

The impact of ecological variability on the reproductive endocrinology of wild female African elephants

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Received 29 August 2006; revised 1 December 2006; accepted 1 December 2006

Available online 10 January 2007

Abstract

Non-invasive endocrine methods enable investigation of the relationship between ecological variation and ovarian activity and how this impacts on demographic processes. The underlying physiological factors driving high variation in inter-calving intervals among multi-parous African elephants offer an interesting system for such an investigation. This study investigates the relationship between Normalized Differential Vegetation Index (NDVI), an ecosystem surrogate measure of primary productivity, and fecal progesterin concentrations among wild female elephants. Matched fecal samples and behavioral data on reproductive activity were collected from 37 focal individuals during the two-year study. Linear mixed models were used to explore the relationship between fecal 5 α -pregnane-3- α -20-one concentrations and the independent variables of NDVI, calf sex, female age, gestation day, and time since last parturition. Among both non-pregnant and pregnant females, fecal 5 α -pregnane-3- α -20-one concentrations were significantly correlated with time-specific NDVI indicating a strong relationship between ecological conditions and endocrine activity regulating reproduction. In addition, the age of a female and time since her last parturition impacted hormone concentrations. These results indicate that the identification of an individual's reproductive status from a single hormone sample is possible, but difficult to achieve in practice since numerous independent factors, particularly season, impact fecal hormone concentrations. Regardless of season, however, fecal 5 α -pregnane-3- α -20-one concentrations below 1 μ g/g were exclusively collected from non-pregnant females, which could be used as a threshold value to identify non-pregnant individuals. Collectively the information generated contributes to a better understanding of environmental regulation of reproductive endocrinology in wild elephant populations, information salient to the management and manipulation of population dynamics in this species.

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Keywords: Non-invasive; Hormone; Feces; Progesterogens; NDVI; Demography; Population management; Ecology; African elephant; Reproduction

Introduction

The timing of reproduction is shaped by selective forces, acting to maximize lifetime reproductive success (Kennish, 1997; Pianka, 1976). In many species, reproductive activity and cessation (anoestrus) are highly seasonal and relate to environmental variability, such as resource availability and photoperiod

length (Lincoln and Short, 1980; Wayne et al., 1989). The degree of correlation between reproductive activity and ecological conditions is related to the amplitude and consistency in timing of seasonal fluctuations (Colwell, 1974). In this context, the timing of reproductive activity in north temperate regions is relatively stable across years in relation to strong seasonal variation (Stearns, 1992), while less predictable in equatorial regions where the interaction between ecological variability and life history constraints plays a greater role in timing reproductive events (Sinclair et al., 2000; Wingfield et al., 1992). Thus, increased knowledge of the relationship between physiological parameters and timing of reproduction,

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particularly in equatorial regions, can offer novel insights into the factors influencing reproductive phenology and the strategies employed to maximize fitness.

Fecal hormone analysis to monitor gonadal and adrenal endocrine function has advanced the use of hormone measurements in studies of the reproductive biology of wild and captive animals (Brown et al., 1997; Heistermann et al., 1996). These techniques allow novel exploration of the proximate cues related to the initiation or cessation of hormonal activity leading to ovulation and, thus, the mechanisms controlling the timing of conception. For example, seasonal variation in endocrine function of *Hanuman langurs* in Southern Nepal was found to be driven by food availability, causing birthing peaks to occur during the dry season when resources were poor (Ziegler et al., 2000). Research on the factors influencing endocrine activity in domestic animals demonstrates that the timing of ovulation and anoestrus for north temperate species of goats is driven by photoperiod though marginally impacted by nutrition (Zarazaga et al., 2005). In contrast, equatorial species of goats do not respond to photoperiod differentiation but are impacted by variation in nutrition (Rondina et al., 2005). Similarly, results from research on captive African elephants (*Loxodonta africana*) held in north temperate regions demonstrate that photoperiod does not influence hormonal activity in this equatorial species (Bechert et al., 1999). Despite its importance for the management of elephants, both in captivity and in the wild, information on the factors influencing the timing of ovarian activity and anoestrus in this species is lacking.

African elephants experience the unique life history characteristic of a 22-month gestation period (Laws, 1969), complicating the timing of reproductive events (Rasmussen et al., 2006). As a result, inter-calving intervals among elephants are more variable than most ungulates (Moss, 2001; Wittemyer et al., 2007). Such characteristics make elephants of interest for exploring the relationship between environmental variability and reproductive activity. Many of the equatorial savanna ecosystems inhabited by elephants experience stochastic rainfall resulting in variable levels of primary productivity within and between seasons. Previous demographic work on wild elephants demonstrated that seasonal conception probabilities are strongly correlated with fluctuations in primary productivity, while the timing of parturition occurred during likely periods of increased food availability (Wittemyer et al., 2007).

The aim of the present study is to investigate the environmental factors impacting endocrine activity in wild African elephants. Using measurements of fecal 5α -pregnane-3-ol-20-one concentrations to monitor ovarian activity (Fieiss et al., 1999; Foley et al., 2001), we describe the influence of resource availability and individual characteristics such as age, post-partum period, and reproductive state on fecal progesterone concentrations. Collectively, our findings not only advance theoretical knowledge on the relationship between hormonal cycling and environmental variation in an equatorial species, but also address applied objectives regarding the conservation and management of this species. Insight is offered on the causes of anoestrus or “flatlining”, a major concern for the long term viability of captive populations of African elephants

(Brown et al., 2004; Schulte et al., 2000). Understanding of the factors influencing ovarian activity and anoestrus periods is important for the management of wild populations since overpopulation of elephants impacts biodiversity in some locations while local extirpation threatens other populations.

Methods

Study area and population

The study area lies within the 220 km² Samburu and Buffalo Springs National Reserves in northern Kenya (37.5°E, 0.5°N). These semi-arid parks are dominated by acacia-comiphora savanna and scrub bush and located along the Ewaso N'giro River, the major permanent water source in the region (Barkham and Rainy, 1976). As a result, the reserves are a focal area for wildlife. Rainfall averages approximately 350 mm per year and occurs during biannual rainy seasons generally taking place in April/May and November/December. The elephant population using these reserves numbers approximately 900 individuals (Wittemyer et al., 2005a) and constitutes part of the larger Samburu/Laikipia elephant population comprising over 5000 individuals (Omondi et al., 2002). All individuals observed within the reserves are individually identified, following well established methods (Moss, 1996, 2001). All individuals are aged via known birth dates or physical characteristics (as described in Moss, 1996) using individuals accurately aged by molar progression during immobilization as reference points. Age estimates of 80% of individuals over 20 years of age were found to be ± 3 years of their actual age (Rasmussen et al., 2005). The behavior and reproductive activity of the entire population have been closely monitored since 1997 (Wittemyer, 2001; Wittemyer et al., 2005a). In this study, we focus on the reproductive biology of 37 of the best known females in the population. Each of these females gave birth to at least one calf during the four-year period beginning June 2001 and ending June 2005. For a more detailed description of the study population and ecology of the study area, see Wittemyer (2001).

Behavioral observations and sample collection

Elephants range widely and unpredictably, therefore we focused behavioral observation and sample collection on 37 females from 10 well-known social groups, quantitatively defined from point observations of associations in previous research (Wittemyer et al., 2005b). The reproductive states (pregnant vs non-pregnant) of the study females were assessed during all day focal followed by opportunistic ad libitum sampling (Altmann, 1974) from direct observation of estrus events, defined by the observations of mate guarding/mating by males and distinct estrus behavior by females (Moss, 1983) or backdating parturition events. The birth dates of calves less than 1 month old can be estimated accurately to within 1 week (Moss, 1996, 2001). The average age at first observation of the 37 females' calves born during the study period was 9 (range 1 to 36) days. In order to approximate the date of conception for the known parturition events, we subtracted the average gestation period of 656 days (approximately 22 months) from the estimated date of birth (Moss, 1983).

The study elephants are largely habituated to the presence of vehicles, enabling easy observation of behavior and collection of individual specific samples. We analyzed 540 fecal samples collected from the 37 focal individuals (mean \pm standard deviation: 14.6 \pm 9.7 samples per individual; range: 3–37). Samples were collected on a monthly basis, or as close to monthly as possible, between June 2001 and June 2003 directly following defecation to ensure identification of the sample producer. The fecal bolus was homogenized by hand (using rubber gloves), and a 10–15 g portion was stored in a polyethylene tube with approximately 25 ml of 96% ethanol (Wasser et al., 1988). Samples were refrigerated at the field site until transport to the German Primate Centre for analysis (see Fecal extraction and hormone assays, below).

Ecological data

Previous work has demonstrated that reproduction of the study elephants is more closely correlated with remotely sensed NDVI (Normalized Differential Vegetation Index) than rainfall (Rasmussen et al., 2006), thus we compare

hormonal activity in the study elephants to NDVI data as our measure of ecological variability in this study. NDVI is a remote sensing index value, calculated by comparing visible and near-infrared reflectance measurements ($NDVI = (NIR - VIS) / (NIR + VIS)$), where NIR is the near-infrared light, and VIS the visible light reflected by the vegetation), highly correlated with green biomass (Goward and Prince, 1995). Negative values correspond to the absence of vegetation and positive values correspond to variation in vegetative phenology (Justice et al., 1985). To measure time-specific vegetation productivity, we used 10-day composite NDVI values recorded by the SPOT (Satellite Probatoire d'Observation de la Terre; 1998–2004) system. The study area, from which NDVI values were averaged, was defined by buffering permanent water in and adjacent to the national reserves as described in Rasmussen et al. (2006). These NDVI data were used to delineate seasons, as described in Rasmussen et al. (2006), where the beginning and end of the wet season were defined as occurring where NDVI values were greater than or equal to 1 standard deviation above the dry season baseline.

Fecal extraction and hormone assays

Fecal samples were homogenized in the original ethanolic solvent as described by (Ganswindt et al., 2005). Briefly, the ethanolic fecal suspension of each sample (including a 3×5 ml 80% methanolic rinse of the original sample tube) was transferred into two 50 ml tubes, and steroids were extracted by shaking for 15 min on a multi-tube vortex. Following centrifugation at 3300 rpm for 10 min, both supernatants were combined, the total volume recorded, and the extracts finally stored at 4 °C until hormone analysis. The remaining fecal pellets were dried in a vacuum oven at 50 °C, and the dry weight of individual samples was determined. Extraction efficiency, determined in a subset of 10 randomly selected fecal samples by monitoring the recovery of (³H)progesterone added to the samples prior to homogenization, was $86.2\% \pm 4.7\%$ (mean \pm SD).

Fecal extracts were measured for immunoreactive 5 α -pregnane-3-ol-20-one (5 α -P-3-OH), which has previously been shown to provide reliable information on ovarian function and pregnancy in the African elephant (Fiess et al., 1999). The 5 α -P-3-OH enzyme immunoassay used an antibody raised in a rabbit against 5 α -pregnane-3 β -ol-20-one-3HS-BSA and 5 α -pregnane-3 β -ol-20-one-3HS conjugated with biotin as a label (Hodges et al., 1997). Cross-reactivities of the antibody are described in Hodges et al. (1997). Serial dilutions of fecal extracts gave displacement curves which were parallel to the respective standard curve of the assay. Sensitivity of the assay at 90% binding was 18 pg/well, and intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls ranging between 11.8% and 13.9%. In brief, 50 μ l aliquots of 5 α -P-3-OH standard (range 9.8–2500 pg), quality controls, and diluted fecal extracts were pipetted in duplicate into microtiter plate wells. Fifty microliters of label and antiserum was added and the plates incubated overnight at 4 °C. Following incubation, the plates were washed four times and 150 μ l (20 ng) of streptavidin–peroxidase added to each well. Following incubation in the dark for 30 min, plates were washed again before 150 μ l peroxidase substrate solution was added and plates further incubated for 30–60 min. The reaction was terminated by adding 50 μ l of 4 N H₂SO₄ and the absorbance measured at 450 nm.

Statistical analysis

Analysis of the relationship between fecal 5 α -pregnane-3-ol-20-one concentrations and explanatory variables was explored within both a linear mixed-effect model framework (LME; Venables and Ripley, 1999) and a generalized additive model framework (GAM; Venables and Ripley, 1999). Because the data modeled consisted of repeated measures of hormone concentrations from individual females, female identity was incorporated as a random effect in LME models. We use GAMs with smoothing splines to explore possible nonlinear relationships (Dobson, 2002). Nonlinear relationships were then modeled using polynomials in LME models. LME and generalized linear models are thought to be more robust to type I errors than analysis of transformed data, the relationship between the response and explanatory variables does not need to be simple and is widely applied in the statistical literature on non-normally distributed data (McCullagh and Nelder, 1989).

We included several covariates predicted to relate to reproductive state in our LME analysis of 5 α -pregnane-3-ol-20-one concentrations per sample. In the

analysis of concentrations collected from pregnant females, covariates explored were NDVI during sample collection, female age, fetus sex (identified post birth), gestation day at sample collection, and the inter-pregnancy interval calculated as the difference between the parturition date of the individual's previous calf and the conception date of the individual's current pregnancy. As previously shown by Hodges (1998), GAM analysis revealed that the relationship between gestation date and hormone concentrations was nonlinear (Fig. 1). Therefore a nonlinear function for gestation date was included in the model. Covariates used in LME analysis of 5 α -pregnane-3-ol-20-one concentrations per sample collected from non-pregnant females were NDVI during sample collection, female age, previous calf sex, and duration between sample collection and the parturition date of the individual's last calf (duration of non-pregnant period). Step-wise elimination of non-significant variables was conducted and reduced models were compared to the full model using AIC (Burnham and Andersen, 1998) and BIC (a similar but more conservative criterion than AIC; (Miller, 2002)) as a guide for model selection.

Samples were grouped according to the reproductive state of the individual, pregnant ($n=302$ for 34 individuals) or non-pregnant ($n=238$ for 32 individuals), known by backdating from parturition events as described previously. Additionally, samples were categorized by season, wet ($n=257$ for 35 individuals) or dry ($n=283$ for 37 individuals), at the time of sample collection where season was defined using NDVI data. To avoid possible complications of pseudoreplication, non-parametric techniques were used to analyze the median value per individual of 5 α -pregnane-3-ol-20-one concentrations in dung across season and state. Differences in hormone levels between reproductive states and seasons were tested using a Kruskal–Wallis Rank Sum test. Pair-wise comparisons between wet and dry season samples for both pregnant and non-pregnant females were conducted using Wilcoxon Tests. The average and standard error concentrations of 5 α -pregnane-3-ol-20-one in samples collected from females pregnant with male and female calves were compared across the gestation period to assess for differences in hormone concentrations in relation to fetal sex. All statistical analyses were conducted in S-Plus (Venables and Ripley, 1999).

Results

Individual 5 α -pregnane-3-ol-20-one profiles from monthly or bimonthly samples were compared with 10-day composite NDVI. Combined with observed estrus events, known parturition, and estimated conception dates, these profiles offer insight into the relationship between hormonal activity and reproductive events, as well as the continuous effects of seasonal variation in primary productivity (Fig. 2). Fig. 2a shows a complete pregnancy profile including pre-conception and post parturition samples, though behavioral estrus was not observed for this female. 5 α -Pregnane-3-ol-20-one concentrations appear to fluctuate in relation to NDVI, though this relationship is dampened during the later half of gestation. Fig. 2b shows hormonal flatlining (anoestrus) during the dry season progressing into cyclic activity during the first wet season, but without behavioral estrus. Estrus behavior was observed coinciding with the second wet season, but conception did not occur. However, 16 weeks later during the dry season, estrus behavior was observed by the female coinciding with conception followed by an increase in 5 α -pregnane-3-ol-20-one levels during pregnancy. Again, 5 α -pregnane-3-ol-20-one levels fluctuate in relation to NDVI throughout the whole profile. Fig. 2c shows hormonal activity in a primiparous female 8 years of age prior to and including her first estrus and conception event. The relationship between 5 α -pregnane-3-ol-20-one and NDVI is also apparent, but less clear than with mature females. Trends in these profiles illustrate the interaction between 5 α -pregnane-3-

ol-20-one and NDVI values, as well as the impact of gestation period.

The variables impacting 5α -pregnane-3-ol-20-one levels in both non-pregnant (Fig. 3a) and pregnant females (Fig. 3b) were examined in linear mixed-effect (LME) models where female identity was incorporated as a random factor (Table 1). Significant explanatory variables in the LME model of 5α -pregnane-3-ol-20-one concentrations in dung samples collected from non-pregnant females were NDVI during sample collection ($t=5.95$; Fig. 4a) and calving interval ($t=3.68$), calculated as the period between sample collection and the date of birth of the individual's last calf (i.e. the duration of the non-pregnant period). Non-significant variables in this LME model were the age of the female ($t=0.88$) and the sex of a female's previous calf ($t=0.77$). We selected the reduced model based on AIC and BIC comparison (Table 1). Because the relationship between 5α -pregnane-3-ol-20-one concentrations and gestation period did not appear linear (Fig. 1), we used Generalized Additive Models (GAM) to explore the significance of nonlinear relationships between the response variable and independent variables for pregnant females. GAM results found a significant nonlinear relationship between 5α -pregnane-3-ol-20-one concentrations and day of gestation ($df=3$, $F=19.668$, $p<0.001$). Therefore, in subsequent LME analysis we used a quadratic function for gestation. Significant explanatory variables in the LME model of 5α -pregnane-3-ol-20-one concentrations in pregnant females (Table 1) were NDVI during sample collection ($t=6.82$; Fig. 4), age ($t=-4.06$), day of gestation ($t=2.66$), and the square of day of gestation ($t=-4.77$) which in combination represents the identified quadratic relationship between fecal progestins and gestation period (Fig. 1). Fetal sex was not significant ($t=0.35$) and eliminated from the full model. This resulted in a slightly higher AIC for the reduced model as compared to the full model (indicating a worse fit), though the more conservative model selection criteria BIC indicates that

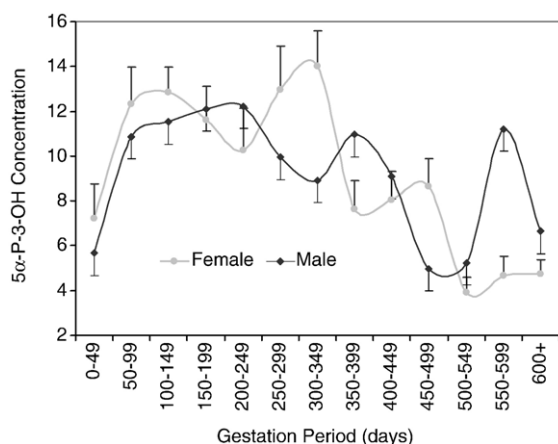


Fig. 1. Grouped fecal 5α -pregnane-3-ol-20-one (5α -P-3-OH) concentrations from 34 pregnant females across their 22-month gestation period. The lines represent the average concentration per 50-day increment with standard error bars and are divided in relation to the sex of the calf (male—black diamonds and female—gray squares). Concentrations are not corrected for impacts of individual female characteristics or ecological conditions at the time of sampling.

the reduced model is not different from the full model (Table 1). Further comparison of 5α -pregnane-3-ol-20-one concentrations from females carrying male and female calves indicates that sex-related differences in average hormone levels occur during mid and late pregnancy (Fig. 1). Therefore, the impact of calf sex appears to be salient periodically during gestation, but not a major factor accounting for differences in hormone concentrations. Because primiparous females were excluded from the analysis when variables based on an individual's previous calf were included in models, we also ran analyses on all samples from pregnant and non-pregnant females including only variables un-related to previous calves, thereby including all samples from all individuals in the analysis. Significant variables were the same for both pregnant and non-pregnant females in these models.

The median 5α -pregnane-3-ol-20-one concentration in samples collected per female when pregnant (median=7.63, IQR 6.41–12.61) was significantly greater than the median concentrations of samples collected when females were not pregnant (median=1.96, IQR=1.39–2.23; Wilcoxon Sign-Rank Test $Z=216.5$, $n=29$, $p<0.001$). Additionally, 5α -pregnane-3-ol-20-one concentrations in samples collected from individual females during wet seasons (median=7.84, IQR=5.90–10.09) were significantly greater than concentrations in their samples collected during dry seasons (median=2.80, IQR=1.73–5.22; Wilcoxon Sign-Rank Test $Z=261.5$, $n=34$, $p<0.001$). Seasonal differences were significant both between paired median 5α -pregnane-3-ol-20-one concentrations from samples collected from non-pregnant females (dry: median=1.37, IQR=1.01–1.75; wet: median=3.65, IQR=3.09–4.90; Wilcoxon Sign-Rank Test $Z=104.5$, $n=21$, $p<0.001$) as well as those from paired pregnant females (dry: median=7.65, IQR=5.49–11.66; wet: median=9.43, IQR=8.15–12.24; Wilcoxon Sign-Rank Test $Z=132.5$, $n=30$, $p<0.002$; Fig. 4). Practically, samples grouped according to season, dry (Fig. 5a) or wet (Fig. 5b), and in relation to the reproductive state of the female indicate that a pregnancy test based on 5α -pregnane-3-ol-20-one concentrations would be most discerning for samples collected during the dry season. Only 5 of 146 samples (~3%) collected during the dry season from non-pregnant females had 5α -pregnane-3-ol-20-one concentrations greater than $3 \mu\text{g/g}$. In contrast, 58 of 92 (~63%) of non-pregnant samples collected during the wet season had concentrations greater than $3 \mu\text{g/g}$. 5α -Pregnane-3-ol-20-one concentration less than $1 \mu\text{g/g}$ was exclusively collected from non-pregnant females, regardless of season. As a minimum, therefore, $1 \mu\text{g/g}$ can serve as a threshold with which to distinguish the pregnancy status of females.

Discussion

This study presents the first time-specific analysis of factors impacting hormone excretion patterns in individual free-ranging adult African elephant females. As a consequence of their extended 22-month gestation period, elephants appear to optimize the timing of the onset of reproduction in order to maximize use of known and future probable resources to

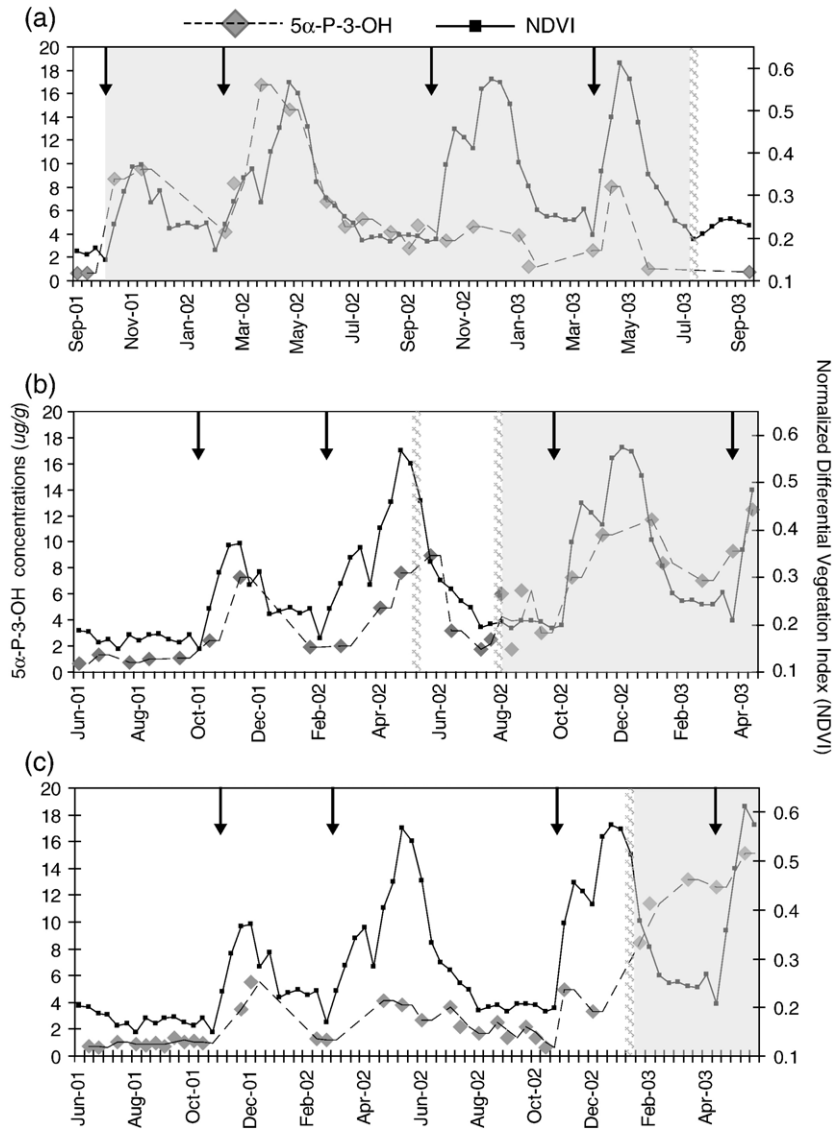


Fig. 2. Individual profiles of 5 α -pregnane-3-ol-20-one (5 α -P-3-OH) concentration extracted from dung samples for three females collected during the two-year study. The 10-day composite Normalized Differential Vegetation Index (NDVI) values (line) are shown over the same period. Ten-day periods containing an observed estrus event are highlighted with a checkered pattern. Ten-day periods containing a known birth date are highlighted with a striped pattern. Pregnant periods are highlighted in gray. Arrows indicate the onset of wet seasons. Profile (a) containing 22 samples covers a complete pregnancy though estrus was not observed for this female (and therefore not indicated). Profile (b) containing 26 samples depicts two behavioral estrus events where only the second resulted in pregnancy. Profile (c) containing 33 samples shows the first estrus and pregnancy of a primiparous female.

coincide with energetic investment in reproduction (Wittemyer et al., 2007). The results from this study demonstrate that progesterone levels in both pregnant and non-pregnant females are closely correlated with the quality of available food resources and impacted by the individual characteristics of age and inter-pregnancy interval. These results advance our understanding of the ecological influence on endocrine regulation of the timing of reproduction and offer insight into the possible relationship between endocrine activity and prenatal mortality.

Seasonal conception probabilities in the Samburu elephant population were found to be strongly correlated with seasonal maximum NDVI, where conception probabilities in seasons with high primary productivity (as indicated by a high NDVI maximum value) were significantly greater than those during

seasons with low primary productivity (Wittemyer et al., 2007). Our results here provide endocrine evidence of the ecological control on elephant reproductive rates. At a course level, 5 α -pregnane-3-ol-20-one concentrations in fecal samples collected during the dry season were significantly lower than wet season samples and impacted by the reproductive state of the female (Fig. 4) corroborating results from the Tarangire elephant population in Tanzania (Foley et al., 2001). To investigate time-specific factors influencing progesterone concentrations in fecal samples, fine scale analysis of the statistical relationship between 5 α -pregnane-3-ol-20-one concentrations of non-pregnant females and multiple independent variables was conducted using linear mixed-effect (LME) models with female identity incorporated as a random variable. LME results demonstrate a

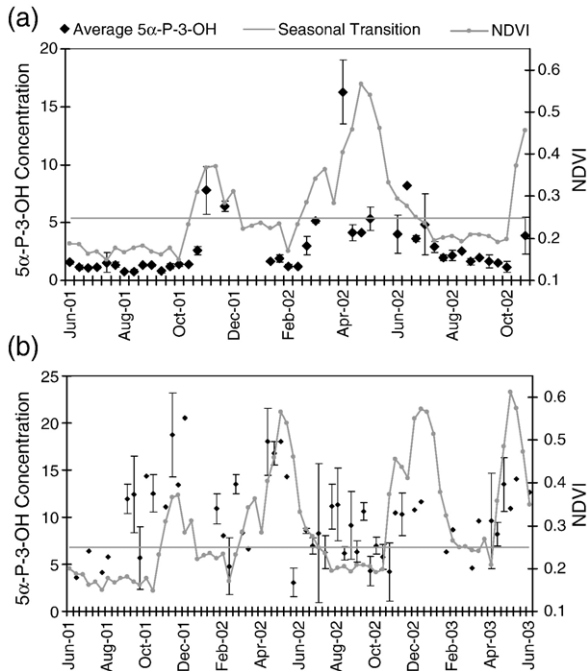


Fig. 3. Fecal 5 α -pregnane-3-ol-20-one (5 α -P-3-OH) concentrations (black diamonds) from (a) non-pregnant and (b) pregnant females plotted in relation to average ecosystem Normalized Differential Vegetation Index (NDVI) values (gray circles) during the study period. The mean and standard error (bars) of 5 α -pregnane-3-ol-20-one concentrations (calculated from different females) are collated within 10-day periods coinciding with NDVI composite values. The transition between wet and dry season is indicated by the dotted line.

positive, significant correlation between 5 α -pregnane-3-ol-20-one levels and NDVI values (a surrogate for primary productivity), indicating that hormonal activity in non-pregnant elephants is, in part, driven by ecological conditions. Results also demonstrate that the time since last parturition is significantly, positively correlated with 5 α -pregnane-3-ol-20-one levels. Thus, elephants appear to need a post-partum recovery period, the duration of which is mediated by ecological conditions, before they are physiologically capable of ovulation. In other words, it appears that the physiological condition of an elephant drives progestin metabolite levels which, in turn, must reach a threshold level to enable ovulation. The onset of the rainy season (Figs. 2 and 3a), when primary productivity and the quality of food sharply increase, appears to serve as the energetic stimulus driving increased progestin levels and the onset of reproductive activity. These results offer insight into the physiological basis for condition dependent estrus and its influence on general population demographic fluctuations in relation to climatic variability (Wittemyer et al., 2007). Seasonal breeders in a variety of other taxa demonstrate similar ecological control of endocrine activity and subsequent ovulation, including langurs (Ziegler et al., 2000), gilts (Virolainen et al., 2004), and mole-rats (Herbst et al., 2004).

Among non-pregnant females, 5 α -pregnane-3-ol-20-one concentrations were not significantly related to either the age of the individual or sex of the individual's previous calf, despite earlier, demographic focused (non-endocrine) elephant studies

which found differentiation in reproductive output across age groups (Moss, 2001). During pregnancy, however, LME model results demonstrate a negative significant correlation between the age of an individual and 5 α -pregnane-3-ol-20-one concentrations, with older females demonstrating lower 5 α -pregnane-3-ol-20-one concentrations than younger females. Visual inspection of this relationship indicates that concentrations decreased linearly with age. This correlation may be indicative of the hormonal factors driving age-related reproductive senescence or menopause, a phenomena found in elephants, humans, and a few other mammals species (Packer et al., 1998).

While demographic analysis in the study population using 265 births indicated that parturition is timed to occur around the likely period of increased primary productivity (Wittemyer et al., 2007), less than half of the females in this study gave birth

Table 1
Results from LME models of the relationship between the dependent variable fecal 5 α -pregnane-3-ol-20-one concentrations and listed independent variables

	Value	SE	df	t-value	p-value	AIC	BIC
<i>Non-pregnant females</i>							
Full model						865.38	896.94
Intercept	-2.803	1.286	133	-2.179	0.031		
NDVI	0.003	2.07	133	5.868	<0.001		
Age	12.146	0.023	133	0.145	0.885		
Time since last birth	0.004	0.001	133	3.578	<0.001		
Previous calf sex	0.175	0.594	25	0.294	0.771		
Reduced model						856.27	881.80
Intercept	-2.624	0.963	134	-2.726	0.007		
NDVI	12.092	2.033	134	5.947	<0.001		
Time since last birth	0.004	0.001	134	3.678	<0.001		
<i>Pregnant females</i>							
Full model						1926.3 ^a	1963.16
Intercept	12.176	1.73	296	7.04	<0.001		
NDVI	16.156	2.315	296	6.979	<0.001		
Age	-0.119	0.027	296	-4.36	<0.001		
Calf sex	-1.42	0.669	296	-2.123	0.346		
Gestation date ^b	0.0169	0.007	296	2.52	0.012		
(Gestation date) ^{2, b}	-5E-05	0.00001	296	-4.771	<0.001		
Reduced model						1929.8 ^a	1963.01
Intercept	11.137	1.67	297	6.668	<0.001		
NDVI	15.842	2.324	297	6.816	<0.001		
Age	-0.111	0.027	297	-4.062	<0.001		
Gestation date ^b	0.0179	0.007	297	2.659	0.008		
(Gestation date) ^{2, b}	-5E-05	0.00001	297	-4.768	<0.001		

^a AIC of the full and best fit model of pregnant females differs in part as a function of sample size differences between the two models. Differences in sample size relate to the number of samples from primiparous females included in analyses. Primiparous females are not included in models using the variable calf sex.

^b Gestation date is included in GLM as a quadratic function (Gestation date+(Gestation date)²) in relation to GAM results of the relationship between fecal progestin concentration and gestation day.

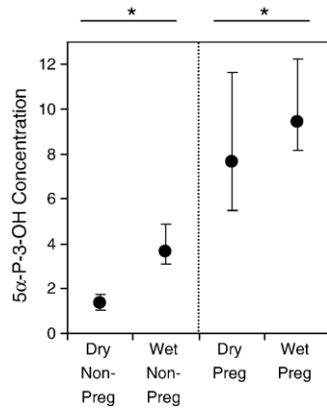


Fig. 4. The median fecal 5α -pregnane-3-ol-20-one (5α -P-3-OH) concentrations (black circles) and inter-quartile ranges (error bars) of all females are presented in relation to reproductive state and season. The asterisks represent statistically significant differences between categorized individually paired median values.

during the study period. As a result, little information about the relationship between endocrine activity and the timing of parturition could be gleaned. Our study, however, did offer novel insight into the influence of ecological stochasticity on endocrine functioning during pregnancy. In addition to variation in 5α -pregnane-3-ol-20-one concentrations related to gestation period (Foley et al., 2001; Hodges, 1998; Hodges et al., 1997), which in the case of our model was incorporated as the quadratic function gestation day + the square of gestation day identified using a GAM, progesterin concentrations in dung samples of pregnant elephants were significantly higher during periods of increased primary productivity (Figs. 3b and 4). Results from LME models demonstrate that 5α -pregnane-3-ol-20-one concentrations were significantly, positively correlated with NDVI while controlling for the effects of gestation period (Table 1). Thus, hormone levels decreased in the dry season, though never below the $1 \mu\text{g/g}$ level associated with non-pregnant flatlining (Fig. 5). To our knowledge, few studies have shown a relationship between progesterin concentrations during pregnancy and ecological variability. Since all pregnant females successfully carried their calves to term in this study, we did not record progesterin levels in relation to prenatal mortality. Our results suggest that periods of ecological constraints drive down 5α -pregnane-3-ol-20-one levels, and a threshold hormonal level may exist below which a pregnancy can no longer be maintained, resulting in prenatal mortality. Starvation-related decline in progestagens has recorded in elk, leading to subsequent abortion of calves though only under extremely harsh conditions (Cook et al., 2002).

In contrast to a previous study using 8 African elephants (Meyer et al., 2004), our results indicate sample progesterin concentrations differed in the latter half of gestation in relation to fetus sex suggesting that pre-partum sexing of fetuses in elephants is possible. Calf sex has been shown to impact progesterin concentration in Asian elephants (Meyer et al., 2004) and lemurs (Shideler et al., 1983), though sex does not appear to impact levels in all primates (Jurke et al., 1997).

Information regarding general reproductive biology of African elephants is important for *in situ* management. Some

increasing elephant populations in southern and eastern Africa are reaching densities that could potentially negatively impact biodiversity (Baxter and Getz, 2005; Buechner and Dawkins, 1961; Dublin et al., 1990). Understanding the factors that stimulate reproduction can serve to increase the efficacy of invasive management actions planned for such populations, such as culling or the administration of birth control, by optimizing the timing of such actions to impose the greatest impact on population growth. The efficacy of harvesting regimes deployed to maximize yield or reduce population densities can be increased if synchronized with salient aspects of a species reproductive biology. Additionally, our finding of seasonal hormonal flatlining in wild elephants offers insight for captive managers on the possible use of dietary stimuli to instigate endocrine activity.

While it is clear from captive studies that the reproductive state of elephants can be readily determined by sequential sampling of 5α -pregnane-3-ol-20-one concentration measured from fecal samples (Brown, 2000), logistics in most wild setting only enable single sample testing. We compiled our data on pregnant and non-pregnant females to assess the utility of a single sample pregnancy test. Results demonstrate that 5α -pregnane-3-ol-20-one concentration less than $1 \mu\text{g/g}$ were exclusively collected from non-pregnant females, however, no other categories could be exclusively related to a single reproductive state. Separating samples collected during the dry season from those collected during the wet season reduced the overlap between concentrations attributed to pregnant and non-pregnant females. During the dry season, 87% of samples

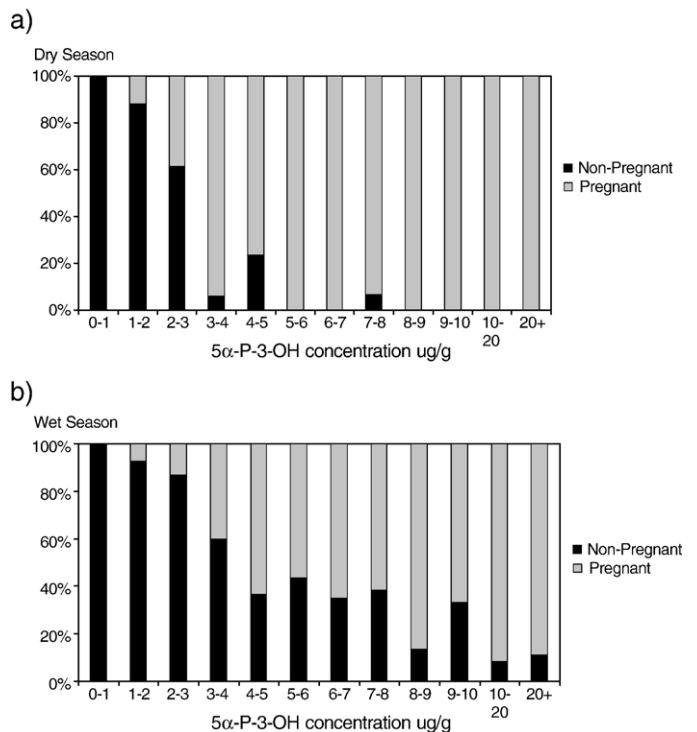


Fig. 5. Vertical bar plot depicting the number of samples collected during the (a) dry or (b) wet season from pregnant (gray) or non-pregnant (black) individuals for each 5α -pregnane-3-ol-20-one (5α -P-3-OH) concentration category.

collected with 5α -pregnane-3-ol-20-one concentrations less than $3 \mu\text{g/g}$ were from non-pregnant females and all samples with concentrations greater than $5 \mu\text{g/g}$ were attributed to pregnant females (Fig. 5). Wet season results overlapped across progesterone concentration classes limiting the ability to distinguish between reproductive states hormonally. Because of the many factors impacting progesterone concentration in elephants, high confidence in a pregnancy test based on single samples of 5α -pregnane-3-ol-20-one is unlikely. If implemented, such a test will be most effective if conducted during the dry season. These results offer novel insight into the factors impacting endocrine functioning and reproductive activity among free-ranging female elephants, providing information important to the development of new methods and directions for improving elephant population management and control.

Acknowledgments

We thank the Office of the President of the Republic of Kenya, the Kenya Wildlife Service, and the Samburu and Buffalo Springs County Councils for permission to conduct this research. Daniel Lentipo and the rest of the Save the Elephants team assisted in the collection of field data for this study. Dr. A. K. Oerke, A. Heistermann, and J. Hagedorn provided expert help in the laboratory techniques. The National Science Foundation IRFP OISE-0502340 (GW), Evangelisches Studienwerk e.V. Villigist (AG), and the German Primate Center provided funding for this work.

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