Schmitt, 1993; Brown et al., 1993] and cyproterone acetate (an antiandrogen) [Niemuller et al., 1998] can reduce testosterone during musth. However, appropriate doses and injection regimens need to be developed, and the long-term effects on fertility and behavior have not been determined.

In pre-pubertal males, testosterone concentrations generally are <0.5 ng/mL. Testosterone production by bulls tends to be age dependent and correlated with testicular weight [McNeilly et al., 1983; Smith et al., 1987]. It also can be related to social rank, with dominant males exhibiting higher average testosterone concentrations than subordinates [Lincoln and Ratnasooriya, 1996]. Evaluating androgen status is an important part of the reproductive health assessment of bulls. However, the variability within and between animals makes it is unwise to assign specific cutoff values to characterize the normalcy of androgen production. Just as the collection of one poor-quality ejaculate does not mean a bull is infertile, so should multiple androgen analyses be conducted before concluding that testicular function is compromised. If consistently low circulating testosterone is detected, however, a problem with steroidogenic function should be suspected. In other mammals, testosterone is essential for maintaining spermatogenesis, promoting normal function of the epididymis and accessory sex glands, and stimulating sexual interest [Knobil and Neill, 1998]. There are indications that many adult bull elephants lack sufficient libido or are not producing spermic ejaculates [Schmidt, 1993; Hildebrandt et al., 2000b]. Whether these problems are associated with low testosterone production or other factors needs to be investigated. It may be that the apparent sub- or infertility in reproductive-age bulls has a common etiology with the "flatliner" problem observed in females. Endocrine monitoring of bulls in conjunction with other reproductive assessments involving ultrasound examinations and semen collection must be initiated and continued if we are to determine the magnitude of these problems, determine causes, and develop treatments.

NON-INVASIVE HORMONE MONITORING

About half of the captive elephant females are bled at a frequency (weekly or biweekly) to permit assessing estrous cyclicity (Table 1). Numbers are undoubtedly lower for bulls, although exact data are not available. It is clear that more compliance is needed, but it also is recognized that routine blood collection may not be

suitable for all animals or institutions. In those cases, monitoring gonadal status non-invasively through the analysis of fecal, salivary, or urinary steroid metabolites is an option. These approaches offer advantages in safety and ease of sample collection, and in general data are comparable to circulating hormone profiles. However, there are disadvantages that should be considered. Urine, collected by midstream catch or off enclosure floors, can be difficult to obtain depending on enclosure access. Urine also requires additional processing steps in the analysis of creatinine to account for variation in fluid intake, and hydrolysis of steroid conjugates to liberate assayable free steroids. Fecal samples often are easier to collect, but analyses are hampered by a more laborious and expensive sample preparation process, the lack of a suitable index (such as creatinine) to standardize results, and a comparatively long excretion lag time. Lag times are important and must be considered when correlating specific events or behaviors with hormone activity. On the basis of radiolabel infusion studies in elephants (tracing the excretion of injected radioactive steroids), lag times from injection to excretion range from a few hours for urine [Asian: Czekala et al., 1992; African: Wasser et al., 1996] to 30–50 hours for feces [Asian: Turczynski et al., 1993; African: Wasser et al., 1996]. Collection of an appropriate sample also is more critical for feces because steroid concentrations are not evenly distributed [Wasser et al., 1996]. Best results for feces are obtained when samples are dried and the fecal powder is separated from fibrous material, although wet sample analysis is valid provided the feces are well mixed before sampling. It is obvious that when blood collection is not possible, non-invasive techniques to monitor endocrine status are invaluable management tools.

Females

Progestogens

No appreciable amounts of progesterone are excreted by the elephant [Niemuller et al., 1993; Brown and Lehnhardt, 1995; Wasser et al., 1996], reflecting the low levels of this steroid in circulation. In addition, excreted metabolites found in other species (e.g., pregnanediol glucuronide, 20α-dihydroprogesterone) are barely detectable in elephant urine or feces [Heistermann et al., 1997; Gual-Sill et al., 1999]. Rather, 5α-DHP and 5α-P-3-OH predominate in feces as they do in the blood, although the ratios are switched in favor of 5α-P-3-OH [African: Heistermann et al., 1997; Fieβ et al., 1999]. These pregnanes also are excreted into urine, again with 5α-P-3-OH predominating and 5α-DHP being present in much lower (nearly insignificant) concentrations [African: Heistermann et al., 1997; Fieβ et al., 1999]. In both urine and feces, these reduced pregnanes can be quantified using group-specific antisera developed for serum, and profiles reflect changes in luteal activity during the estrous cycle and pregnancy [African: Wasser et al., 1996; Heistermann et al., 1997; Fieβ et al., 1999]. Because the same pregnanes are present in Asian elephant blood [Schwarzenberger et al., 1997], it is likely they also are excreted into urine and feces. However, studies on the excretory fate of 5α-pregnanes in Asian elephants have not been conducted. Rather, it was discovered comparatively early that the 17α-OHP metabolite, 5β-pregnanetriol, is abundant in urine [Niemuller et al., 1993], and more recently has been found in feces [Gual-Sill et al., 1999]. Thus, this presently is the metabolite of choice for non-invasive monitoring of reproductive activity in Asian elephants. By contrast, 5β-pregnanetriol concentrations in African elephant urine are

low, and no clear cyclic pattern of excretion is observed [M. Heistermann, B. Tro-horsch, J.K. Hodges, unpublished data]. If pregnanetriol also is absent in African elephant feces, or its precursor, 17α-OHP, is not found in African elephant circulation, it would represent a significant species difference in steroid production and metabolism.

Estrogens

Radiolabel infusion studies determined that estradiol is rapidly conjugated in circulation and excreted primarily (95%) in urine rather than feces as estradiol and estrone conjugates [Asian: Czekala et al., 1992; African: Wasser et al., 1996]. These studies suggest that urinary estrogen analyses may have biological relevance, whereas the low proportion of estrogens excreted into feces no doubt explains why this measurement has failed to provide useful physiological data for assessing cyclicity [Wasser et al., 1996; Fieβ et al., 1999].

Urinary estrogen data have not been consistent, however, perhaps because of sampling or even species differences. Initially, 3-week fluctuations in urinary estrone and estradiol conjugates were observed during an 8.5-week period [Asian: Ramsay et al., 1981]. Subsequent analyses of urinary total estrogens for more than a year revealed a 15–16-week cyclic pattern in the Asian elephant [Mainka and Lothrop, 1990], but no clear cyclicity in the African elephant [Fieβ et al., 1999]. Urinary estrogens in both species increase during pregnancy, but the time course differs. In the Asian elephant, urinary estrogens increase during the first year of gestation and remain elevated until parturition [Mainka and Lothrop, 1990], whereas in the African elephant, estrogens are elevated only between weeks 30 and 65 of gestation [Fieβ et al., 1999]. To add to the confusion, fecal total estrone (but not total estradiol) in the African elephant does increase during the second half of gestation, differing from the urinary profile for that species [Fieβ et al., 1999]. Altogether, it may be that monitoring excreted estrogens is a potentially useful tool for confirming pregnancy, care must be taken to consider the species as well as the material being analyzed for accurate diagnosis.

Cortisol

Although not a reproductive hormone per se, the potential impact of cortisol (i.e., stress) on reproduction cannot be overlooked. Stress refers to physiological and behavioral responses elicited by aversive stimuli (stressors) and their consequences on general health. The negative effects of stress include impaired immune function, increased disease susceptibility, abnormal behavior, and suppressed reproduction [Moberg, 1985, 1990]. However, it also is recognized that not all "stress" is deleterious, and that some level of stimulation may be necessary for normal functioning [Weiss et al., 1989; Moodie and Chamove, 1990]. The ultimate impact of a stressor depends on subjective perceptions and response options. Thus, although a variety of potential stressors exist in zoos, the deleterious effects occur only if an animal is unable to respond with appropriate behavioral and physiological responses (i.e., coping) [Weiss, 1971]. Recognizing whether conditions facilitate or compromise coping is key to ensuring animal well-being and maximizing reproductive potential.

Stress responses involve the release of glucocorticoids, such as cortisol, from the adrenal glands, and a change in cortisol concentrations commonly is used as a physiological indicator of stress [Moberg, 1985]. Cortisol can be measured in blood, but because secretion is dynamic (serum concentrations fluctuate diurnally as well as

in pulses), analysis of excreted corticoid metabolites often is a better indicator of overall adrenal activity. Assays for measuring cortisol or its metabolites in female elephants have been validated for urine [African and Asian: Brown and Lehnhardt, 1995; Brown et al., 1995], feces [African: K. Burks, J. Mellen and J.L. Brown, unpublished data; Wasser et al., in press] and saliva [Asian: Dathe et al., 1992]. Native cortisol in blood is excreted into urine and both can be quantified using a commercial cortisol RIA kit [Brown et al., 1995]. In our laboratory, fecal corticoids are measured in a variety of species using a commercial RIA kit for corticosterone (ICN Biomedicals, Inc., Costa Mesa, CA), although neither cortisol nor corticosterone has been shown to be excretory products [Graham and Brown, 1996; Monfort et al., 1998; Wasser et al., in press]. Using these techniques, increased glucocorticoid excretion in elephants has been observed after ACTH challenge [Brown et al., 1995; Wasser et al., in press], during altered social and management conditions [Dathe et al., 1992; K. Burks, J. Mellen and J.L. Brown, unpublished data], and at parturition (Fig. 5) [Brown and Lehnhardt, 1995]. Preliminary data suggest a diurnal rhythm of cortisol secretion in the elephant [J.L. Brown, unpublished data]. Concentrations of urinary cortisol throughout the day in three Asian elephants (n = 10 days of sample collection) averaged 15.2 ± 1.6 ng/mg Cr from 0800 to 1000 hours, 11.5 ± 4.4 ng/mg Cr from 1000 to 1200 hours, 10.4 ± 0.7 ng/mg Cr from 1200 to 1400 hours, and 6.5 ± 0.9 ng/mg Cr from 1400 to 1600 hours. Although concentrations varied considerably, urinary cortisol was greater ($P < 0.05$) in the morning (0800–1000 hours) than in the afternoon (1400–1600 hours). Thus, when designing "stress monitoring studies," timing of urine collection should be standardized for accurate data interpretation. Continued studies should focus on developing behavioral indices of stress for elephants, used in conjunction with physiological measures (e.g., corticoid analyses). This information is needed to determine whether stress plays a role in poor reproductive performance or abnormal behaviors in both bulls and cows. If so, the obvious next step will be to develop mitigating actions to improve environment or management conditions.

Males

After injection of ^{14}C-labeled testosterone into an Asian elephant bull, 89% of the radioactivity was excreted into feces and 11% into urine [Turczynski, 1993]. Circulating testosterone was at least partially excreted in its native form; however, other excreted radioactive peaks also were identified in both urine and feces. Most of the radioactivity in urine was in a conjugated form (70%), whereas nearly all of the fecal metabolites were unconjugated. Androgen immunoactivity was associated with several of the radioactive peaks in both hydrolyzed urine and feces. Although the correspondence between serum and excreted immunoactivity was significant, it was not as high as expected ($r = 0.37$ for urine; $r = 0.49$ for feces) [Turczynski, 1993]. For urine, perhaps because most of the metabolites are conjugates resistant to enzyme hydrolysis, a better approach might be to use direct androgen conjugate assays. We recently validated a 3α,17β-androstanediol glucuronide assay for elephant urine, although longitudinal studies have yet to be conducted [J.L. Brown, unpublished data]. For feces, a better correspondence with serum might be obtained by taking excretion lag time into consideration. Regardless, the biological relevance of urinary and fecal androgen monitoring of testicular activity has been demonstrated by showing a significant increase in excreted testosterone immunoactivity during musth in captive Asian bulls [urine and feces: Turczynski, 1993] and in free-ranging

African elephants [urine: Poole et al., 1984]. Musth also has been associated with increased urinary androstenedione and LH excretion [Brannian et al., 1989]. Because bulls often are too dangerous for routine blood collection, especially during musth, non-invasive monitoring of testicular function by urinary or fecal androgen metabolite analysis provides a safe means of obtaining these data. Just as with females, long-term endocrine monitoring is important, especially as more bulls are being diagnosed with reproductive problems.

USE OF ENDOCRINE TECHNIQUES TO SUPPORT ASSISTED REPRODUCTION

Conservation management programs for endangered species are enhanced by assisted reproductive technologies such as artificial insemination (AI), in vitro fertilization/embryo transfer, and gamete cryopreservation. For elephants, these technologies would be especially valuable for overcoming logistical problems, such as ensuring reproduction between behaviorally incompatible pairs and eliminating the risks and stress of animal transport. They also are an efficient means of facilitating gene flow without having to remove animals from native habitats, especially if semen from wild bulls can be used with AI.

For more than 2 decades, attempts to impregnate elephants by AI were unsuccessful. Then, within just a couple of years, success was achieved in two Asian [Dickerson Park Zoo: Schmitt, 1999; National Zoological Park: J.L. Brown and T.B. Hildebrandt, unpublished data] and three African [Indianapolis Zoo: Hildebrandt et al., 1999a,b; Vienna: T.B. Hildebrandt, unpublished data] elephants. This dramatic turnaround is due primarily to two recent advances: 1) a refined insemination technique using ultrasound and endoscopy, which places semen closer to the site of fertilization [Hildebrandt et al., 1999a,b]; and 2) an improved ability to estimate the time of ovulation. For the latter, inseminations can be timed on the basis of two endocrine criteria: 1) the initial increase in circulating progestogens, just before the transitory progestogen decrease during the luteal phase [Carden et al., 1998; Schmitt, 1999]; and 2) 3 weeks after the first, anLH surge [Hildebrandt et al., 1999a,b; J.L. Brown and T.B. Hildebrandt, unpublished data]. There are advantages and disadvantages to each approach. Both methods require the collection of daily blood samples. The "progesterone" assay has a 1-day turnaround and is run routinely by several laboratories. The disadvantage is that there is little lead time between the increase in progestogens and when inseminations need to be performed. In using LH, the analysis takes 2–3 days for results, but identifying the anLH surge in a batch of samples provides nearly 3 weeks of preparation time, a must when bulls, cows, and insemination teams are not present at the same facility. A disadvantage is that few laboratories run this assay; it is not available as a commercial kit and it requires the ability to prepare an iodinated LH label. It also is a heterologous system, requiring antisera that cross-react with elephant LH (only a few of which are available). Simplifying this technique will be essential to expanding its use for AI, as well as for aiding natural mating.

In captivity, libido problems frequently are encountered, with females refusing to stand for mating or bulls having no sexual interest [Schmidt, 1993]. No known therapies exist to alleviate these problems. However, because a cow is more likely to stand for mating when in estrus, and it is when she is most fertile, it is important to

precisely time introductions around this event. This can be done by identifying the anLH surge and scheduling breeding for 3 weeks later, to coincide with the ovLH surge and estrus. This approach has been tried on two occasions, but unfortunately mating did not occur [J.L. Brown, unpublished data]. Thus, it is clear there is more to breeding elephants than getting males and females (or their germplasm) together, and even the most sophisticated technology cannot solve all problems. Still, substantial progress has been made in recent years and there is no reason to doubt our continued ability to improve the breeding management of elephants.

CONCLUSIONS

From a practical perspective, endocrine monitoring is key to assessing the reproductive status of elephants, and can be done using commercial immunoassays [(RIA or enzyme immunoassay] or more elephant-specific analyses. Profiles can be generated using serum, plasma, urine, feces, and perhaps even saliva. Still, although we now have a fairly broad understanding of elephant endocrinology, a number of information gaps still exist. Future studies must be multifaceted, focusing on research to improve basic knowledge while using technology to support in situ and ex situ conservation projects. Specific research and management efforts should focus on the following.

1. Committing to a long-term reproductive assessment program, involving longitudinal endocrine monitoring in association with periodic ultrasound examinations to identify causes of sub- or infertility (i.e., "flatlining" in cows; poor semen quality and low libido in bulls).
2. Monitoring behavior in conjunction with reproductive assessments to determine how social factors impact general health and reproduction, and whether stress plays a role in reproductive dysfunction.
3. Confirming the relevance of the proposed ovarian cycle model by continued studies in both species. To date, ultrasound visualization of follicular waves has only been done in African elephants, whereas data on inhibin and FSH profiles are only available for Asian elephants.
4. Conducting additional non-invasive studies to characterize: a) pregnane metabolites in urine and feces (Asian); b) 17α-OHP in blood, and its metabolite 5β-pregnanetriol, in feces (African); c) daily urinary estrogen analysis to characterize follicular waves during the non-luteal phase (Asian and African); and d) androgen metabolites in urine and feces (African). Completing these studies will allow determining which assay systems are best for non-invasive monitoring of gonadal function in both species.
5. Investigating whether analysis of conjugated estrogens in circulation will improve the ability to monitor follicular activity.
6. Determining the function of the anLH surge, and whether it is associated with preestrous pheromonal or behavioral signals.
7. Simplifying the LH assay (and/or converting it to an enzyme immunoassay) to make the technique more widely available, and exploring the potential of developing a urinary assay for ovulation detection.

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REFERENCES

Bechert US, Swanson L, Wasser SK, Hess DL, Stormshak F. 1999. Serum prolactin concentrations in the captive female African elephant (*Loxodonta africana*): potential effects of season and steroid hormone interactions. Gen Comp Endocrinol 114:269–78.

Brannian JD, Griffin F, Papkoff H, Terranova PF. 1988. Short and long phases of progesterone secretion during the oestrous cycle of the African elephant (*Loxodonta africana*). J Reprod Fertil 84:357–65.

Brannian JD, Griffin F, Terranova PF. 1989. Urinary androstenedione and luteinizing hormone concentrations during musth in a mature African elephant. Zoo Biol 8:165–70.

Brown JL. 1999. Difficulties associated with diagnosis and treatment of ovarian dysfunction in elephants—the flatliner problem. J Elephant Managers Assoc 10:55–61.

Brown JL, Lehnhardt J. 1995. Serum and urinary hormones during pregnancy and the peri- and postpartum period in an Asian elephant (*Elephas maximus*). Zoo Biol 14:555–64.

Brown JL, Lehnhardt J. 1997. Secretory patterns of serum prolactin in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants during different reproductive states: comparison with concentrations in a non-cycling African elephant. Zoo Biol 16:149–59.

Brown JL, Citino SB, Bush M, Lehnhardt J, Phillips LG. 1991. Cyclic patterns of luteinizing hormone, follicle-stimulating hormone, inhibin, and progesterone in the Asian elephant (*Elephas maximus*). J Zoo Wildl Med 22:49–57.

Brown JL, Bush M, Wildt DE, Raath JP, de Vos V, Howard JG. 1993. Effects of GnRH analogues on pituitary-testicular function in free-ranging African elephants (*Loxodonta africana*). J Reprod Fertil 99:627–34.

Brown JL, Wemmer CM, Lehnhardt J. 1995. Urinary cortisol analyses for monitoring adrenal activity in elephants. Zoo Biol 14:533–42.

Brown JL, Hildebrandt T, Theison W, Neiffer DL. 1999a. Endocrine and ultrasound evaluation of a non-cycling African elephant: identification of a follicular ovarian cyst. Zoo Biol 18:223–32.

Brown JL, Schmitt DL, Bellem A, Graham LH, Lehnhardt J. 1999b. Hormone secretion in the Asian elephant (*Elephas maximus*): characterization of ovulatory and anovulatory LH surges. Biol Reprod 61:1294–9.

Campbell BK, Dobson H, Baird DT, Scaramuzzi RJ. 1999. Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. J Reprod Fertil 117:355–67.

Carden M, Schmitt D, Tomasi T, Bradford J, Moll D, Brown JL. 1998. Utility of serum progesterone and prolactin analysis for assessing reproductive status in the Asian elephant (*Elephas maximus*). Anim Reprod Sci 53:133–42.

Chappel SC, Schmidt MJ. 1979. Cyclic release of luteinizing hormone and the effects of luteinizing hormone-releasing hormone injection in Asiatic elephants. Am J Vet Res 40:451–3.

Cooper KA, Harder JD, Clawson DH, Fredrick DL, Lodge GA, Peachey HC, Spellmire TJ, Winstel DP. 1990. Serum testosterone and musth in captive male African and Asian elephants. Zoo Biol 9:297–306.

Czekala NM, Roocroft A, Bates M, Allen J, Lasley BL. 1992. Estrogen metabolism in the Asian elephant (*Elephas maximus*). Zoo Biol 11:75–80.

Dathe HH, Kuckelkorn B, Minnemann D. 1992. Salivary cortisol assessment for stress detection in the Asian elephant (*Elephas maximus*): a pilot study. Zoo Biol 11:285–9.

Doyle C, York B, Whitely A. 1999. A survey of Asian elephant births from 1962–1998. J Elephant Managers Assoc 10:146–8.

Eisenberg JF, McKay GM, Jainudeen MR. 1971. Reproductive behaviour of the Asiatic elephant (*Elephas maximus maximus L.*). Behaviour 34:193–225.

Fieβ M, Heistermann M, Hodges JK. 1999. Patterns of urinary and faecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). Gen Comp Endocrinol 115:76–89.

Ginther OJ, editor. 1992. Endocrinology of the ovulatory season. In: Reproductive biology of the mare. Basic and applied aspects. 2nd edition. Cross Plains, WI: Equiservices. p 233–90.

Graham LG, Brown JL. 1996. Cortisol metabolism in the domestic cat and implications for developing a non-invasive measure of adrenocortical activity in non-domestic felids. Zoo Biol 15:71–82.

Gual-Sill F, Pickard AR, Holt WV, Green D. 1999. Preliminary results of non-invasive monitoring of the estrous cycle in female Asian elephants (*Elephas maximus*) through fecal steroid analysis. Proceedings of American Association of Zoo Veterinarians Annual Conference, Columbus, OH. p 87–92.

Hall-Martin AJ, van der Walt LA. 1984. Plasma testosterone levels relative to musth in the male African elephant. Koedoe 27:147–9.

Heistermann M, Trohorsch B, Hodges JK. 1997. Assessment of ovarian function in the African elephant (*Loxodonta africana*) by measurement

of 5α-reduced progesterone metabolites in serum and urine. Zoo Biol 16:273–84.

Hermes R, Olson D, Göritz F, Brown JL, Schmitt DL, Hagan D, Peterson JS, Fritsch G, Hildebrandt TB. 2000. Ultrasonography of the sexual cycle in female African elephants (*Loxodonta africana*). Zoo Biol 19:369–82.

Hess DL, Schmidt AM, Schmidt MJ. 1983. Reproductive cycle of the Asian elephant (*Elephas maximus*) in captivity. Biol Reprod 28:767–73.

Hildebrandt TB, Goeritz F, Pratt NC, Schmitt DL, Lehnhardt J, Hermes R, Quandt S, Raath J, West G, Montali RJ. 1997. Assessment of health and reproductive status in African elephants by transrectal ultrasonography. Proceedings of American Association of Zoo Veterinarians Annual Conference, Houston, TX. p 207–11.

Hildebrandt TB, Goeritz, F, Schnorrenberg A, Hermes R, Schmitt DL, Hagan D, Peterson JS, Brown JL, Loskutoff N, Pratt NC, Lehnhardt J, Miller G, Montali RJ, Olson D. 1999a. Successful artificial insemination of African nulliparous elephants at the Indianapolis Zoo. Thirty-ninth International Symposium on Diseases of Zoo and Wildlife Animals, Vienna.

Hildebrandt TB, Göritz F, Hermes R, Schmitt DL, Brown JL, Schwammer H, Loskutoff N, Pratt NC, Lehnhardt JL, Montali RJ, Olson D. 1999b. Artificial insemination of African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. Proceedings of American Association of Zoo Veterinarians Annual Conference, Columbus, OH. p 83–6.

Hildebrandt TB, Göritz F, Pratt NC, Brown JL, Montali RJ, Schmitt DL, Fritsch G, Hermes R. 2000a. Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): an important tool for assessing female reproductive function. Zoo Biol 19:321–32.

Hildebrandt TB, Hermes R, Pratt NC, Fritsch G, Blottner S, Schmitt DL, Ratanakorn P, Brown JL, Rietschel W, Göritz F. 2000b. Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): an important tool for assessing male reproductive function. Zoo Biol 19:333–46.

Hodges JK. 1998. Endocrinology of the ovarian cycle and pregnancy in the Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephant. Anim Reprod Sci 53:3–18.

Hodges JK, Henderson C, McNeilly AS. 1983. Circulating oestrogen concentrations during pregnancy in the African elephant (*Loxodonta africana*). J Reprod Fertil 67:121–7.

Hodges JK, McNeilly AS, Hess DL. 1987. Circulating hormones during pregnancy in the Asian and African elephants (*Elephas maximus* and *Loxodonta africana*): a diagnostic test based on the measurement of prolactin. Int Zoo Yearbook 26:285–9.

Hodges JK, Heistermann M, Beard A, van Aarde RJ. 1997. Concentrations of progesterone and the 5α-reduced progestins, 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one, in luteal tissue and circulating blood and their relationship to luteal function in the African elephant, *Loxodonta africana*. Biol Reprod 56:640–6.

Jainudeen MR, Eisenberg JF, Tilakeratne N. 1971. Oestrous cycle of the Asiatic elephant, *Elephas maximus*, in captivity. J Reprod Fertil 27:321–8.

Jainudeen MR, Katongole CB, Short RV. 1972. Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elephas maximus*. J Reprod Fertil 29:99–103.

Kapustin N, Critser JK, Olson D, Malven PV. 1996. Nonluteal estrous cycles of 3-week duration are initiated by anovulatory luteinizing hormone peaks in African elephants. Biol Reprod 55:1147–54.

Knobil E, Neill J, editors. 1998. In: Encyclopedia of reproduction, Vols. 1–4. New York: Academic Press.

Lincoln GA, Ratnasooriya WD. 1996. Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator. J Reprod Fertil 108:107–13.

Mainka SA, Lothrop CD Jr. 1990. Reproductive and hormonal changes during the estrous cycle and pregnancy in Asian elephants (*Elephas maximus*). Zoo Biol 9:411–9.

McNeilly AS, Martin RD, Hodges JK, Smuts GL. 1983. Blood concentrations of gonadotrophins, prolactin and gonadal steroids in males and in non-pregnant and pregnant female African elephants (*Loxodonta africana*). J Reprod Fertil 67:113–20.

Mikota SK, Sargent EL, Ranglack GS, editors. 1994. The reproductive system. In: Medical management of the elephant. Oak Park, MI: Indira Publishing House. p 159–86.

Moberg G. 1985. Influence of stress on reproduction: measure of well-being. In: Moberg GP, editor. Animal stress. Bethesda, MD: American Physiological Society. p 245–67.

Moberg GP. 1990. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. J Dairy Sci 74:304–11.

Monfort SL, Mashburn KL, Brewer BA, Creel SR. 1998. Evaluating adrenal activity in African wild dogs (*Lycaon pictus*) by fecal corticosteroid analysis. J Zoo Wildl Med 29:129–33.

Moodie EM, Chamove AS. 1990. Brief threatening events are beneficial for captive tamarins. Zoo Biol 9:275–86.

Niemuller CA, Liptrap RM. 1991. Altered androstanedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant. J Reprod Fertil 91:139–46.

Niemuller CA, Shaw HJ, Hodges JK. 1993. Non-invasive monitoring of ovarian function in Asian elephants (*Elephas maximus*) by measurement of urinary 5β-pregnanetriol. J Reprod Fertil 99:243–51.

Niemuller CA, Shaw HJ, Hodges JK. 1997. Pregnancy determination in the Asian elephant (*Elephas maximus*): a change in the plasma progesterone to 17α-hydroxy-progesterone ratio. Zoo Biol 16:415–26.

Niemuller C, Brown JL, Hodges JK. 1998. Re-

production in elephants. In: Knobil E, Neill J, editors. Encyclopedia of reproduction, Vol. 1. New York: Academic Press. p 1018–29.

Niemuller C, Gray C, Cummings E, Liptrap RM. 1998. Plasma concentrations of immunoreactive relaxin activity and progesterone in the pregnant Asian elephant (*Elephas maximus*). Anim Reprod Sci 53:119–31.

Olsen JH, Chen CL, Boules MM, Morris LS, Coville BR. 1994. Determination of reproductive cyclicity and pregnancy in Asian elephants (*Elephas maximus*) by rapid radioimmunoassay of serum progesterone. Zoo Wildl Med 25:349–54.

Olson, D, Wiese RJ. 2000. State of the North American African elephant population and projections for the future. Zoo Biol 19:311–9.

Plotka ED, Seal US, Zarembka FR, Simmons LG, Teare A, Phillips LG, Hinshaw KC, Wood DG. 1988. Ovarian function in the elephant: luteinizing hormone and progesterone cycles in African and Asian elephants. Biol Reprod 38: 309–14.

Poole JH. 1994. Sex differences in the behaviour of African elephants. In: Short RV, Balaban E, editors. The differences between the sexes. Cambridge: Cambridge University Press. p 331–46.

Poole JH, Kasman LH, Ramsay EC, Lasley BL. 1984. Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*). J Reprod Fertil 70:255–60.

Ramsay EC, Lasley BL, Stabenfeldt GH. 1981. Monitoring the estrous cycle of the Asian elephant (*Elephas maximus*), using urinary estrogens. Am J Vet Res 42:256–260.

Rasmussen LE, Buss IO, Hess DL, Schmidt MJ. 1984. Testosterone and dihydrotestosterone concentrations in elephant serum and temporal gland secretions. Biol Reprod 30:352–62.

Rasmussen LEL, Hess DL, Haight JD. 1990. Chemical analysis of temporal gland secretions collected from an Asian bull elephant during a four-month musth period. J Chem Ecol 16:2167–81.

Schmidt MJ. 1993. Breeding elephants in captivity. In: Fowler M, editor. Zoo and wild animal medicine, current therapy 3. Philadelphia: WB Saunders. p 445–8.

Schmitt DL. 1999. Moola and Haji at the Dickerson Park Zoo. J Elephant Managers Assoc 10:199–202.

Schwarzenberger F, Strauss G, Hoppen H-O, Schaftenaar W, Dieleman SJ, Zenker W, Pagan O. 1997. Evaluation of progesterone and 20-oxo-progestagens in the plasma of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Zoo Biol 16:403–13.

Turczynski CJ. 1993. The endocrinology of musth in the male Asiatic elephant (*Elephas maximus*): serum estradiol, serum LH and serum, fecal and urinary testosterone. PhD dissertation. Texas A&M University, College Station, TX.

Turczynski CJ, Pernikoff D, Garcia R, Gross TS, Kraemer DC. 1992. Ovarian cycle influence in the Asian elephant (*Elephas maximus*). Biol Reprod 46(Suppl 1):95.

Wasser SL, Papageorge S, Foley C, Brown JL. 1996. Excretory fate of estradiol and progesterone in the African elephant (*Loxodonta africana*) and pattern of fecal steroid concentrations throughout the estrous cycle. Gen Comp Endocrinol 102:255–62.

Wasser SL, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL. A generalized fecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. Gen Comp Endocrinol (in press).

Watson PF, D'Souza F. 1975. Detection of oestrus in the African elephant (*Loxodonta africana*). Theriogenology 4:203–9.

Wiese RJ. 2000. Asian elephants are not self-sustaining in North America. Zoo Biol 19:299–310.

Weiss JM. 1971. Effects of coping behaviour with and without a feedback signal on stress pathology in rats. J Comp Physiol Psychol 77:22–30.

Weiss JM, Sundar SK, Becker KJ. 1989. Stress-induced immunosuppression and immunoenhancement; cellular immune changes and mechanisms. In: Goetzl EJ, Spector NH, editors. Neuroimmune network: physiology and diseases. New York: Wiley-Liss. p 193–206.