



Using a simplified field progestagen method to assess ovarian activity in female African elephants

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ABSTRACT

Non-invasive endocrine monitoring is an important conservation tool frequently used to support the management of wildlife species. Investigating ovarian activity and pregnancy rates of free-ranging African elephants can enhance our knowledge about the physiology of this highly social and long-lived species. The utility of a new, field-friendly progestagen enzymeimmunoassay (EIA) method was tested by monitoring ovarian activity of 40 females in Addo Elephant National Park (AENP), South Africa. Fecal samples were collected opportunistically from known individuals (June 2007–September 2009) and fecal progestagen metabolites (FPM) were extracted in the field and analyzed on-site using a visual, qualitative EIA. Those results were compared to the laboratory quantitative EIA assessment of hormone concentrations in the U.S. Qualitative FPM concentrations were strongly correlated with quantitative concentrations ($r_s = 0.72$). Fecal samples collected from pregnant elephants ($n = 22$) had significantly higher FPM concentrations than non-pregnant individuals, and month of gestation was the strongest predictor of FPM concentrations. Endocrine profiles suggested that five older females were no longer cycling normally. Simplified field endocrine techniques could transform endangered species research under field conditions and provide reliable and economical new tools for making sensible conservation management decisions based on real-time hormone results.

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1. Introduction

Hormone analyses are frequently used to support *in situ* and *ex situ* management of wildlife. Steroid metabolites excreted in urine or feces can provide a non-invasive measure of hormone activity in free-ranging species without having to capture, tranquilize or otherwise disturb the animals (Lasley and Kirkpatrick, 1991; Monfort, 2003). Moreover, urine and feces provide a 'pooled' measure of gonadal and/or adrenal activity, dampening the pulsatile and diurnal fluctuations of many circulating hormones (Monfort, 2003). In field settings, feces are typically easier and less risky to collect than urine (Graham et al., 2001; Monfort, 2003; Wasser et al., 1996). The ease of fecal collection facilitates longitudinal (even daily) analyses, which enhance statistical power when

monitoring hormone activity, such as estrous cyclicity and pregnancies (Monfort, 2003).

Endocrine techniques have been used as a tool to monitor the physiological state of *in situ* (Foley et al., 2001; Gobush et al., 2008; Hodges et al., 1997; Wittemyer et al., 2007) and *ex situ* (Brown, 2000) female African elephants (*Loxodonta africana*). In fact, the first study to apply non-invasive endocrine techniques to a free-ranging wildlife species was Poole et al. (1984), who measured testosterone in urine from male African elephants to investigate biological mechanisms associated with musth. Endocrinology continues to play a role in conservation-related issues as they apply to elephants, including, but not limited to, the impacts of poaching and human-elephant conflict on elephant welfare (Ahlering et al., 2011), and the need for population control in some areas (Delsink et al., 2006; Fayrer-Hosken et al., 2000; Kerley and Shrader, 2007; Kirkpatrick, 2007). However, traditional sample processing and hormone assay techniques often require specialized and expensive equipment (Kirkpatrick et al., 1993) and make it nearly impossible to do on-site endocrine assessments in the field. Conservation managers could benefit from a quick endocrine test that provides information on the reproductive status of elephants and/or the effectiveness of contraceptive techniques (Lasley

Abbreviations: AENP, Addo Elephant National Park; CV, coefficient of variation; EIA, enzymeimmunoassay; FPM, fecal progestagen metabolites; PPD, postpartum duration (in years); SCBI, Smithsonian Conservation Biology Institute; 5 α -DHP, 5 α -pregnane-3,20-dione; 5 α -P-3-OH, 5 α -pregnane-3-ol-20 one.

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and Kirkpatrick, 1991). Thus, a new method was developed to qualitatively monitor progestagen concentrations in elephant feces under field conditions (Freeman et al., 2010).

Elephants have the longest reported estrous cycle of 14–16 weeks in duration, with an 8–12 week luteal phase (Brown, 2000; Hodges, 1998; Plotka et al., 1988). Additionally, the elephant also differs from most other mammals in that the major circulating luteal progestagen is not progesterone, but reduced progestagens like 5α -pregnane-3,20-dione (5α -DHP) and 5α -pregnane-3-ol-20-one (5α -P-3-OH) (Hodges et al., 1997). Elephants also have the longest gestation of any animal, 22 months (Laws, 1969). Gestation in African elephants is characterized by a sustained elevated progestagen concentration beyond the 8–12 week luteal phase (Meyer et al., 2004; Moss, 1983; Wittemyer et al., 2007). Fifty-five percent of metabolized progestagens in elephants is excreted in feces (Wasser et al., 1996) and fecal concentrations closely correspond to serum levels with a 2-day lag time (Graham et al., 2001; Wasser et al., 1996). This tight correspondence between serum and FPM concentrations was used to validate the field method of Freeman et al. (2010) on samples collected from *ex situ* elephants.

This study describes the first analysis of FPM in free-ranging female African elephants in Addo Elephant National Park (AENP), South Africa. The primary goal was to compare FPM results generated onsite using the field qualitative EIA method (Freeman et al., 2010) with quantitative progestagen concentrations of the same samples measured at the Endocrine Laboratory of the Smithsonian Conservation Biology Institute (SCBI) in Front Royal, VA, USA. A secondary goal was to investigate the relationship between female age and FPM concentrations. We hypothesized that field and laboratory FPM results would be positively correlated and that a decline in FPM concentrations would be found with advanced age. Combining field FPM analyses with visible physiological indicators and behavioral analyses could enhance the conservation management of free-ranging elephant populations. For instance, comparing hormone results with visible signs of pregnancy (e.g., swollen breasts) would lead to reasonable estimates of miscarriage rates, something that has never been studied in wild elephants (Foley et al., 2001).

2. Methods

2.1. Study site

The study site was located in the Eastern Cape of South Africa at the Addo Elephant National Park (AENP), and was founded in 1931 to protect the remaining 11 elephants left in the area (Whitehouse and Hall-Martin, 2000). The study area consists of 13,500 ha of habitat that ranges from sub-tropical succulent thicket to open, grassy plains (Whitehouse and Hall-Martin, 2000). Water within the park is pumped from a reservoir, ensuring a continuous supply to seven main waterholes.

2.2. Study animal and fecal sample collection

The elephant population in AENP consisted of approximately 415 individuals comprising six matrilineal lines when the study was conducted between July 2007 and September 2009. Research has shown that the social structure and behavior of elephants in AENP is comparable to elephants from other regions of Africa (Loizi et al., 2009; Schulte et al., 2007; Whitehouse and Hall-Martin, 2000). A family tree of the elephant population has been constructed from intense studies dating back to 1976–79 and has been updated regularly since 1996 (Esposito, 2008; Loizi, 2004; Whitehouse et al., 2001). Elephants were identified by ear tears and vein patterns, eye wrinkles, and other physical features that

were compared to files consisting of photographs and descriptions of the individuals (Whitehouse and Hall-Martin, 2000; Whitehouse et al., 2001). For most individuals under age 12, the date of birth could be estimated to the month and in many cases, the week or day based on observations (Loizi et al., 2009; Meyer et al., 2009; Whitehouse and Hall-Martin, 2000).

Elephants were easiest to locate and observe at five pumped waterholes throughout AENP. Waterholes serve as areas where elephants congregate, socialize, and exchange information (Merte et al., 2010). Observations were conducted from a vehicle for safety; the elephants were habituated to vehicles being in close proximity. Fecal samples were collected only when the individual was seen defecating, to ensure proper identification of the sample, and as close to monthly from each individual as possible.

2.3. Fecal extraction

A field method previously validated for elephants was used for extracting fecal hormones on site (Freeman et al., 2010). In brief, 0.50 ± 0.02 g of fresh feces were weighed on a Mettler–Toledo battery-powered balance (#PL202-S/00, accurate to 0.01 g) and placed into an 8-ml o-ring, plastic tube (#60.542.077, Sartstedt Inc., Nümbrecht, Germany). A total of 5 ml of 70% isopropyl alcohol was added; tubes were capped and then shaken vigorously by hand for 30 sec. The tubes were allowed to sit overnight at room temperature; the next day they were shaken by hand again and allowed to settle for 5 min. The shaken extract was immediately diluted in water and analyzed in the field using a qualitative assessment (see Section 2.4). Additionally, 1 ml aliquots were taken from the top of the vial and placed into separate 12×75 mm polypropylene tubes (#2332 and 2305, Perfector Scientific, Atascadero, CA). These tubes were air-dried and stored until heated to 72°C for 30 min prior to being shipped to the Endocrine Laboratory at the SCBI for quantitative analysis.

2.4. Field qualitative EIA technique

Field progestagen EIA plates were prepared at the SCBI and shipped to AENP following the protocol previously validated for *ex situ* elephants by Freeman et al. (2010). Microtitre plates (Nunc MaxiSorp, flat wells, #439454, Thermo Scientific, Waltham, MA) were coated with a monoclonal progesterone antibody (1:10,000 CL425, C. Munro, University of California-Davis, CA), stored at 4°C overnight and then washed once. A commercially available buffer (Coating Stabilizer and Blocking Buffer, #J16430D, Biodesign International, Saco, ME) was added to each well and the plate incubated at room temperature for 30 min. Excess buffer was shaken from the plates without washing; the plates were placed upside down on top of a layer of drierite (#23001, W.A. Hammond Drierite Company, Xenia, OH) in a sealed tub placed under vacuum for 2 h. The dried, buffered microtitre plates bound with the progesterone antibody were sealed in 7.6×12.7 cm Ziploc bags with two desiccant packets until shipped to the AENP for hormone analyses.

For the field qualitative assay, progesterone standards (100, 50, 25, 12.5 and 0 pg/ml; #P 0130, Sigma Aldrich, Inc., St. Louis, MO) or diluted sample were added to the microtitre wells, followed immediately by a horseradish peroxidase-conjugated label (1:40,000; C. Munro). After an hour of incubation at room temperature, the plates were washed using a 0.02% solution of Tween 20 (#P 2287, Sigma Aldrich, Inc.) and substrated with a phosphate-citrate buffer (#P4560, Sigma Aldrich, Inc.) and TMB (#T3405, Sigma Aldrich, Inc.). After 5 min, the standards and samples developed into distinct shades of blue (ranging from nearly clear to dark royal blue). The qualitative hormone concentrations of the unknown

samples were visually determined by comparing color intensity to corresponding known standards with a similar intensity, which provided a qualitative ranking (0–5) based upon the number of that standard. The color intensity and qualitative ranking (0–5) were directly related to the FPM concentration; thus, higher qualitative rankings had higher FPM concentrations (Freeman et al., 2010).

2.5. Quantitative hormone analysis

In the SCBI Endocrine Laboratory, dried fecal extracts were reconstituted with dilution buffer (0.2 M NaH_2PO_4 , 0.2 M Na_2HPO_4 , NaCl) and 80% methanol by vortexing the plastic tubes for 1 hour and then sonicating for 30 min to completely dissolve the extract. Reconstituted extracts were diluted in buffer and analyzed using the methods of Graham et al. (2001). The laboratory EIA FPM analyses were the same as the field qualitative method with the following exceptions: the microtitre plates were not treated with the commercial stabilizing buffer; the assay used a 10-point standard curve (200–0.78 pg/ml); and fecal hormone concentrations were quantified using a spectrophotometer. Assay sensitivity was 0.78 pg/well and intra- and inter-assay coefficients of variation (C.V.) were <10%.

2.6. Data analyses

Results of the visual qualitative, 'field' analyses (rank, 1 = low concentration to 5 = high concentration) were compared to quantitative FPM values obtained using the standard laboratory assay method with the 10-point curve and a portable spectrophotometer (Graham et al., 2001) using a Spearman rank order correlation. Differences in the average quantitative FPM concentrations between the qualitative hormone levels were assessed with a Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks.

Samples from pregnant female elephants ($n = 190$, from 22 elephants; range: 3–21 samples per female) were identified by backdating from dates of parturition, i.e. by subtracting the average gestation period of 22 mo (Laws, 1969) from the estimated date of birth. Kruskal–Wallis ANOVA on ranks was used to compare qualitative and quantitative FPM concentrations with respect to month of gestation and sex of the offspring. Postpartum duration (PPD) was calculated for each female by calculating the difference between the sample collection date and the parturition date of the elephant's previous calf. FPM concentrations for each pregnant female and the six elephants with a long PPD (≥ 5 years) were averaged by month and analyzed over time using Kruskal–Wallis ANOVA on ranks. Five years was selected as the cut-off for determining a long PPD because it is 1 year more than the mean range for the inter-calf interval (2.6–4.0 years) documented for females in AENP (Gough and Kerley, 2006).

Age of the individual elephants changed over the study period, thus we placed each female into age categories (in 10-year intervals) to analyze the affect of age. Comparisons were made among the age category and mean values calculated for each female (PPD, qualitative FPM levels, and quantitative FPM concentrations), to avoid pseudoreplication, using ANOVA, or Kruskal–Wallis ANOVA on ranks when data were not normally distributed or did not exhibit equal variance.

A Kolmogorov–Smirnov test was used for normality assumption testing and the Levene median test for equal variance assumption testing. Comparisons of means among treatments were performed using Tukey's or Dunn's test, as appropriate. Data were analyzed using SigmaStat (v. 11.0 2008, Systat Software, Inc.) and Systat (v. 12.02 2007, Systat Software, Inc.). For all analyses, $P < 0.05$ was considered significant and data were reported as mean $\pm$ SEM.

3. Results

We analyzed 608 fecal samples (15.2 ± 0.7 samples per individual; range: 5–24) collected from 40 focal elephants (age range: 17–60 years). We determined that at least 22 of the 40 females were pregnant during the study by backdating parturition events. Because some of the females conceived before the onset of the study and others gave birth after the study was over, complete pregnancy hormonal profiles were not assessed for all of the elephants. Due to possible lost pregnancies (e.g., miscarriages) and females that gave birth after the study ended, the percentage of pregnant females (55%) in AENP is probably underestimated.

3.1. Comparison of 'Field' and laboratory Pg results

The quantitative, laboratory analyzed, FPM concentrations of samples collected from female African elephants varied significantly with respect to the visual, qualitative levels assigned in AENP ($H_4 = 244.882$, $P < 0.001$). Mean FPM concentrations varied between all qualitative levels except for standards (colors) 1 and 2 (Fig. 1; Dunn's $P < 0.05$). Additionally, the laboratory quantitative FPM concentrations were correlated with the visual, qualitative FPM levels determined in the field ($r_s = 0.72$, $P < 0.001$).

3.2. Excretory FPM profiles

Feces from 22 pregnant elephants (138.19 ± 8.23 ng/g feces) had higher FPM concentrations ($T_{171,433} = 62045.0$, $P < 0.001$) than feces from 18 non-pregnant females (113.65 ± 7.07 ng/g feces), and higher qualitative FPM levels ($T_{171,433} = 68411.0$, $P < 0.001$; pregnant: 3.44 ± 0.08 non-pregnant: 2.63 ± 0.05). Although elephants carrying female calves had on average 23% higher FPM concentrations (144.23 ± 14.95 ng/g feces, $n = 14$) than those carrying males (116.93 ± 12.25 ng/g feces, $n = 8$), the difference was not significant ($T_{76,108} = 6511.0$, $P = 0.15$). Additionally, the qualitative FPM levels did not vary with respect to sex of the fetus ($T_{20} = -1.30$, $P = 0.21$; female: 3.47 ± 0.09 , male: 3.18 ± 0.14). The quantitative pregnancy FPM concentrations of female African elephants in AENP varied over the months of gestation ($H_{24} = 41.01$, $P < 0.05$); however, posthoc analyses did not identify any significant differences in the pairwise comparisons between months (Fig. 2a; Dunn's, $P > 0.05$). Similarly, the average qualitative FPM levels also varied with respect to months of gestation

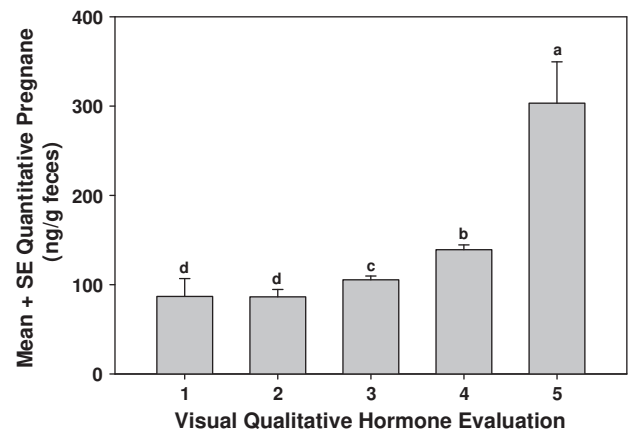


Fig. 1. The quantitative, laboratory analyzed, FPM concentrations of samples collected from female African elephants vary significantly with respect to the visual, qualitative analyses conducted in Addo Elephant National Park, South Africa ($H_4 = 244.882$; $P < 0.01$). Letters denote significant differences (Dunn's post-hoc, $P < 0.05$).

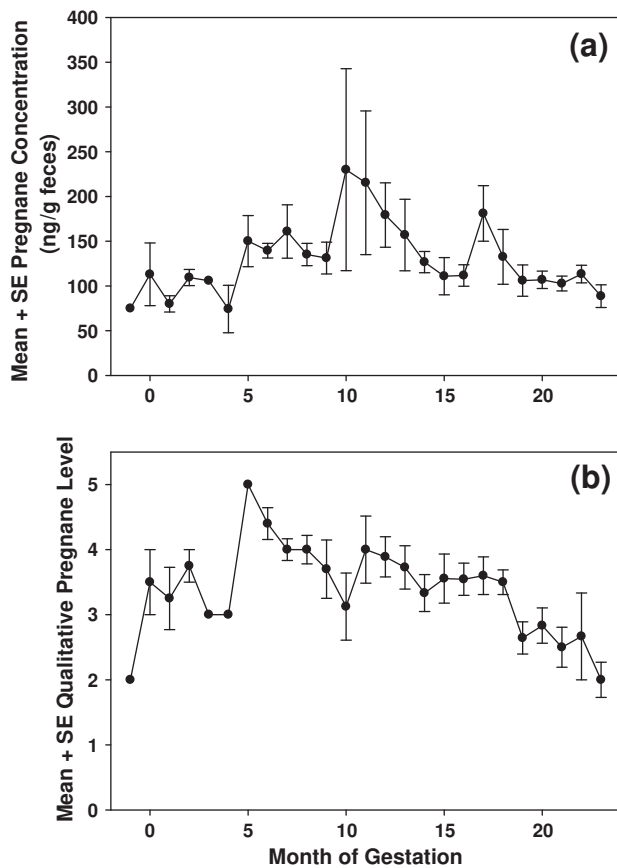


Fig. 2. Pregnancy profiles for female African elephants in Addo Elephant National Park, South Africa. (a) Average quantitative FPM concentrations (ng/g feces) varied between months of gestation ($H_{24} = 41.01$, $P < 0.05$). (b) Average qualitative progesterone levels also varied between months of gestation ($H_{24} = 58.24$, $P < 0.001$). However, no significant differences were found between months during post-hoc analyses (Dunn's $P > 0.05$).

($H_{24} = 56.24$, $P < 0.001$), yet no significant pair-wise differences were found during post-hoc analyses (Fig. 2b; Dunn's $P > 0.05$). There was a strong correlation between quantitative and qualitative FPM concentrations from pregnant elephants ($r_s = 0.71$, $P < 0.001$).

Of the 40 females studied in AENP, several ($n = 6$) had not had a calf in 5 years or more (range: 5–27 years). Fig. 3a depicts the average monthly FPM concentrations for these adult females. The most extreme case was a 46-year-old female that had not had a calf in over 25 years (Fig. 3b). The second oldest female in the study (58 years of age) had not had a calf in over 17 years (Fig. 3c). Based on the fecal FPM profiles, none of these females exhibited regular ovarian cycles. Although the PPD of these females was large, there was not a significant relationship between average PPD and mean FPM concentrations (quantitative: $r = -0.16$, $P = 0.33$; qualitative: $r = -0.19$, $P = 0.26$; baseline: $r = -0.08$, $P = 0.64$) for the studied AENP elephants.

3.3. Effect of age

Postpartum duration varied among the age categories ($H_4 = 12.050$, $P < 0.05$; Table 1). Although the oldest females had the largest PPD, posthoc analyses did not find any significant pair-wise variation (Dunn's $P > 0.05$). There was a trend for females in older, 10-year age categories to have lower qualitative FPM levels ($F_{4,35} = 3.572$, $P < 0.05$), with the oldest females having significantly lower values than those in their prime breeding years

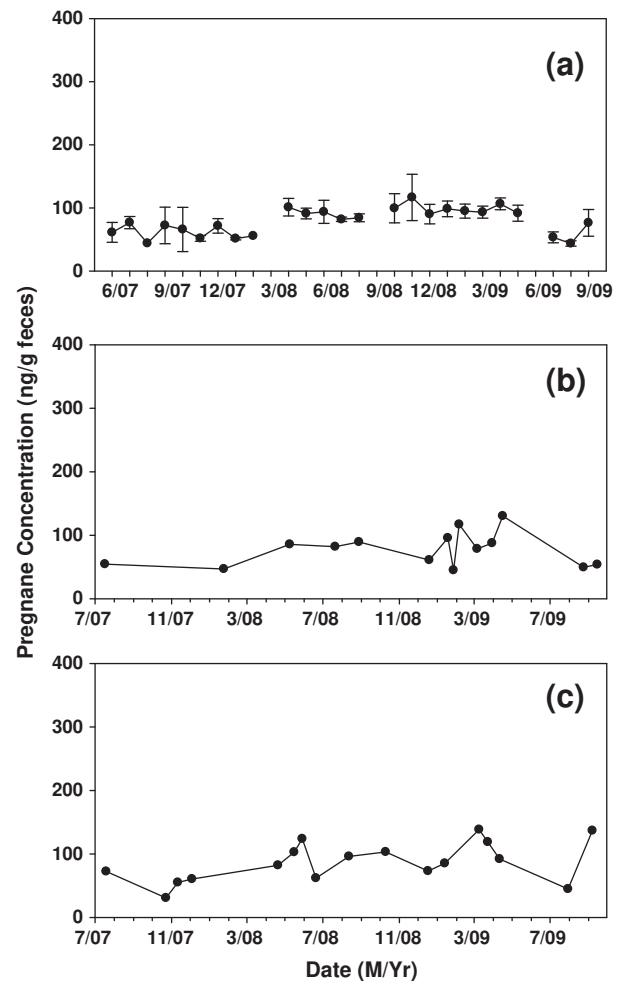


Fig. 3. Hormone profiles for female African elephants in Addo Elephant National Park, South Africa. (a) Average monthly FPM concentrations for adult females that have not had a calf in over 5 years. (b) FPM profile for a matriarch (mean: 45.5 yr of age) that has not had a calf in over 27 years. (c) FPM profile for a grand matriarch (mean: 57.2 yr of age) that has not had a calf in over 17 years.

(ages 30–39; Tukey's $P < 0.01$). While there was a similar trend for females in older, 10-year age categories to have lower mean quantitative FPM concentrations (83.29 ± 4.65 compared to 105.19 ± 6.89 ng/g feces; Table 1), there were no significant differences among the ages ($H_4 = 7.12$, $P = 0.13$).

4. Discussion

This study is the first to investigate the effectiveness of a field-friendly EIA for monitoring FPM concentrations in free-ranging African elephants. Results indicate that the qualitative field method provides FPM profiles comparable to the gold standard, quantitative laboratory technique, and similar to tests on feces from zoo elephants (Freeman et al., 2010). Pregnancy profiles generated using these techniques also were similar to previously published results for both *ex situ* and *in situ* elephants (Foley et al., 2001; Meyer et al., 2004; Wittemyer et al., 2007). There was some indication of ovarian acyclicity among female elephants in AENP based on FPM concentrations that remained relatively stable; a phenomenon that is commonly observed in North American, *ex situ*, African elephants (Brown et al., 2004; Proctor et al., 2010). The oldest females in AENP exhibited the lowest FPM levels and the longest interval since the birth of their last calf. Our results may support

Table 1

Comparisons among adult female African elephants in Addo Elephant National Park based upon their age (categorized in decades). All results are reported as mean $\pm$ SEM. Letters denote significant differences among the family categories (Tukey's or Dunn's; $P < 0.05$).

Age category	10–19	20–29	30–39	40–49	50–59
Postpartum years	1.47 $\pm$ 0.21	2.78 $\pm$ 0.60	2.02 $\pm$ 0.25	7.38 $\pm$ 5.02	9.08 $\pm$ 2.77
Qualitative FPM score	2.68 $\pm$ 0.11 ^{a,b}	3.01 $\pm$ 0.11 ^{a,b}	3.01 $\pm$ 0.10 ^a	2.59 $\pm$ 0.14 ^{a,b}	2.42 $\pm$ 0.1 ^b
Quantitative FPM (ng/g feces)	103.61 $\pm$ 5.32	105.22 $\pm$ 4.19	105.19 $\pm$ 6.89	98.88 $\pm$ 14.82	83.29 $\pm$ 4.65

the occurrence of menopause in free-ranging elephants (Freeman et al., submitted for publication).

The field fecal extraction and EIA technique offers the ability to produce real-time results on-site without the need for expensive instrumentation or sample exportation. Thus, it can provide data on the reproductive state of individuals in a short period of time, allowing managers to make informed decisions based on sound scientific data (Freeman et al., 2010). Unlike the rhinoceros, where field assays for progestagens can readily diagnose pregnancy using a single fecal sample (MacDonald et al., 2008), elephants require monthly longitudinal sample collection for at least 4 months (the length of a normal estrous cycle) to distinguish between pregnant and non-pregnant luteal phases. While the present study demonstrates that pregnancy can be detected with longitudinal sampling, field applications such as these cannot rapidly predict parturition as they can in rhinos (MacDonald et al., 2008).

Slightly over half of the 40 adult females from which feces were collected were pregnant during the course of the study. Conception in African elephants occurs once every 3–5 years (Gough and cKerley, 2006; Hall-Martin, 1987; Laws, 1969; Moss, 2001; Whitehouse and Hall-Martin, 2000), or potentially up to 10 times in a lifetime (Hall-Martin, 1987). At any given time, about a third of wild females are believed to be pregnant, a third are lactating, and a third are available for breeding (Hall-Martin, 1987). Overall FPM concentrations in the pregnant elephants in the present study were higher than FPM concentrations from non-pregnant elephants, due to the averaging of follicular and luteal phase samples in cycling elephants. Additionally, a significant variation in FPM concentration with respect to month of gestation was demonstrated, and month of gestation was the strongest predictor of FPM levels in pregnant AENP females. These results are similar to those demonstrated previously for *ex situ* (Meyer et al., 2004) and *in situ* (Foley et al., 2001; Wittemyer et al., 2007) African elephants. Similar to Meyer et al. (2004) there was no difference in mean progestagen concentrations with respect to sex of the fetus. However, Wittemyer et al. (2007) found that elephants carrying male calves have higher FPM concentrations in mid to late pregnancy than those carrying females. Due to a limited number of complete pregnancy profiles, stage of the pregnancy was not analyzed with respect to fetal gender in the present study.

In spite of the high percentage of pregnant (22 out of 40) females demonstrated in the present study and historically high pregnancy rates in AENP (Gough and Kerley, 2006), there is evidence of females with low reproductive rates that are potentially acyclic. Six of the AENP females monitored had not had a calf in 5 years or more; the majority of those females were in their 40s ($n = 1$) or 50s ($n = 4$). One elephant (Fig. 3b) appeared to be no longer cycling; only two of her FPM concentrations rose above baseline. Another female (Fig. 3c) either had irregular cycles, lasting more than 16 weeks, or was also acyclic (only five of her FPM concentrations exceeded baseline). Of the remaining AENP elephants with long PPD, three appeared to be acyclic and one possibly pregnant (profiles not shown). There was one younger female (~19 years of age) in the present study that had never successfully produced a calf. She was not included in the analyses of females with long PPD because her actual PPD could not be determined. The majority

of her FPM concentrations were low ($n = 16$; range: 59.2–256.2 ng/g feces) and physiological signs (e.g., swollen breasts that later diminished with declining FPM concentrations) suggested she may have experienced a miscarriage during the course of the study. Anestrus is harder to detect using these fecal extraction and EIA techniques than serum RIA analyses, as there is more natural fluctuation in the amount of metabolized and excreted hormone (Freeman, pers. obs). This makes it more difficult to diagnose acyclicity in free-ranging elephants than *ex situ* females.

High rates of ovarian acyclicity are a serious problem among *ex situ* African elephants in North America. More than one third of reproductive-aged African savannah elephants in North American facilities exhibit irregular or acyclic ovarian activity (Brown et al., 2004; Proctor et al., 2010). While the exact cause of this physiological phenomenon is not known, rank within the captive herd and a high body condition index are related to a female being acyclic (Freeman et al., 2004, 2009). Wittemyer et al. (2007) were the first to refer to anestrus periods in free-ranging African elephants. They found that poor vegetative quality during the dry season is correlated with ovarian inactivity (low FPM concentrations) in elephants in the Samburu and Buffalo Springs Reserves of northern Kenya. Wittemyer et al. (2007) also found a negative relationship between age and FPM concentrations in pregnant African elephants. One female African elephant in Tarangire National Park, Tanzania whose FPM concentrations were monitored was determined to be infertile (Foley et al., 2001); however, the authors never explained what hormone results led to this conclusion. The consistently low FPM concentrations among some African elephants in ANEP indicate that they likely were not cycling. Their potential lack of ovarian activity could be functionally similar to what is observed in North America, albeit at an older age than what is typically observed among the *ex situ* elephants. Discovering that ovarian acyclicity is not solely a captivity-related phenomenon and gaining knowledge about the factors that naturally control estrous cycle activity will assist *ex situ* managers in finding ways to maximize reproduction and create self-sustaining elephant populations. With legal and political limitations on importing wild-caught elephants (Hutchins and Keele, 2006), promotion of self-sustaining *ex situ* populations are important because zoo animals play a crucial role as stewards for promoting education about wildlife conservation and aid worldwide scientific research efforts (Riddle et al., 2003; Schulte, 1999; Smith and Hutchins, 2000).

Non-invasive endocrine techniques have the potential to help to optimize conservation initiatives with little additional resources (MacDonald et al., 2008). Integrating field hormone monitoring techniques with behavioral and ecological research will enhance our understanding of reproductive strategies (Ganswindt et al., 2002; Lasley and Kirkpatrick, 1991), evolutionary biology (Santymire and Armstrong, 2010) and life-history requirements of endangered species (Monfort, 2003). More specifically, progestagen data can enable wildlife managers to monitor individual reproductive status and population level reproductive rates in free-ranging populations. Combining progestagen analyses with visible signs of pregnancy (e.g., swollen breasts) would lead to reasonable estimates of miscarriage rates, something that has never been studied in wild elephants (Foley et al., 2001). When coupled

with infant counts, a broader picture of the reproductive health of elephant populations would become evident (Foley et al., 2001). We are particularly interested in determining conclusively if ovarian acyclicity is strictly limited to *ex situ* elephants. The goal of these types of studies ultimately is to develop better long-term management strategies to ensure the success of all elephants, both *in situ* and *ex situ*.

Additionally, field endocrine techniques could be used in a more applied way to determine the impact of culling and contraceptives on population management (Foley et al., 2001; Wasser et al., 1996). Endocrine techniques also can be used to measure the impact of poaching on reproductive success of recovering populations (Gobush et al., 2008). Determining which females are still cycling would be useful for managers as they determine the best way to regulate population growth. Elephant overpopulation in some areas of Africa is a growing problem, and is fueling the debate over how best to manage these populations (Owen-Smith et al., 2006) – through resuming culling (Dickson and Adams, 2009), the use of contraceptives (Kerley and Shrader, 2007), or other means (van Aarde and Jackson, 2007). Non-invasive endocrine monitoring will provide managers with biological data to identify the best candidates for translocation or contraception as well as the success of contraceptive measures. Furthermore, many populations, particularly those of forest elephants (Eggert et al., 2008; Munshi-South et al., 2008), are difficult to monitor due to dense vegetation and rugged terrain. However, when non-invasive endocrine methods are combined with DNA analyses of gender and individual identity of fecal samples (Gobush et al., 2008), the reproductive health of individuals can be monitored in these more elusive populations. Field endocrine techniques could transform endangered species research under field conditions and provide new tools for making sensible conservation management decisions based on real-time hormone results.

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