Hormones and Behavior 125 (2020) 104804

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Ovarian cyclicity and prolactin status of African elephants (*Loxodonta africana*) in North American zoos may be influenced by life experience and individual temperament

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ARTICLE INFO

Keywords:
Temperament
Prolactin
Life experience
Cyclicity
Zoo
Elephant

ABSTRACT

Hyperprolactinemia is an endocrine disorder associated with infertility in many species, including elephants. In a recent survey of zoos accredited by the Association of Zoos and Aquariums (AZA), over half of African elephant females (N = 101) were not cycling normally, 30% of which exhibited hyperprolactinemia. We examined whether life experience and temperament predict ovarian cyclicity and circulating prolactin status in individual African elephant females. We hypothesized that, similar to humans, acyclicity and hyperprolactinemia in elephants will be associated with an apprehensive or fearful, anxious temperament, and an increased number of potentially challenging life events (transfers, deaths and births). Ninety-five adult African elephant females housed at 37 AZA institutions were included in this study. Blood samples were collected twice a month for 1 year to determine ovarian cycle (cycling, n = 44; irregular, n = 13; non-cycling, n = 38) and prolactin (normal, n = 44; low; n = 23; high; n = 28) status. Keeper ratings on a 6-point scale were obtained on 32 temperament traits in 85 of these elephants. We determined that giving birth and being exposed to herd mates entering the facility were positively associated with normal ovarian cycle and prolactin profiles. By contrast, age, serum cortisol, and an increased number of herd mates leaving a facility were negatively associated with both. Contrary to our hypothesis, hyperprolactinemia was associated with a popular and caring temperament rating, whereas consistently low prolactin was associated with a fearful, apprehensive temperament. These findings indicate that pituitary-ovarian function may be impacted by life history (cyclicity) and temperament (prolactin), which should be taken into consideration when making management decisions.

1. Introduction

Decades of research on the biology of elephants have identified several distinct aspects of reproduction compared to other mammals, such as having the longest spontaneous estrous cycle (13–17 weeks in duration) and gestational (20–22 months) durations (Brown, 2014, 2019). Compared to other mammals, the major circulating luteal steroid in elephants is not progesterone, but various 5α-reduced pregnanes (e.g., 5α-pregnane-3,20-dione, 5α-pregnane-3-ol-20 one, 17α-hydroxyprogesterone) (Heistermann et al., 1997; Hodges, 1998: Hodges et al., 1997; Schwarzenberger et al., 1997; Short and Buss, 1965; Wierer et al., 2012), which we term “progestagens”. Discovery of a unique double luteinizing hormone surge, which can predict time of ovulation (Brown et al., 1999; Kapustin et al., 1996), and development of an artificial insemination technique (Brown et al., 2004b) were promising advances in the breeding management of both Asian and African elephants (Brown, 2014). It is troubling then, that neither population in the US is currently self-sustaining (Hildebrandt et al., 2006; Olson, 2011). For Asian elephants, a recent report by the Association of Zoos and Aquariums (AZA) found that low reproduction is primarily related to advancing age; 57% are > 40 years old and considered post-reproductive (Population Analysis and Breeding and Transfer Plan, 2014), whereas for African elephants both advancing age and reproductive problems like ovarian acyclicity are major concerns (Brown et al., 2016; Brown, 2014, 2019). Through intensified breeding efforts, zoo African elephant births have increased over the past 10 years, but it

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https://doi.org/10.1016/j.yhbeh.2020.104804
Received 15 January 2020; Received in revised form 21 April 2020; Accepted 5 June 2020
Available online 10 July 2020
0018-506X/

has not been enough to offset deaths, and growth in the population is still maintained primarily through imports. Demographic modeling of the population emphasized that reproduction, even more than imports, is key to sustainability (Faust and Marti, 2011; Population Analysis and Breeding and Transfer Plan, 2017).

A major challenge in meeting population goals is that only ~25% of African elephant females in U.S. zoos are considered suitable breeders because of reproductive problems and advancing age (Population Analysis and Breeding and Transfer Plan, 2017). In the most recent AZA reproductive survey, 53% of African females were not cycling normally (Brown et al., 2016). Furthermore, 55% of those were hyperprolactinemic, with 45% exhibiting consistently low prolactin (Brown et al., 2016). Hyperprolactinemia, or excess production of prolactin by the anterior pituitary, is a significant health disorder in humans and other mammals and associated with ovarian acyclicity and hypogonadism (Faje and Nachtigall, 2013; Serri et al., 2003; Zacur, 1999). It was first described in elephants in 1997 (Brown and Lehnhardt) and later confirmed in subsequent studies (Brown et al., 2004a, 2004b; Dow and Brown, 2012; Prado-Oviedo et al., 2013). Several physiological factors known to be related to hyperprolactinemia-induced infertility in women (Serri et al., 2003), such as elevated cortisol concentrations (Brown et al., 2004a), hyperandrogenism (Mouttham et al., 2011), hyperestrogenism (Prado-Oviedo et al., 2013) and disruptions in thyroid homeostasis (Brown et al., 2004a), do not appear to be definitive causes in African elephants. Thus, a key component to ensuring population sustainability of zoo African elephants is understanding the underlying etiology of fertility issues related to pituitary-gonadal dysfunction.

Studies in women have shown that life experience can play a role in the etiology of prolactin perturbations (Assies et al., 1992; Sobrinho et al., 1984; Sobrinho, 2003; Sonino et al., 2004). External stressors, such as social conflict, a new job, death of a loved one, divorce, and academic and work place pressures increase the secretion of prolactin in predisposed individuals (Assies et al., 1992; Sobrinho et al., 1984; Sobrinho, 2003; Sonino et al., 2004). Compared to controls, patients with hyperprolactinemia experienced a significantly higher number of stressful events in the months and years leading up to disease manifestation (Sonino et al., 2004). Studies also found that hyperprolactinemic women exhibit signs of anxiety (Sobrinho et al., 1984; Sobrinho, 2003; Sonino et al., 2004), including depression, hostility, and phobias (Yavuz et al., 2003). Thus, it is possible that zoo elephants may be similarly susceptible to challenging life events that manifest as hyperprolactinemia.

Based on a large study in North America, elephants had varied life histories that could influence how they respond to challenges in the zoo environment (Prado-Oviedo et al., 2016). Females experienced an average of 2.80 transfers in their lifetime, ranging from 0 to 10 transfer events, and most were over 30 years of age and had not yet reproduced. Zoo African females generally reach puberty at around 8–12 years of age (Brown, 2014; Brown et al., 2016); however, the average age at first conception was 21 years, and few have had more than one calf (Prado-Oviedo et al., 2016). This contrasts with reproduction in the wild, where although females attain puberty at about the same time, reproduction occurs shortly thereafter (at ~14 years), and most reproduce regularly (5–7 years) throughout their lifetime (Lee et al., 2016; Laws, 1967). Additionally, in contrast to wild females, most zoo elephants have limited exposure to birth events or infants, which could result in limited behavioral repertoires for successful calf rearing (Lee, 1987; Schulte, 2000). Finally, females experienced an average of 2.42 herd mate deaths in their lifetime, ranging from 0 to 15, that could have disrupted group cohesion.

The physiological stress response is complex, but primarily involves activation of the hypothalamic-pituitary-adrenal axis. It can also include the release of prolactin, which induces adrenal hypertrophy and increases the adrenal cortex's sensitivity to ACTH, resulting in higher glucocorticoid release (Levine and Muneyyirci-Delale, 2018). It may also disrupt the dopamine negative feedback loop that regulates prolactin production by directly suppressing dopamine secretion, or by upregulating other releasing factors, such as serotonin, that compete with dopamine's inhibitory action (Calogero et al., 1998; Gambarana et al., 1999; Wuttke et al., 1987; Levine and Muneyyirci-Delale, 2018). Some studies suggest that stress induced prolactin secretion is a mechanism to maintain homeostasis within the immune system. Glucocorticoids are also released during a stress response and act as suppressive immunemodulators (Levine and Muneyyirci-Delale, 2018). However, while prolactin may induce a short-term protective proinflammatory state, chronic exposure can lead to pituitary dysregulation (Levine and Muneyyirci-Delale, 2018).

The degree to which transfer, birth and death events negatively impact individual elephants likely is influenced by social factors (e.g., group dynamics, relatedness, how long individuals have been together). Coping with stressful events is aided by the presence of supportive conspecifics or can be compromised by poor social situations (Baranyi et al., 2005; Christensen et al., 2011; Bartolomucci et al., 2001; Ljungberg and Westlund, 2000). Recently, Brown et al. (2016) found that female African elephants that spent time in a greater number of different social groups had increased odds of being hyperprolactinemic, and that social isolation, either with or without the ability to interact with herd mates through a barrier, was associated with an increased risk of ovarian acyclicity. These findings suggest that for female African elephants, not being in a stable social group may be a stressor that negatively effects ovarian function and elicits a heightened prolactin response similar to that observed in human females (Sobrinho et al., 1984; Sobrinho, 2003; Sonino et al., 2004).

Individual differences in temperament - consistent individual differences in behavior that are biologically based and independent of learning - can influence coping styles within a changing environment (Réale et al., 2007). Temperament is the filter that shapes perception of the social and physical environment (Henry, 1986; von Holst, 1998), and is associated with negative and positive emotional states (Boissy and Blache, 2009). Thus, temperament likely plays a role in how zoo elephants adapt to varied husbandry and environmental factors, most of which are not under their control (e.g., habitat dimensions, herdmates, proximity to other species, lighting, exposure to sounds, entrance/exit of conspecifics) (Morgan and Tromborg, 2007). It follows then, that individual temperament could have a major impact on how social groups function (Tetley and O'Hara, 2012). Together, differences in life history events and individual temperaments could have major impacts on the functioning of the hypothalamo-pituitary-adrenal and ovarian axes, and potentially play a role in reproductive success of zoo elephants, as well as their resilience to stress (Kurosi et al., 2012; Pechtel and Pizzagalli, 2011; Suderman et al., 2012; Tetley and O'Hara, 2012).

The objective of this study was to determine if differences in individual female African elephant life histories, temperament, and serum cortisol, as a proxy of stress, impact ovarian cyclicity and circulating prolactin concentrations. We hypothesized that reproductive acyclicity and hyperprolactinemia in African elephants will be associated with a more apprehensive or fearful-anxious temperament, an increased number of potentially challenging life events (transfers, births and deaths), and thus higher concentrations of serum cortisol. Understanding relationships between life events and elephant temperament, and how they are influenced by extrinsic forces, is important information needed to optimize management of elephants in zoo settings.

2. Materials and methods

2.1. Ethics statement

All data included in this study were from elephants enrolled in the Using Science to Understand Zoo Elephant Welfare study (Carlstead et al., 2013). This study was authorized by the management at each

Table 1
Summary of population demographics including number of elephants (N) and age, prolactin and cortisol distribution (mean, SEM, minimum and maximum) by cyclicity and prolactin status.

Cyclicity status	Prolactin status	N	Age (years)				Prolactin (ng/ml)				Cortisol (ng/ml)			
			Mean	SEM	Min	Max	Mean	SEM	Min	Max	Mean	SEM	Min	Max
Cycling	Normal	44	33.35	0.94	22.92	52.92	12.22	1.42	4.45	64.53	16.01	0.74	7.24	30.56
Irregular	High	5	32.52	3.17	22.92	41.25	40.59	15.17	15.77	98.29	22.69	3.74	13.68	31.24
	Low	8	33.43	2.28	24.92	42.24	9.57	1.20	5.13	14.36	18.25	1.35	12.87	23.50
	Total	13	33.08	1.78	22.92	42.24	21.50	7.00	5.13	98.29	19.96	1.68	12.87	31.24
Non-cycling	High	23	36.58	1.16	27.92	47.92	34.57	4.65	15.27	105.24	18.94	1.38	9.22	37.26
	Low	15	34.77	1.20	28.92	43.09	8.44	0.85	2.44	12.97	19.91	1.29	10.56	32.82
	Total	38	35.86	0.85	27.92	47.92	24.26	3.51	2.44	105.24	19.67	1.10	9.22	37.26
All Elephants	High	28	35.85	1.12	22.92	47.92	35.65	4.55	15.27	105.24	19.61	1.32	9.22	37.26
	Low	23	34.30	1.09	24.92	43.09	8.84	0.69	2.44	14.36	19.91	1.29	10.56	32.82
	Normal	44	33.35	0.94	22.92	52.92	12.22	1.42	4.45	64.53	16.01	0.74	7.24	30.56
	Total	95	34.32	0.61	22.92	52.92	18.30	1.89	2.44	105.24	18.02	0.63	7.24	37.26

participating zoo and, where applicable, was reviewed and approved by zoo research committees. In addition, approval was obtained from the Smithsonian National Zoological Park's ACUC (#11–10).

2.2. Animals and blood sample collection

Female elephants (n = 95 individuals at 37 institutions) were from the study of Prado-Oviedo et al. (2016) and averaged 34.32 ± 0.61 years of age (range, 22–53 years); none were pregnant or lactating at the time of the study. Blood samples were collected from an ear or leg vein every other week for 1 year without anesthesia (26 serum samples per elephant). Protocols requested that blood draws occur before 12 noon. Blood was allowed to clot at room temperature, centrifuged (~1500 ×g) and the serum stored frozen at −20 °C or colder until analysis. An overview of the study population is provided in Table 1.

2.3. Hormonal analyses

We utilized a solid-phase I^{125} radioimmunoassay (RIA) (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with an antibody that crossreacts with several progestogen metabolites, and has been shown to be effective in characterizing temporal patterns in elephants (Brown et al., 1991, 2000; Brown and Lehnhardt, 1995; Brown et al., 2004a, 2004b). Antibody cross-reactivity is 100% with progesterone, 43.8% with 5α-pregnane-3β-ol-20-one, 31.1% with 5β-pregnane-3α-ol-20-one, 23.3% with 5β-pregnane-3β-ol-20-one, 16% with 5α-pregnane-3α-ol-20-one, 3.5% with5β-pregnane-3β,20α-diol, 2% with 20α-dihydroprogesterone, 1.3% with 5β-pregnane-3,20-dione, and < l% with 5β-pregnane-3α,l7α-diol, 5α-pregnane-3α,20α-diol, 5α-pregnane-3β,20a-diol, l7α-hydroxyprogesterone and 5β-pregnane-3α17, 20α-triol (kit literature). Assay sensitivity was 0.05 ng/ml, and the intra- and interassay coefficients of variation were < 10%.

Serum cortisol was measured using a solid-phase I^{125} radioimmunoassays (RIA) (Siemens Medical Solutions Diagnostics, Los Angeles, CA) validated for elephants (Brown and Lehnhardt, 1995). Assay sensitivity was 2.5 ng/ml, and the intra- and interassay coefficients of variation were < 10%. Serum prolactin was analyzed by a validated heterologous RIA that utilized an anti-human prolactin antisera (NIDDK-anti-hPRL-3) and ovine prolactin label and standards (NIDDK-oPRL-I-2) (Brown and Lehnhardt, 1995; Brown and Lehnhardt, 1997). Assay sensitivity was 5.0 ng/ml, and the intra- and inter-assay coefficients of variation for all assays were < 10% and < 15%, respectively.

2.4. Determination of reproductive cyclicity and prolactin status

Estrous cycle characteristics were calculated as described by Glaeser et al. (2012). Data were used to categorize the ovarian cycle status of each elephant as normal cycling (regular 12- to 18-week progestogen cycles), irregular cycling (cycle durations outside ± 2 ∗ SD of the population mean (Glaeser et al., 2012) or acyclic (baseline progestogens, < 0.1 ng/ml, throughout) (Brown et al., 2004a, 2004b). Representative profiles for cycling, irregular cycling and acyclic female elephants are shown in Fig. 1.

Females determined to have an irregular (n = 13) or acyclic (n = 38) ovarian cycle pattern were further categorized into two abnormal prolactin groups based on temporal patterns (Fig. 2) and average prolactin concentrations for the 1-year collection period (Prado-Oviedo et al., 2013; Brown et al., 2016). The cut-off value of abnormal prolactin was calculated using ROC curve analyses and the concordance statistic (Hosmer et al., 2013; Gönen, 2007) and determined to be 15 ng/ml (AUC = 0.959, 95% CI 0.919 to 0.999). Irregular cycling and non-cycling elephants with an average prolactin concentration of 15 ng/ml or greater and no temporal pattern were considered HIGH (n = 28), non-cycling elephants with average prolactin concentrations below 15 ng/ml and no temporal pattern were considered LOW (n = 23), and elephants with normal temporal patterns of both progestagen (i.e., cycling) and prolactin secretion were considered NORMAL (n = 44) (Fig. 2).

2.5. Life experience variables

Social life event histories were extracted from the North American Studbook African elephant studbook (Olson, 2012) and organized chronologically for all 95 elephants (Table 2). All events that occurred after an elephant entered a US facility (via importation or birth) were counted and incorporated into the analyses. Events that occurred prior to importation were unknown and could not be included in these analyses. In addition, because some elephant herds are managed as subgroups and not all interact physically with each other, we could not confirm that elephants were physically present for all events involving births, deaths or transfers of herd-mates, except in cases of females who gave birth to offspring. Therefore, individual accounts of zoo-level events may or may not have directly impacted the focal elephant. Life history-related variables are similar to those described in Prado-Oviedo et al. (2016) and described below.

2.5.1. Transfers

Transfer events (TR) were the number of physical location changes the focal animal experienced once it was in captivity. This event excluded wild capture but included transfer to the first facility in which an elephant was housed after capture (i.e., importation). Transfers in ownership that did not involve a change in physical location were confirmed with the studbook managers and not counted.

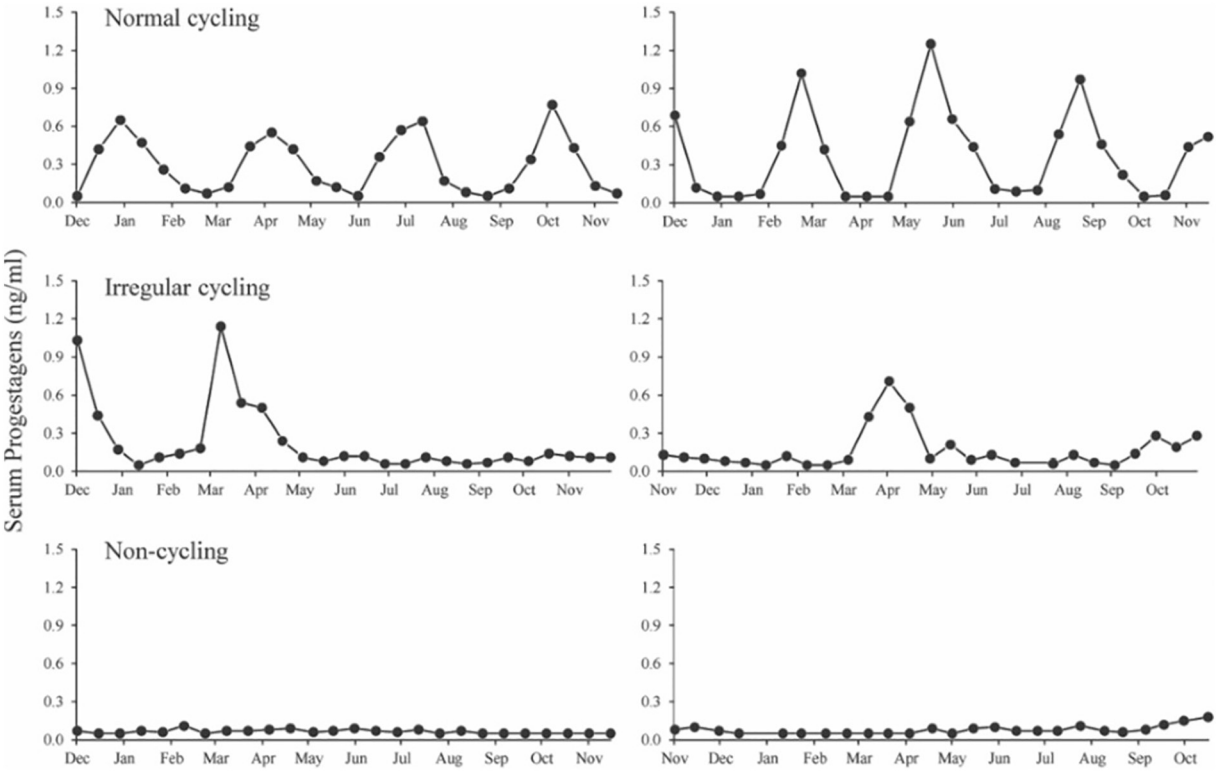

Fig. 1. Representative serum progestagen profiles for normal cycling (upper panels), irregular cycling (middle panels) and acyclic (lower panels) female African elephants.

2.5.2. Transfer-In exposures

Transfer-In exposures ($ExpTR_{in}$) were the number of non-focal elephants (i.e. herd mates) transferred into the same facility as the focal animal. For an $ExpTR_{in}$ event to be included as a variable, the non-focal and focal elephant had to overlap at the same facility for a minimum of 1 day. If a focal and non-focal elephant from different facilities were transferred to a new facility on the same day it was counted as an $ExpTR_{in}$ event for the focal animal. However, if elephants were transferred in together from the same facility, it was not counted.

2.5.3. Transfer-Out exposures

Transfer-Out exposures ($ExpTR_{out}$) were the number of non-focal elephants that were transferred out of the same facility as the focal animal. For an $ExpTR_{out}$ event to be included as a variable, the non-focal and focal elephant had to overlap at the same facility for a minimum of 1 day. Events where non-focal and focal elephants transferred out of the same facility together to a new facility were not included in this variable.

2.5.4. Offspring births

Offspring birth events (OffB) were the number of recorded births from the time those females entered the studbook system to the end of the study period. All offspring births were counted regardless of how long the offspring lived.

2.5.5. Birth exposures

Birth exposures (ExpB) were the number of recorded births that occurred at the same facility as the focal animal from the time of birth or importation. The ExpB events were included regardless of how long the calf survived.

2.5.6. Death exposures

Death exposures (ExpD) were the number of recorded deaths that occurred at the same facility as the focal animal from the time of birth or importation, including death of offspring. All deaths were counted regardless of how long the animal lived.

2.6. Individual elephant temperament

Full-time keepers who had worked with an elephant for at least 1 year and at least 2 days per week throughout the year rated temperament using 32 trait adjectives (Supplemental Data Table 1) based on previous studies in elephants (Lee and Moss, 2012; Horback et al., 2013; Grand et al., 2012). Keepers were asked to rate elephants independently on their overall impressions of each elephant's behavior patterns over time, not on a particular day or event. Temperament trait adjectives were rated for each elephant on a 6-point scale: 0 = trait is not present at all; 1 = trait is weakly represented; 2 = trait is present but falls below the average; 3 = individual falls just about halfway between the extremes or average for elephant behavior as you know it; 4 = trait is strong although not outstanding; 5 = trait is very strong and conspicuous, approaching the extreme. All elephants were rated by two or more keepers. For the final analysis, keeper ratings were averaged across each trait for each elephant.

3. Statistical analyses

The life event history variables (total counts) were strongly positively skewed for each event type due to the presence of "0" events (i.e., an event never occurred in the lifetime of the focal animal). Zero events were included in the subsequent analyses where appropriate because a lack of experience/exposure was considered biologically relevant (Prado-Oviedo et al., 2016).

Temperament scores were evaluated for interrater reliabilities by intra-class correlation coefficients (ICC) (Shrout and Fleiss, 1979). ICC values were interpreted as follows: 0–0.2 = poor agreement; 0.3–0.4 = fair agreement; 0.5–0.6 = moderate agreement and > 0.8 = almost perfect agreement (Cicchetti, 1994) (Supplemental Data

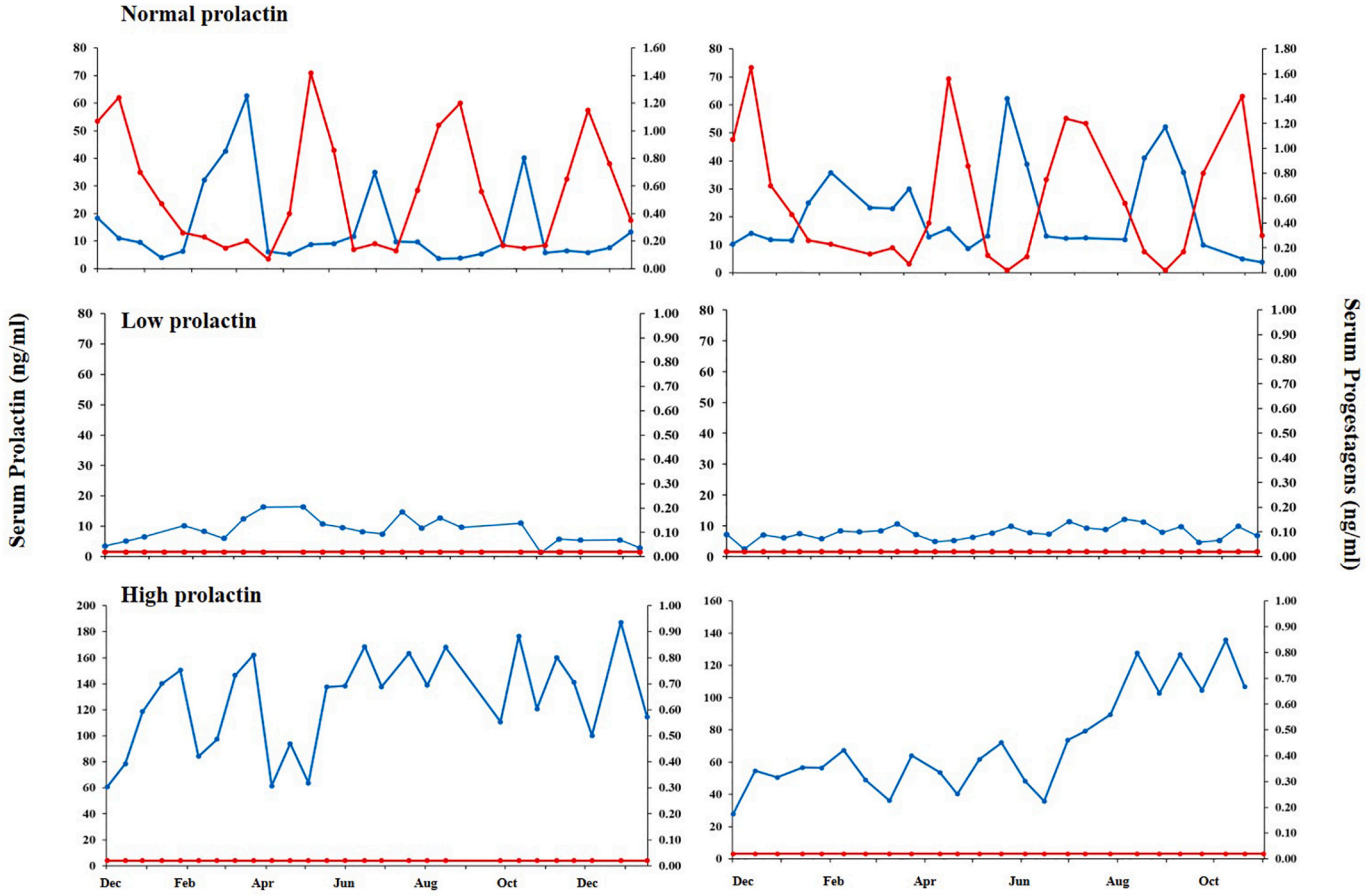

Fig. 2. Representative serum progestagen (red lines) and prolactin profiles (blue lines) of a normal prolactin (upper panels), non-cycling low prolactin (middle panels) and non-cycling high prolactin (lower panels) female African elephants. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1). We used a 0.5 "cut off" point, eliminating any personality trait averages that fell below this reliability estimate. Principal Component Analyses (PCA) (JMP vs. 10) followed by an orthogonal transformation was used to assess the temperament trait ratings. PCA allowed orthogonal transformation to convert potentially correlated variables (traits) into a set of values of linearly uncorrelated variables (traits). The factor scores (i.e. variables) generated by the PCA were used in subsequent analyses (Supplemental Data Table 2). Five main components resulted and an orthogonal Varimax rotation was applied to arrive at the final factors (Supplemental Data Table 3). Three main factors emerged explaining 79% of the observed variation: fearful-apprehensive (41%), effective-aggressive (26%) and popular-caring (12%). Only factors with Eigenvalues over 1 were used for the individual factor score calculation. The factors were labeled according to the two adjectives that showed the strongest positive loading for each factor.

Multinomial logistical regressions were used to determine the relationship between age, average serum cortisol, life history event variables (Model 1) and individual temperament (Model 2) scores (independent variables), with cyclicity and prolactin status (dependent variables). Age has been shown to be associated with higher prolactin concentrations (Brown et al., 2016; Prado et al., 2019a) and was therefore included in Model 1, but because temperament has been shown to be an innate individual quality, it was not included in Model

Table 3
Re-coding of trichotomous dependent variables into dichotomous variables for ROC curve analyses.

		ROC curve fit 1	ROC curve fit 2	ROC curve fit 3
Cyclicity Status	Cycling	1	0	0
	Irregular	0	1	0
	Non-cycling	0	0	1
Prolactin Status	Normal	1	0	0
	Low	0	1	0
	High	0	0	1

Table 2
Life history event abbreviations and definitions.

Event name	Abbreviation	Description
Transfers	TR	Recorded physical facility transfers.
Transfer-In exposures	$ExpTR_{in}$	Recorded entrance of non-focal elephants into same facility as focal animals.
Transfer-Out exposures	$ExpTR_{out}$	Recorded exit of non-focal elephants from the same facility as focal animals.
Offspring births	OffB	Recorded births of offspring to reproductively aged females.
Birth exposures	ExpB	Recorded non-offspring births at the same facility as focal animals.
Death exposures	ExpD	Recorded deaths at the same facility as focal animals.

2. Cortisol was significantly associated with cyclicity and prolactin status in a univariate analysis of the data (Supplement Table 4), and therefore was included in both models. Multinomial logistic regression models allow for the prediction of the trichotomous dependent variables (i.e., cycling, irregular, and non-cycling; normal, low, and high prolactin) from one or more independent variables. The overall test of relationships among the independent and dependent variables was based on the reduction in the likelihood values for a model that did not contain any independent variables versus a model that did. This difference in likelihood followed a chi-square distribution. The significance test for the final model chi-square was our statistical evidence of the presence of a relationship between the dependent variable and a combination of the independent variables. All independent variables were included in the initial model. Factors that did not improve the overall predictive power of the models (based on the Akaike's Information Criterion) of the reduced model were dropped sequentially. All statistics reported (*p*-values) are based on this final minimal model, and non-significant variables were re-entered individually into the minimal model to determine levels of non-significance. The minimal models were reported as estimated odds ratios (EOR) of life events where β was the estimated regression coefficient for the model (reference). The β coefficient estimate was the natural logarithm of the EOR of exposure to the event. EOR was the estimated likelihood, where a value < 1 indicated that an increase by 1 event experience/exposure or temperament score was associated with a decreased odds of predicting cyclicity or prolactin status, relative to the reference category. A value > 1 indicated that an increase by 1 event experience/exposure or temperament score was associated with increased odds of predicting cyclicity or prolactin status, relative to the reference category. The reference categories were cycling and normal prolactin elephants for each of the regression models.

To test the utility of the multinomial logistic regression models, Receiver Operating Characteristic (ROC) curve analyses were conducted (Bewick et al., 2004). Area under the curve (AUC) analysis was used to determine which of the models predicts cyclicity or prolactin status the best. The AUC of the ROC curve ranged from 0.5 to 1.0, with larger values indicative of better fit. The accuracy of AUC measures was classified as: 1.0 = perfect test; 0.9–0.99 = excellent test; 0.8–0.89 = good test; 0.7–0.79 = fair test; 0.51–0.69 = poor test; 0.5 = failed test (Hastie et al., 2009; Zhou et al., 2002). Trichotomous variables (cyclicity and prolactin status) were re-coded into dichotomous (1 or 0) variables for ROC curve fitting (Table 3). A ROC curve was separately fit for each dependent variable using the estimated cell probabilities for the response category generated from the minimal models. All values were reported as mean ± SEM and statistical significance was assumed at a $P < 0.05$. Statistical analysis, except PCA, were carried out on the software program SPSS version 25 (IBM Corp. Released, 2013). The PCA was performed using the software program SAS version 9.3 (SAS Institute Inc, 2011).

4. Results

4.1. Model 1 – age, cortisol, cyclicity, prolactin and life experience variables

Table 4 summarizes the life event data. Every year increase in age was associated with a 12% increase in the odds of being non-cycling as

Table 4
Summary of life events for the study population by cyclicity and prolactin status. Data include the number of elephants that were included in each variable (N), and the number of events including mean (# events/elephant), SEM, minimum and maximum.

	Full population				
	N	Mean	SEM	Min	Max
TR	95	3.17	0.14	0	7
$ExpTR_{in}$	95	12.20	1.23	0	53
$ExpTR_{out}$	95	7.24	0.92	0	48
OffB	95	0.37	0.07	0	3
ExpB	95	1.57	0.26	0	10
ExpD	95	2.53	0.28	0	15

	Cyclicity status														
	Cycling					Irregular					Non-cycling				
	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max
TR	44	3.55	0.23	1	7	13	3.08	0.46	2	7	38	2.76	0.17	0	5
$ExpTR_{in}$	44	15.52	2.01	1	50	13	5.05	0.85	1	11	38	10.79	1.82	0	53
$ExpTR_{out}$	44	7.48	1.32	0	48	13	3.46	1.02	0	12	38	8.26	1.66	0	37
OffB	44	0.45	0.11	0	3	13	0.77	0.28	0	3	38	0.13	0.08	0	2
ExpB	44	1.34	0.37	0	9	13	2.08	0.77	0	10	38	1.66	0.4	0	8
ExpD	44	2.77	0.44	0	15	13	1.85	0.85	0	6	38	2.47	0.43	0	10

	Prolactin status														
	Normal					Low					High				
	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max
TR	44	3.55	0.23	1	7	23	3.04	0.33	0	7	28	2.68	0.16	1	4
$ExpTR_{in}$	44	15.52	2.01	1	50	23	7.61	1.81	0	37	28	10.75	2.08	1	53
$ExpTR_{out}$	44	7.48	1.32	0	48	23	5.26	1.60	0	30	28	8.50	1.94	0	37
OffB	44	0.45	0.11	0	3	23	0.09	0.60	0	1	28	0.46	0.17	0	3
ExpB	44	1.34	0.37	0	9	23	1.61	0.51	0	8	28	1.89	0.50	0	10
ExpD	44	2.77	0.44	0	15	23	2.65	0.61	0	10	28	2.04	0.39	0	9

TR = transfer rate; $ExpTR_{in}$ = exposure to non-focal transfers in; $ExpTR_{out}$ = exposure to non-focal transfers out; OffB = offspring births; ExpB = exposure to births; ExpD = exposure to deaths.

Table 5
Summary of parameter estimates for estimated odds ratios (EOR) of cyclicity, prolactin status and life events, including 95% Wald confidence intervals for EOR.

		Age						Serum cortisol					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity status	Cycling	44	Reference					44	Reference				
	Irregular	13	0.11	1.11	0.96	1.29	0.15	13	0.17	1.18	1.03	1.35	0.01*
	Non-cycling	38	0.12	1.12	1.01	1.24	0.03*	38	0.14	1.15	1.04	1.28	0.01*
Prolactin status	Normal	44	Reference					44	Reference				
	Low	23	0.08	1.09	0.97	1.22	0.16	23	0.14	1.15	1.03	1.29	0.01*
	High	28	0.14	1.15	1.03	1.28	0.02*	28	0.15	1.17	1.04	1.31	0.01*

		Transfers						Offspring births					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	44	Reference					44	Reference				
	Irregular	13	0.06	1.07	0.68	1.68	0.78	13	0.46	1.58	0.72	3.45	0.25
	Non-cycling	38	−0.28	0.76	0.49	1.19	0.23	38	−0.97	0.38	0.15	0.95	0.04*
Prolactin Status	Normal	44	Reference					44	Reference				
	Low	23	0.02	1.02	0.65	1.60	0.94	23	−1.65	0.19	0.04	0.86	0.03*
	High	28	−0.44	0.64	0.37	1.11	0.12	28	0.01	1.01	0.47	2.17	0.99

		Transfer-In exposures						Transfer-Out exposures					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	44	Reference					44	Reference				
	Irregular	13	−0.29	0.75	0.60	0.94	0.01*	13	0.05	1.06	0.88	1.27	0.57
	Non-cycling	38	−0.09	0.92	0.84	1.01	0.07	38	0.11	1.12	1.00	1.24	0.04*
Prolactin Status	Normal	44	Reference					44	Reference				
	Low	23	−0.15	0.86	0.76	0.97	0.02*	23	0.11	1.11	0.98	1.26	0.10
	High	28	−0.08	0.05	0.83	1.02	0.11	28	0.12	1.13	1.01	1.27	0.04*

		Birth exposures						Death exposures					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	44	Reference					44	Reference				
	Irregular	13	0.48	1.62	1.11	2.37	0.01*	13	−0.41	0.67	0.45	0.99	0.05*
	Non-cycling	38	0.21	1.24	0.96	1.59	0.10	38	−0.30	0.74	0.56	0.98	0.03*
Prolactin Status	Normal	44	Reference					44	Reference				
	Low	23	−0.16	1.17	0.88	1.56	0.28	23	−0.18	0.84	0.62	1.12	0.24
	High	28	0.34	1.40	1.07	1.84	0.02*	28	−0.49	0.61	0.44	0.86	0.004*

* $p < 0.05$.

compared to cycling elephants, and a 15% increase in the odds of being a HIGH prolactin elephant compared to NORMAL prolactin (Table 5). Every unit (ng/ml) increase in average serum cortisol was associated with a 18% and 15% increase in the odds of being an irregular or non-cycling elephant, respectively, compared to cycling elephants (Table 5). Similarly, every unit (ng/ml) increase in average serum cortisol was associated with a 15% and 17% increase in the odds of being a LOW or HIGH prolactin elephant, respectively, compared to NORMAL prolactin individuals (Table 5).

An increase in the number of OffB events was associated with a 62% decrease in the odds of being non-cycling compared to cycling, and an 81% decrease in the odds of having LOW compared to NORMAL prolactin (Table 5). Compared to cycling elephants, an increase in the number of $ExpTR_{in}$ events was associated with a 25% decrease in the odds of being an irregular cycling elephant, and a nearly significant (EOR 0.92, 95% C.I. 0.84–1.01) 8% decrease in the odds of being a non-cycling elephant (Table 5). An increase in the number of $ExpTR_{in}$ events was also associated with a 14% decrease in the odds of being a LOW prolactin elephant (Table 5). Compared to cycling and NORMAL prolactin elephants, an increase in the number of $ExpTR_{out}$ events was associated with a 12% increase in the odds of being a non-cycling elephant and a 13% increase in the odds of being a HIGH prolactin elephant (Table 5). An increase in the number of ExpB events was associated with a 62% increase in the odds of being irregular compared to cycling; and a 40% increase in the odds of being HIGH compared to NORMAL prolactin (Table 5). Finally, an increase in the number of ExpD events is associated with a decreased odds of being an irregular, non-cycling, or HIGH prolactin elephant by 34%, 26%, and 39%, respectively, compared to cycling and NORMAL prolactin elephants (Table 5). After adjusting for the other factors, we did not find a statistically significant association between the cyclicity (Irregular: EOR 1.07, 95% C.I. 0.68–1.68; Non-cycling: EOR 0.76, 95% C.I. 0.49–1.19), and prolactin (Low: EOR 1.02, 95% C.I. 0.65–1.60; High: EOR 0.64, 95% C.I. 0.37–1.11) status groups and the number of TR events

Table 6
Summary of temperament scores for the study population and by cyclicity and prolactin status. The number of elephants that were included in each variable (N), the mean, SEM, minimum and maximum score data are presented.

	Full population				
	N	Mean	SEM	Min	Max
Fearful-apprehensive	85	2.85	0.11	0.69	4.81
Aggressive-effective	85	3.23	0.12	0.69	6.02
Popular-caring	85	2.97	0.09	0.68	4.52
Active-trainable	85	3.83	0.08	2.42	5.39
Patient-slow	85	5.20	0.07	3.81	6.82

	Cyclicity status														
	Cycling					Irregular					Non-cycling				
	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max
Fearful-apprehensive	39	2.72	0.15	0.69	4.40	11	3.06	0.38	1.21	4.73	35	2.94	0.17	1.34	4.81
Aggressive-effective	39	3.20	0.18	1.10	6.02	11	3.10	0.29	1.39	4.35	35	3.30	0.20	0.69	5.14
Popular-caring	39	2.94	0.12	1.24	4.44	11	3.06	0.35	0.68	4.52	35	2.97	0.14	1.38	4.46
Active-trainable	39	3.92	0.11	2.44	5.39	11	3.71	0.21	2.42	4.69	35	3.77	0.14	2.45	5.32
Patient-slow	39	5.15	0.11	4.11	6.82	11	5.21	0.23	4.01	6.46	35	5.25	0.11	3.81	6.76

	Prolactin status														
	Normal					Low					High				
	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max	N	Mean	SEM	Min	Max
Fearful-apprehensive	39	2.72	0.15	0.69	4.40	20	3.40	0.23	1.45	4.81	26	2.64	0.19	1.21	4.76
Aggressive-effective	39	3.20	0.18	1.10	6.02	20	3.06	0.26	1.39	4.86	26	3.40	0.21	0.69	5.14
Popular-caring	39	2.94	0.12	1.24	4.44	20	2.48	0.20	0.68	3.88	26	3.38	0.14	1.76	4.52
Active-trainable	39	3.92	0.11	2.44	5.39	20	3.91	0.18	2.45	5.26	26	3.63	0.15	2.42	5.32
Patient-slow	39	5.15	0.11	4.11	6.82	20	5.22	0.17	3.81	6.76	26	5.26	0.11	3.98	6.46

individuals experienced throughout their life (Table 5).

4.2. Model 2 - cortisol, cyclicity, prolactin and individual temperament

Table 6 summarizes the temperament score data. Every unit increase (ng/ml) of serum cortisol was associated with a 16% and 13% increase in the odds of being an irregular or non-cycling elephant, respectively (Table 7). Similarly, very unit (ng/ml) increase in serum cortisol was associated with a 14% increase in the odds of having both LOW and HIGH prolactin status compared to NORMAL prolactin individuals (Table 7). After adjusting for the other factors, we did not find a statistically signficant association in temperament scores between cycling, irregular, and non-cycling elephants (Table 7). Compared to elephants with NORMAL prolactin, a 1-point increase on the fearful-apprehensive factor scale was associated with a 91% increase in the odds of being a LOW prolactin elephant (Table 7). A 1-point increase on the popular-caring factor scale was associated with an 194% increase in the odds of having abnormally high prolactin concentrations compared to elephants with NORMAL prolactin (Table 7).

4.3. ROC curve analyses

Life history events (TR, OffB, $ExpTR_{in}$, $ExpTR_{out}$) were fair classifiers for cycling and non-cycling elephants and were good classifiers for irregular cycling elephants (Table 8). Life history events (OffB, $ExpTR_{in}$, $ExpTR_{out}$) were also fair classifiers for NORMAL, LOW and HIGH prolactin status (Table 8). Temperament (fearful-apprehensive and popular-caring) was a poor classifier of normal prolactin and was a fair classifier of LOW and HIGH prolactin. Because temperament did not show predictability with cyclicity status in the logistical regression, ROC curve analysis was not conducted for cyclicity status with temperament as a classifier. Comparing across classifiers, temperament was better at classifying elephants with regards to prolactin status, while life experience events were better at classifying elephants in relation to cyclicity status (Table 8).

5. Discussion

This study provides new information regarding associations among life history events, temperament, and pituitary-ovarian disorders in female African elephants. Overall, having a calf or exposure to herd mates entering the facility were positively associated with normal ovarian function and prolactin status. By contrast, exposure to an increased number of herd mates leaving a facility were negatively associated with both. Additionally, abnormally low prolactin was related to a more fearful and apprehensive temperament, whereas contrary to our hypothesis, hyperprolactinemia was positively associated with a more popular and caring temperament. Finally, age and serum cortisol were positively associated with an increased likelihood of having abnormal cycles and prolactin status compared to normal controls. These findings indicate that acyclicity and hyperprolactinemia might be affected by social/management factors and, additionally, may be further influenced by individual elephant temperament.

In normal cycling females, prolactin is cyclic and increases during the non-luteal phase of the estrous cycle (Brown et al., 2004a, 2004b; Prado-Oviedo et al., 2013). It likely is involved in normal follicular development, as it is in other mammalian species (Bjurulf et al., 1994; Frasor and Gibori, 2003; Gagvels et al., 1992; Grosdemouge et al., 2003; Smith et al., 1976). However, in African elephants, both low (e.g., a non-cycling pattern) and high prolactin secretion is associated with ovarian acyclicity (Prado-Oviedo et al., 2013; Brown et al., 2016). In elephants with low prolactin, LH and FSH are at basal levels without the normal temporal patterns of cycling elephants, although non-cycling elephants administered GnRH exhibit normal pituitary LH and

Table 7
Summary of parameter estimates for estimated odds ratios (EOR) of cyclicity and prolactin status and temperament scores, including 95% Wald confidence intervals for EOR.

		Serum cortisol						Aggressive-effective					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	39	Reference					39	Reference				
	Irregular	11	0.15	1.16	1.03	1.32	0.02*	11	−0.01	0.99	0.51	1.91	0.97
	Non-cycling	35	0.13	1.13	1.03	1.25	0.01*	35	0.14	1.15	0.74	1.79	0.53
Prolactin Status	Normal	39	Reference					39	Reference				
	Low	20	0.13	1.14	1.02	1.28	0.02*	20	0.06	1.06	0.62	1.81	0.83
	High	26	0.13	1.14	1.02	1.27	0.02*	26	0.17	1.19	0.72	1.98	0.50

		Fearful-apprehensive						Popular-caring					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	39	Reference					39	Reference				
	Irregular	11	0.43	1.53	0.72	3.28	0.27	11	0.61	1.85	0.70	4.85	0.21
	Non-cycling	35	0.29	1.33	0.79	2.24	0.29	35	0.33	1.39	0.72	2.67	0.33
Prolactin Status	Normal	39	Reference					39	Reference				
	Low	20	0.65	1.91	1.02	3.59	0.04*	20	−0.48	0.62	0.26	1.49	0.29
	High	26	0.04	1.04	0.56	1.91	0.91	26	1.08	2.94	1.25	6.91	0.01*

		Active-trainable						Patient-slow					
		N	β	EOR	95% C.I.		P-Value	N	β	EOR	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity Status	Cycling	39	Reference					39	Reference				
	Irregular	11	−0.11	0.90	0.33	2.48	0.84	11	0.06	1.06	0.35	3.24	0.92
	Non-cycling	35	−0.004	1.00	0.49	2.02	0.99	35	0.17	1.19	0.56	2.53	0.65
Prolactin Status	Normal	39	Reference					39	Reference				
	Low	20	0.39	1.48	0.62	3.51	0.38	20	0.35	1.42	0.55	3.64	0.47
	High	26	−0.28	0.75	0.33	1.74	0.51	26	0.01	1.01	0.44	2.31	0.99

* $p < 0.05$.

FSH responses (Brown et al., 1999; Brown, unpublished). In humans, prolactin deficiency with no other perturbations in pituitary hormones is rare and does not compromise fertility (Iwama et al., 2013; Kauppila, 1997), so this may indicate a difference in etiology between African elephants and humans with respect to chronically low prolactin.

In a recent study, Prado et al. (2019a) demonstrated that the chance of an elephant developing hyperprolactinemia increases the longer it exhibits baseline progestagen concentrations, and there is a positive association between the duration of acyclicity and mean prolactin concentrations. Thus, the chance an elephant will develop hyperprolactinemia increases the longer progestagen concentrations are baseline. Additionally, long periods of low prolactin that lack a normal cyclic pattern may be insufficient to support normal follicular function and could represent an important transitory state in the etiology of infertility in some elephants (Prado et al., 2019b). A clinical trial using cabergoline, a dopamine agonist, to decrease prolactin was effective, but only during treatment (Morfeld et al., 2014). In contrast to women, where cabergoline treatment successfully resolves ovarian cycle

Table 8
Summary of area under the curve (AUC) fits for receiver operator characteristics (ROC) curve analyses for cyclicity and prolactin status with life events and temperament models as classifiers. Hyperprolactinemia, or excess prolactin production, is a significant reproductive disorder in many species, including African elephants. Temperament did not show predictability with cyclicity status in the logistical regression, therefore ROC curve analysis was not conducted for cyclicity status with temperament as a classifier.

		Life history events						Temperament scores					
		N	AUC	SEM	95% C.I.		P-Value	N	AUC	SEM	95% C.I.		P-Value
					Lower	Upper					Lower	Upper	
Cyclicity status	Cycling	44	0.70	0.05	0.60	0.81	0.001*						
	Irregular	13	0.81	0.05	0.72	0.90	< 0.001*						
	Non-cycling	38	0.71	0.05	0.60	0.81	0.001*						
Prolactin status	Normal	44	0.74	0.05	0.64	0.84	< 0.001*	39	0.65	0.06	0.53	0.77	0.016*
	Low	23	0.73	0.06	0.62	0.84	0.001*	20	0.76	0.06	0.64	0.88	< 0.001*
	High	28	0.71	0.06	0.60	0.82	0.001*	26	0.74	0.06	0.62	0.85	0.001*

AUC: 1.0 = perfect test; 0.9–0.99 = excellent test; 0.8–0.89 = good test; 0.7–0.79 = fair test; 0.51–0.69 = poor test; 0.5 = failed test.
* $p < 0.05$.

problems associated with hyperprolactinemia (Faje and Nachtigall, 2013), no resumption of ovarian activity was observed in treated African elephants. But, because prolactin responded to cabergoline as expected, it appears the pituitary is functional. Taken together, these data suggest that ovarian cycle problems may not be the result of hypothalamic-pituitary deficiencies, but rather hyperprolactinemia may manifest from the loss of feedback from the ovaries to the pituitary over time, perhaps through an increase in neural stimulatory factors, like serotonin or oxytocin (Prado et al., 2019b).

Giving birth even once is associated with decreased odds of a female elephant becoming acyclic and exhibiting abnormal prolactin secretion. In many species, pregnancy affords long-term benefits for later reproduction and health (Baird and Dunson, 2003; Guzman et al., 1999; Han et al., 2013; Kobayashi et al., 2012). This finding also has been confirmed in elephants (Hildebrandt and Goritz, 1995; Hildebrandt et al., 2000). The protective effect of pregnancy on uterine health is associated with the remodeling process of post-partum uterine involution, where early neoplastic lesions are selected for and undergo apoptosis (Baird and Dunson, 2003; Walker et al., 2001). Full-term pregnancies early in life have been found to be the most effective natural protection against breast and ovarian cancer in women (Guzman et al., 1999; Han et al., 2013; Kobayashi et al., 2012), due in part to the protective effects of progesterone (Han et al., 2013). Moreover, studies have found that the age at first conception is more important than the number of full-term pregnancies throughout a woman's life (Gierach et al., 2005; Pelucchi et al., 2007; Adami et al., 1994). Additionally, it has been shown in a variety of species that early reproduction is associated with increased reproductive output throughout their lives (Nussey et al., 2006; Robinson et al., 2012; Desprez et al., 2014). Over a 40-year monitoring period, Lee et al. (2016) found African elephants that began to reproduce early (< 12.5 yrs. old) had a greater reproductive output compared to those that began reproducing later in life. By contrast, the average age at which African elephant females first gave birth in the US was 21 years (Prado-Oviedo et al., 2016), over 12 years after the onset of puberty (Brown, 2014; Perry, 1953; Laws, 1967). Given that only 25.7% of females in the study had produced a calf (Prado-Oviedo et al., 2016), it should not be surprising that reproductive problems are widespread in the captive population. In a related finding, relationships were found between age and ovarian acyclicity and hyperprolactinemia in African elephant females (Brown et al., 2016; Prado et al., 2019a). As in humans, the delay in reproduction in captive elephants could negatively impact the long-term health and reproduction of this valuable population. Taken together, our data suggest that the longer female African elephants go without reproducing, the more likely they are to develop reproductive problems like acyclicity and hyperprolactinemia later in life. Given the current age and parity of the reproductively-aged North American population, we may have already set these females down a path of ovarian and pituitary dysfunction, which would help explain why these conditions have persisted in the population (Brown et al., 2004a, 2004b; Dow and Brown, 2012; Prado-Oviedo et al., 2013; Brown et al., 2016; Prado et al., 2019a).

We also found a social component to pituitary-ovarian functionality in this population. Specifically, elephants that experienced more offspring births and arrivals (i.e. transfers-in), and fewer departures (i.e. transfers-out) of herd-mates throughout their life were more likely to be cycling and to exhibit normal prolactin temporal patterns. Because elephants are a highly intelligent and social species, life events related to social experiences (e.g., offspring births and new herd mate arrivals) likely play a crucial role in elephant behavioral development and physiology. For example, poaching and culling operations have had long-term impacts on wild African elephant populations (Gobush et al., 2008; Shannon et al., 2013). Females from geographical areas disturbed by human activities have been found to exhibit higher fecal glucocorticoid concentrations, lower reproductive output, and reduced social knowledge compared to similarly aged females of intact groups (Gobush et al., 2008; Shannon et al., 2013). In other species, it has also been demonstrated that early life experiences (e.g., parental separation/death, social deprivation) can affect several aspects of temperament, cognition, and resilience to stress later in adult life (Kurosi et al., 2012; Pechtel and Pizzagalli, 2011; Suderman et al., 2012).

Given their innate sociality, elephant calves can provide a main avenue through which strong familial bonds are built, providing long-term social support (Rault, 2012). Greco et al. (2016) found that the presence of calves in herds reduced the risk of stereotypic behavior in zoo elephants, indicating that the addition of juveniles into existing herds through successful breeding programs may provide an important protective effect from the development of abnormal behavior in the future. In rewilded Asian tourist elephants in Thailand, unrelated females formed stronger social bonds in the presence of a calf (Thitaram et al., 2015). Sociality is believed to have evolved as an adaptive strategy to buffer against environmental challenges (Rault, 2012). When experiencing a stressor, social partners can help to mitigate the impact of the event and promote healthy coping strategies (Cohen and Wills, 1985; Rault, 2012). Elephants form close bonds with herdmates (Schulte, 2000) and likely rely on those to buffer them from environmental and social stressors, as in other species (Cohen and Wills, 1985; Rault, 2012). Therefore, the departure of a herd member may be a particularly stressful event for a species that has evolved to maintain long-term social bonds. However, in contrast to that hypothesis, herd mate deaths were not negatively associated with abnormal ovarian and pituitary activity. Perhaps elephants interpret the two types of departure events differently and are better equipped to cope with the more natural event of a death as compared to a transfer out, where elephants did exhibit disruptions in pituitary-ovarian function.

The temperament analysis found that on average hyperprolactinemic elephants have higher average popular-caring scores and have experienced an increased number of herd mate births, as compared to low and normal prolactin status elephants. It is possible that hyperprolactinemic females are over-compensating to maintain social bonds at the expense of reproduction. In fact, Freeman et al. (2004) found that non-cycling African elephant females were ranked higher in the dominance hierarchy and characterized as 'peace-keepers' by their caretakers. In the wild, elephants live in socially complex, hierarchical groups of related females with a matriarch at the top of the hierarchy (Wittemyer et al., 2005). By contrast, captive herds are usually made up of unrelated, same aged individuals (Olson and Wiese, 2000) and typically lack a clear matriarch (Freeman et al., 2004). However, that does not mean a social structure is not important for social harmony among herd mates, or that social disruptions may not have a negative impact on reproductive function. Social dynamics of group living may involve competition, aggression or affiliative interactions (Rubenstein and Shen, 2009), and can change over time. As females are added or removed from an existing herd, social hierarchy changes can occur that alter the relative effects of cooperation or competition among individual elephants (Rubenstein and Shen, 2009; Freeman et al., 2004). For highly social animals like elephants, the relatively small herd sizes in zoos and the absence of a strong matriarch may contribute to behavior problems that subsequently negatively impact reproduction (Freeman et al., 2004). Our findings lend further support to the belief that ovarian inactivity and the observed hyperprolactinemia in captive African female elephants is mediated in part by social influences (Freeman et al., 2004; Brown et al., 2016).

In humans, hyperprolactinemic patients show a higher prevalence of depressive disorders, hostility, phobias and anxiety (Yavuz et al., 2003). Therefore, we hypothesized that hyperprolactinemia in zoo African elephants would be associated with an apprehensive-fearful temperament. We found, however, that it was the non-cycling, low prolactin elephants that on average had higher apprehensive-fearful scores. Those elephants also exhibited elevated serum cortisol concentrations compared to normal cycling herd-mates and had previously been found to exhibit chronically low dopamine, oxytocin, and

serotonin concentrations (Prado et al., 2019b). Although contradicting our initial hypothesis, these results may be explained by prolactin's function as a 'social hormone' in maintaining and promoting social bonds (Eberhardt et al., 1983; Mathew et al., 2001; Snowdon and Ziegler, 2015). Studies in other species have demonstrated that animals with low prolactin levels engage in less affiliative behavior (e.g. food sharing and grooming) and spend less time in passive body contact with conspecifics Eberhardt et al., 1983; Mathew et al., 2001; Snowdon and Ziegler, 2015), while simultaneously spending more time alone, showing higher incidences of fearful scanning of their social environment and engaging in more antagonistic behaviors (Eberhardt et al., 1983; Mathew et al., 2001; Snowdon and Ziegler, 2015). Our data suggest a similar role of prolactin in maintaining social bonds in African elephants, with low prolactin females being more fearful and apprehensive towards herd mates, and possibly less social, on average. Taken together, we postulate that the observed prolactin perturbations in this species could be the result of a maladaptive response to social stress or incompatibility (Kurosi et al., 2012; Pechtel and Pizzagalli, 2011; Suderman et al., 2012).

Overall, those elephants that experienced at least one full-term pregnancy were more likely to have normal cyclicity and prolactin status. Altogether, all indications suggest that reproduction, particularly early reproduction, provides long-term endocrine, health and behavioral benefits (Brown et al., 2016; Greco et al., 2016). Based on these findings, we propose that life history may have enabled females with normal prolactin levels to gain sufficient social experience and social bonds to allow them to more effectively cope with the challenges they encounter in zoo environments. As observed in other species, early life experience can influence an individual's resilience to stress and predetermine available coping strategies (Kurosi et al., 2012; Pechtel and Pizzagalli, 2011; Suderman et al., 2012). The resulting temperament/coping mechanism may in turn predispose individual elephants to future reproductive and hormonal (i.e., prolactin) abnormalities in response to social stress. Indeed, several studies have shown that high prolactin individuals are more likely to engage with their environment and social partners (Eberhardt et al., 1983; Mathew et al., 2001; Snowdon and Ziegler, 2015), and thus could be regarded as showing active coping strategies (Ledoux and Gorman, 2001). Low prolactin individuals are more withdrawn from their surroundings (Eberhardt et al., 1983; Mathew et al., 2001; Snowdon and Ziegler, 2015) and could be interpreted as having more passive coping mechanisms (Ledoux and Gorman, 2001). We propose that the differences in prolactin status between the two non-cycling elephant groups could be due to differences in coping. Thus, individual elephant temperament and coping strategies should be taken into consideration, and management tailored to ensure optimal welfare.

In sum, we conclude that life history events and temperament play a role in the observed ovarian acyclicity and prolactin perturbations in the North American African elephant population. Furthermore, temperament appears to be the best predictor of prolactin status of a female African elephant. Taken together, prolactin may play a role in supporting social relationships in elephants, as in other species. Therapies to treat hyperprolactinemia by modulating neurohormones, like dopamine, oxytocin, and serotonin, should be considered carefully in light of the important role those females appear to play in maintaining social cohesion, especially if those females are unlikely to breed and have no other clinical symptoms (e.g., spontaneous milk production). With increased emphasis on breeding reproductive aged females, and building socially stable, multi-generational family herds, these conditions may well diminish over the next few decades.

Acknowledgements

The authors would like to gratefully acknowledge the participating zoos that contributed serum samples and temperament data for this study: African Safari, Albuquerque Biological Park, Audubon Institute, Birmingham Zoo, BREC's Baton Rouge Zoo, Buffalo Zoological Gardens, Busch Gardens, Buttonwood Park Zoo, Caldwell Zoo, Calgary Zoo, Cameron Park Zoo, Cheyenne Mountain Zoological Park, Cincinnati Zoo & Botanical Garden, Cleveland Metroparks Zoo, Columbus Zoo, Dallas Zoo, Denver Zoo, Dickerson Park Zoo, Disney's Animal Kingdom, El Paso Zoo, Fresno Chaffee Zoo, Greenville Zoo, Honolulu Zoo, Houston Zoological Gardens, Indianapolis Zoological Society, Inc., Jacksonville Zoological Gardens, Knoxville Zoological Gardens, Lee Richardson Zoo, Little Rock Zoological Garden, Los Angeles Zoo and Botanical Gardens, Louisville Zoological Garden, Lowry Park Zoological Garden, Maryland Zoo, Memphis Zoological Garden and Aquarium, Metropolitan Toronto Zoo, Milwaukee County Zoological Gardens, Montgomery Zoo, Nashville Zoo, National Zoo, Niabi Zoo, North Carolina Zoological Park, Oakland Zoo, Oklahoma City Zoological Park, Oregon Zoo, Parque Zoologico de Leon, Phoenix Zoo, Point Defiance Zoo and Aquarium, Reid Park Zoo, Riverbanks Zoological Park, Roger Williams Park Zoo, Rosamond Gifford Zoo at Burnet Park, San Antonio Zoological Gardens & Aquarium, San Diego Safari Park, San Diego Zoo, Santa Barbara Zoological Gardens, Sedgwick County Zoo, Seneca Park Zoo, Saint Louis Zoo, The Kansas City Zoo, Toledo Zoo, Topeka Zoological Park, Tulsa Zoological Park, Utah's Hogle Zoo, Virginia Zoological Park, Wildlife Conservation Society - Bronx Zoo, Wildlife Safari, Woodland Park Zoo, Zoo Atlanta, Zoo de Granby, Zoo Miami.

In addition, special thanks to the AZA Elephant TAG and TAG Chair Martha Fischer for logistical support. Special thanks to the SCBI interns who helped compile life event data for this study: Joanna Wright, Kelsey Schoenemann, Kristina Ridge and Ukachi Ugorji.

Funding disclosure

This work was funded through and Institution of Museum and Library Services: grant number LG-25-10-0033-10 to co-authors Carlstead and Brown. This work received additional support by the Shared Earth Foundation, Susan Fulton Fund (FBB Captial, Washington, DC), The David and Joan Challinor Graduate Student Fellowship, and The Took Crowell Graduate Student Fellowship. The funders had no role in the study design, data collection, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104804.

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