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Fecal 20-oxo-pregnane concentrations in free-ranging African elephants (*Loxodonta africana*) treated with porcine zona pellucida vaccine

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Abstract

Because of overpopulation of African elephants in South Africa and the consequent threat to biodiversity, the need for a method of population control has become evident. In this regard, the potential use of the porcine zona pellucida (pZP) vaccine as an effective means for population control is explored. While potential effects of pZP treatment on social behavior of African elephants have been investigated, no examination of the influence of pZP vaccination on the endocrine correlates in treated females has been undertaken. In this study, ovarian activity of free-ranging, pZP-treated African elephant females was monitored noninvasively for 1 yr at Thornybush Private Nature Reserve, South Africa, by measuring fecal 5α-pregnan-3β-ol-20-on concentrations via enzyme immunoassay. A total of 719 fecal samples from 19 individuals were collected over the study period, averaging 38 samples collected per individual (minimum, maximum: 16, 52). Simultaneously, behavioral observations were made to record the occurrence of estrous behavior for comparison. Each elephant under study showed 5α-pregnan-3β-ol-20-on concentrations rising above baseline at some period during the study indicating luteal activity. Average 5α-pregnan-3β-ol-20-on concentrations were 1.61 $\pm$ 0.46 μg/g (mean $\pm$ SD). Within sampled females, 42.9% exhibited estrous cycles within the range reported for captive African elephants, 14.3% had irregular cycles, and 42.9% did not appear to be cycling. Average estrous cycle duration was 14.72 $\pm$ 0.85 wk. Estrous behavior coincided with the onset of the luteal phase and a subsequent rise in 5α-pregnan-3β-ol-20-on concentrations. Average 5α-pregnan-3β-ol-20-on levels positively correlated with rainfall. No association between average individual 5α-pregnan-3β-ol-20-on concentrations or cyclicity status with age or parity were detected. Earlier determination of efficacy was established via fecal hormone analysis with no pregnancies determined 22 mo post-treatment and onward. Results indicate the presence of ovarian activity amongst pZP-treated female African elephants in 2 yr after initial immunization. Further study should now be aimed toward investigating the long-term effects of pZP vaccination on the reproductive function of female African elephants.

Keywords: pZP-immunocontraception; Elephant; Estrous cycle; Progestagen; Feces; Enzyme-immunoassay

1. Introduction

Rapidly expanding elephant populations in the Republic of South Africa has led to widespread concern over the resulting destruction of habitat and consequent threats to biodiversity [1–4]. Currently, various approaches have been explored in regard to managing elephant numbers, all with different advantages and disadvantages, including culling [5,6], translocation [5,7], range expansion [5,7,8], sterilization [9,10], and contraception [10,11]. In this regard, the use of the porcine zona pellucida (pZP) vaccine for immunocon-

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traception of African elephant cows is a fairly recent development. The method has shown promise as an effective and acceptable means for management [12,13]. Zona pellucida glycoproteins isolated from pig ovaries have been shown to be homologous to those of the African elephant [1,14]. After treatment intramuscularly, the vaccine triggers anti-zona pellucida antibodies which bind to the zona pellucida capsule, including sperm receptor sites and so prevent fertilization from taking place [1,15]. Since the initial studies, pZP-immunocontraception has been implemented in seven private game reserves where it has been shown to be 100% effective in over 100 cows treated [10]. Nevertheless, pZP vaccination was not fully embraced primarily due to a lack of knowledge regarding potential adverse changes in behavior. However, Delsink et al. [11,16] found no significant change in core and total range use, matriarchal status, cow/calf interaction, herd fragmentation/isolation, musth occurrence, bull hierarchy, and no aberrant or unusual behavior witnessed among the herds, and found pZP treatment to be reversible in the short-term. Theoretically, pZP treatment should not interfere with luteal activity and ovarian cycles should remain intact as with untreated populations. To date, there has been no attempt to examine endocrine correlates associated with pZP vaccine use in African elephants.

In the female African elephant (*Loxodonta africana*), estrous cycle length is reported to range between 13 and 17 wk [17–25]. The cycle is divided into a 4 to 6 wk follicular phase after which an increase in progesterone levels, believed to trigger ovulation, marks the onset of an 8 to 11 wk luteal phase [17,20–22]. Progesterone metabolites have been widely accepted as the major luteal and circulating progestagens in the African elephant and are thus considered a reliable indicator for monitoring ovarian function [19,20,22]. However, the knowledge acquired regarding the reproductive endocrinology of the African elephant is predominantly from studies on captive individuals. Amongst wild populations, the information gleaned regarding estrous cycles has been inconclusive due to the common occurrence of pregnancy or lactation anestrus among females [26–28].

Monitoring luteal endocrine function via fecal hormone analysis has made it possible to examine the reproductive biology of wild and captive animals [1,18]. Fecal samples can be easily collected and noninvasive methods have already been developed and successfully tested to enable the measurement of progestagen metabolites in elephant feces [22,29]. These techniques allow novel exploration of the pZP vaccination effect on the frequency and duration of estrous cycles as well as integration of behavioral and endocrine mechanisms which will aid in a better understanding of the elephant's reproductive physiology.

In this publication, we aim to provide detailed information regarding the 20-oxo-pregnane concentrations in free-ranging African elephant (*Loxodonta africana*) treated with pZP vaccine. Such information will promote a better understanding of the underlying impact of utilizing pZP immunocontraception on ovarian function of treated elephant cows.

2. Materials and methods

2.1. Study site and animals

The study was carried out at Thornybush Private Nature Reserve (TPNR) (24° 23′ to 24° 33′ S, 31° 05′ to 31° 13′ E) in the Limpopo Province of South Africa. The area consisted of moderately dense tree savanna comprised predominantly of *Acacia* and *Combretum* species. Annual rainfall fluctuates greatly from year to year with a mean of 601 mm falling within the months of October to April with the remainder of the year being predominantly dry [30]. Therefore, seasons were defined as wet (October through April) and dry (May through September) [30]. Field work took place during a drought where rainfall recorded in both 2007 (405.4 mm) and 2008 (333 mm) fell well below the average. In May 1994, eight elephants (all females) were relocated from the northern portion of the Kruger National Park (KNP) known as the Shingwedzi area. In June 1998, one 40-yr-old bull was added to the total population, also from Kruger National Park. To date, the TPNR population consists of 29 elephant cows and 11 bulls, two of which were physiologically mature bulls [31] that periodically associated with the herds. Nineteen of the 40 elephants were females of breeding age (range, 8 to 20 yr; average, 11 to 12 yr) [32] treated with pZP and thus included in the study analysis. Identification kits, incorporating tusk shapes and sizes, ear markings, and ear venation patterns, were made for all individuals in the TPNR population before the study, which allowed for individual recognition (Ahlers, unpublished data; 11]. Age distribution was determined at the beginning of the study using a combination of known dates of birth, and rough estimates on shoulder height and age correlation as compared with known adults in the population [33]. Age groups were defined as adult (12 years and older; N = 10), or subadult (6 to 11 yr; N = 9) (Table 1). The management of TPNR were aiming to achieve zero population growth; therefore no

Table 1
Age, calving history, reproductive status, and baseline 5α-pregnan-3β-ol-20-on concentrations ± SD of pZP-treated African elephant cows at Thornybush Private Nature Reserve, South Africa.

Cow	Age (yr)	Age class	Calves (N)	Last calf (mo)	Samples (N)	Reproductive status	Baseline 5a-P-3βOH (μg/g DW)
Hook	30–35	Adult	3	36*	52	Cyclic	1.06 ± 0.44
Flo	25–30	Adult	3	36*	42	Irregular cycling	1.20 ± 0.51
Kombela	25–30	Adult	3	8	31	Noncyclic	0.86 ± 0.49
Mandy	25–30	Adult	2	12	47	Cyclic	1.19 ± 0.36
One Tusk	25–30	Adult	3	7	51	Cyclic	1.04 ± 0.40
Thembisa	25–30	Adult	4	6	35	Cyclic	1.43 ± 0.52
Madam M	20–25	Adult	3	6	33	Noncyclic	1.00 ± 0.54
Dana	12–15	Adult	1	24	41	Cyclic	1.22 ± 0.54
Khala	12–15	Adult	2	14	45	Noncyclic	1.70 ± 0.58
Umkhonto	25–30	Adult	3	24	29	NA	1.07 ± 0.47
Hannah	9–12	Subadult	0	—	42	Irregular cycling	2.19 ± 0.80
No Tusks	9–12	Subadult	0	—	42	Noncyclic	0.71 ± 0.22
Rex	9–12	Subadult	0	—	37	Cyclic	1.14 ± 0.45
Suka	9–12	Subadult	0	—	52	Noncyclic	1.29 ± 0.56
Ziggy	8–10	Subadult	0	—	46	Noncyclic	0.94 ± 0.18
Skew	9–12	Subadult	0	—	28	NA	0.9 ± 0.35
Nkanu	6–9	Subadult	0	—	25	NA	1.29 ± 0.43
Ulwazi	6–9	Subadult	0	—	16	NA	1.25 ± 0.54
Zula	6–9	Subadult	0	—	25	NA	0.87 ± 0.38

DW, dry weight; NA, not applicable; pZP, porcine zona pellucida.
* Estimated birth of youngest calf is Mar/Apr 2005.

untreated control animals could be used in this study. To include an untreated population as a control group would have required access to another population living under the same environmental and social conditions and without any mature bulls present to ensure no pregnant or lactating cows which at the time of the study was not attainable. Therefore, already published information on the behavioral patterns and reproductive endocrinology of untreated elephant females will be used for a comparative interpretation of the results.

2.2. pZP treatments

From 2005 onward, all cows of reproductive age (N = 19) [32] at TPNR received three initial immunizations to build up pZP antibody titer concentrations in 2005 [16]. These were delivered remotely from helicopter using methods modified from Delsink et al. [34]. The vaccination protocol for the TPNR population was as previously described by Bertschinger [35]. Briefly, a primary dose consisting of 400 μg pZP protein in 1 mL PBS plus 0.5 mL of Freund's modified complete adjuvant was administered in May 2005. This was followed by two boosters of 200 μg pZP protein each in 1 mL PBS plus 0.5 mL of Freund's incomplete adjuvant administered in June 2005 and August 2005, respectively. The TPNR breeding population received its first annual booster of 200 μg pZP protein in 1 mL PBS plus 0.5 mL of Freund's incomplete adjuvant in September 2006 and an equivalent booster in September 2007.

2.3. Behavioral data and fecal sample collection

Elephants were tracked daily from March 2007 to February 2008 by means of radio contact from field guides giving last reported location of herd, as well as fresh footprints and dung. Behavioral observations were recorded regularly (a minimum of 3 times weekly per individual) for an average of 3.5 h per day using focal animal sampling [36–38]. Estrous behavior observed was recorded characterized by loud vocalizations, wariness toward bulls, estrous walk (head held high, back arched, and tail raised), olfactory and tactile interactions between bulls and cows, estrous chase, mounting, and consort behavior [39].

During each observation session, approximately 50 g of feces was collected from the study animal shortly after it had defecated and moved away. Using rubber gloves, well homogenized aliquots of each fecal sample were taken and stored in glass vials [18,40]. Samples were placed on ice and frozen at the end of the workday (maximum 6 h after collection), and stored at −20°C until hormone analysis.

The total time spent in the field with behavioral observations and fecal sample collections was 984 h distributed over 234 days. A total of 719 fecal samples were collected over the study period with the aim of obtaining 1.5 samples per week per individual. Mean sample number collected per individual was 38 (minimum, maximum: 16, 52).

2.4. Fecal sample processing and 5α-pregnan-3β-ol-20-on immunoassay

Fecal samples were lyophilized, pulverized, and sieved through a nylon mesh to remove fibrous material [18,22,41–43]. Approximately 50 mg of fecal powder was extracted with 3 mL 80% ethanol in water [43]. The mixture was vortexed for 15 min before centrifugation at 1500 × *g* for 10 min. The resulting supernatant fluid was transferred to Eppendorf tubes for hormone analysis [29,41,44]. The extracts were measured for immunoreactive progesterone metabolites using an enzyme immunoassay (EIA) for 5α-pregnan-3β-ol-20-on which has been shown to provide reliable information on reproductive steroid hormone pattern in elephant cows [45]. Enzyme immunoassays were performed following Ganswindt et al. [41]. Sensitivity of the assay at 90% binding was 3 pg per well. Inter- and intra-assay coefficients of variation ranged between 8.0% and 17.6% for the progestagen measurements. To adjust for water content variations, fecal hormone concentrations were expressed as mass/g dry weight.

2.5. Statistical analysis

Of the 19 elephant females sampled during the study period, five cows were excluded from all analysis due to low sample size (N < 30; Table 1), because interpretation of luteal and follicular phases was rendered unreliable. For the remaining focal animals, 5α-pregnan-3β-ol-20-on concentrations were expressed as μg/g dried feces and plotted against time (weeks) for each pZP-treated cow. Baseline values of 5α-pregnan-3β-ol-20-on concentrations were ascertained for each female using an iterative process as described by Brown et al. [46,47]. The average was subsequently recalculated and the elimination process was repeated until there were no values greater than the mean plus two standard deviations (SD). The remaining values yielded the baseline 5α-pregnan-3β-ol-20-on concentration. Ovarian cycle length and periodicity was determined by measuring weekly concentrations of progesterone metabolites where the first point rise in progestagen concentrations above baseline and remaining above baseline for at least two consecutive weeks marked the beginning of the luteal phase (LP) [46,48]. The commencement of the follicular phase (FP) was defined as the first of two consecutive progestagen concentrations falling below baseline concentration [46,48]. Phase length estimates as well as ovarian cycle length estimates are given as mean ± SD. Females were categorized as having an irregular cycle when overall cycle length exceeded or fell short of the reported norm of 13 to 17 wks [10,17–25]. The noncycling category was reserved for females who had no identifiable pattern in 5α-pregnan-3β-ol-20-on concentrations throughout the study period [47]. Due to small sample sizes, irregularly cycling females were combined with noncycling females for statistical analysis comparing cyclicity status with baseline 5α-pregnan-3β-ol-20-on levels and age. Individuals were categorized as demonstrating periods of anestrus if they had a follicular phase lasting longer than twice the duration of an average normal follicular phase (≥10 wks) [25,48]. Luteal activity was indicated by a >2 SD rise above baseline concentrations in 5α-pregnan-3β-ol-20-on [18]. Determination of pregnancy within treated cows was based on luteal phase lengths persisting longer than 3 to 5 mo [21,22]. Occurrence of behavioral estrus was compared with the time of increasing progesterone metabolite concentrations as behavioral estrus has been reported to last from 2 to 6 days [10]. Increases in progestagen concentrations following the end of the follicular phase confirm that ovulation has taken place with the formation of an active corpus luteum [21].

To determine the effect of age, reproductive status, and seasonal influences on fecal 5α-pregnan-3β-ol-20-on concentration, individual averages were first tested for normality using Shapiro-Wilks ($P > 0.05$ = normal). Normally distributed data were analyzed using Student *t* test and data failing the normality test were analyzed using Wilcoxon rank-sum test (with continuity correction of 0.5). Spearman rank correlation was used to assess the effects of seasonal rainfall on progestagen concentrations. A two-tailed Fisher's exact test was used to test cyclicity status correlation to age, parity, and lactational status [49,50]. Statistical significance was assumed when $P < 0.05$. Statistical analyses were performed using the software programs OpenStat [51] and KyPlot (KyensLab Inc., Tokyo, Japan, Version 2.0 beta 13 1997). Data are presented as means ± SD.

3. Results

3.1. Luteal activity

In all females (N = 14) where more than 30 fecal samples were collected, 5α-pregnan-3β-ol-20-on con-

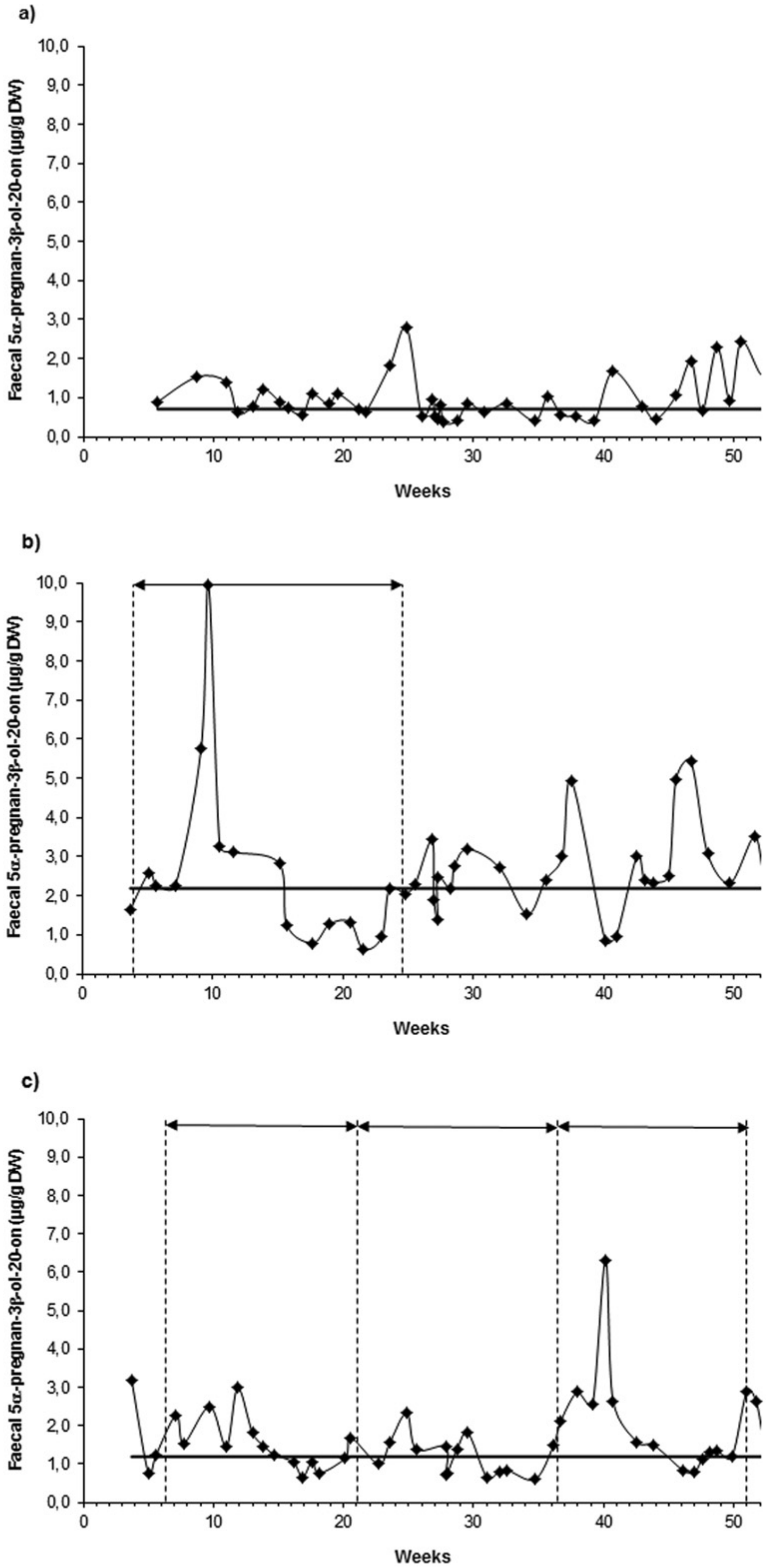

Fig. 1. Fecal 5α-pregnan-3β-ol-20-on concentrations (dots) of three adult African elephant females (No Tusks, Hannah, and Mandy) treated with porcine zona pellucida (pZP) vaccine depicting a) no cyclic pattern, b) irregular cyclic pattern, and c) cyclic estrous pattern. Dotted lines represent onset of successive luteal phases and two-way arrows represent the length of one complete estrous cycle. Solid line illustrates baseline 5α-pregnan-3β-ol-20-on concentrations.

Table 2
Number of cycles, phase lengths and baseline of fecal 5α-pregnan-3β-ol-20-on concentrations ± SD in cycling pZP-treated African elephant females at Thornybush Private Nature Reserve, South Africa.

Cycling cows	Number of cycles found	Luteal phase		Follicular phase		Cycle length (weeks)
		Weeks	5α-pregnan-3β-ol-20-on (μg/g DW)	Weeks	5α-pregnan-3β-ol-20-on (μg/g DW)	
Dana	1	8.29	2.08 ± 0.47	8.00	0.91 ± 0.18	16.29
Hook	2	8.72 ± 1.01	2.32 ± 0.99	5.43 ± 1.82	0.94 ± 0.36	14.14 ± 0.81
Mandy	3	10.33 ± 0.30	1.91 ± 1.07	4.81 ± 0.36	0.94 ± 0.25	15.14 ± 0.38
One Tusk	1	8.14	2.27 ± 1.11	5.71	0.90 ± 0.32	13.86
Rex	1	8.86	2.03 ± 0.61	5.43	0.89 ± 0.20	14.29
Thembisa	1	6.29	2.11 ± 0.01	8.00	0.96 ± 0.41	14.29
Mean ± SD		8.89 ± 1.38	2.07 ± 0.95	5.82 ± 1.44	0.95 ± 0.31	14.72 ± 0.85

DW, dry weight; pZP, porcine zona pellucida.

centrations exceeded 2 SD of individual baseline levels more than once during the study period, indicating luteal activity (Fig. 1). All remaining cows (Table 1) also showed signs of luteal activity with elevations in 5α-pregnan-3β-ol-20-on levels mainly occurring in the second wet season of 2008.

3.2. *Ovarian cyclic pattern*

In eight of the 14 elephant cows a cyclic pattern in 5α-pregnan-3β-ol-20-on levels could be identified. Of these eight individuals, two cows showed an irregular cyclic pattern lasting longer than the reported maximum of 17 wk (17.43 and 20.43 wk, respectively), and for the remaining six females at least one complete estrus cycle could be identified (Fig. 1b, c). Acyclic periods lasting 18.68 ± 5.94 wk (range, 13.14 to 24.57 wk) were detected in four females.

Within the 5α-pregnan-3β-ol-20-on profiles of regular cycling females (N = 6) a total of nine complete estrous cycles were identified (Table 2). For four elephant cows one cycle, falling within the normal 13 to 17 wk estrous cycle length, were identified. For the remaining two females, two and three complete estrous cycles were identified, respectively. Mean estrous cycle length determined from fecal 5α-pregnan-3β-ol-20-on measurements was 14.72 ± 0.85 wk with a luteal phase of 8.89 ± 1.38 wk and a follicular phase of 5.82 ± 1.44 wk (Table 2). Three of the cycling females showed longer than expected follicular phase lengths while one cow had a shorter than expected luteal phase. For the six cycling elephant cows, baseline 5α-pregnan-3β-ol-20-on concentrations ranged between 1.04 ± 0.40 and 1.43 ± 0.52 μg/g dry weight (DW) (Table 1). 5α-pregnan-3β-ol-20-on concentrations during peak luteal phase ranged from 2.71 to 9.96 μg/g. The highest concentrations were found in the two females that showed irregular cycle lengths (Table 1). No difference in baseline 5α-pregnan-3β-ol-20-on levels were found between cycling and noncycling females (t = 2.57; N = 6; P > 0.05).

3.3. *Effect of age on ovarian cycles*

Of the nine adult cows monitored, five showed a regular and one an irregular cyclic pattern. The remaining three were acyclic according to the 5α-pregnan-3β-ol-20-on analyses (Table 1). One each of the subadult cows had a regular or an irregular cyclic pattern, respectively, while the remaining three were acyclic. Age and the occurrence of estrous cycles was not significantly correlated (Fisher's exact test; P = 0.30) and no significant difference could be found between average 5α-pregnan-3β-ol-20-on concentrations of adult and nonadult individuals (t = 1.13; N = 14; P > 0.5).

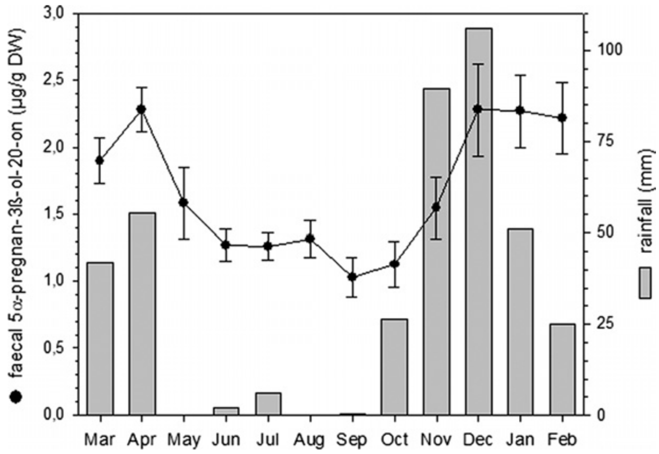

Fig. 2. Mean monthly rainfall (horizontal bars) and overall mean monthly 5α-pregnan-3β-ol-20-on concentrations (dots) ± SE for 14 porcine zona pellucida (pZP)-treated African elephant females from March 2007 to February 2008.

3.4. Effect of calving interval on ovarian cycles

All the adult cows considered in the study (N = 9) had calved at least once (1 to 4 calves) before pZP treatment with the most recent dates of parturition occurring in August 2006 (Table 1). The fecal 5α-pregnan-3β-ol-20-on measurements revealed that no females were pregnant at the time of the study. All adult females except one (Mandy) were lactating for the duration of the study.

Three of six cows that had calved ≤18 mo (range, 6 to 14 mo) failed to show an ovarian cycle while three had a regular cyclic pattern. All three cows that had calved more than 18 mo (range, 24 to 36 mo) before commencement of the study were cyclic, although one of these had an irregular pattern (Table 1).

3.5. Effect of season on 5α-pregnan-3β-ol-20-on concentrations

Average 5α-pregnan-3β-ol-20-on concentrations obtained from individual cows during the wet season (1.98 ± 0.58 μg/g DW) were significantly higher ($t = 3.22$; N = 12; $P < 0.01$) than mean 5α-pregnan-3β-ol-20-on concentrations obtained from females during the dry season (1.25 ± 0.44 μg/g DW). Overall mean monthly 5α-pregnan-3β-ol-20-on concentrations were significantly correlated with total amount of monthly rainfall ($t = 2.00$; N = 12; $P < 0.05$) (Fig. 2).

3.6. Relationship of observed estrus and fecal 5α-pregnan-3β-ol-20-on concentrations

Estrous behavior was noted on three occasions during the study and in each case coincided with the onset of a luteal phase as indicated by a rise in 5α-pregnan-3β-ol-20-on levels above baseline.

4. Discussion

This study is the first to examine the effects of pZP treatment on fecal progestagen concentrations in female African elephants while further demonstrating the usefulness of noninvasive fecal endocrine monitoring in assessing reproductive status in wild populations of African elephants [18,22,26,29]. The results generated in this study underline earlier efficacy of pZP vaccination [10] with regard to managing elephant numbers by detecting no pregnant focal animals 22 mo after treatment and onward. Observed estrous behavior was associated with a rise in progestagen concentration indicating the initiation of the luteal phase as confirmed by Hodges [21] and Brown [23] in untreated African elephant females. Concerning seasonality, the generated endocrine correlates in pZP-treated individuals all remained in agreement with findings in literature regarding untreated populations [26,29]. Seasonal influences on reproductive steroid hormones have been widely reported in a variety of species [52–54] as well as African elephants [26,29]. As a consequence of low rainfall experienced during the dry season, quality and availability of food and water decline [26]. These dry season conditions result in a decline in body condition and have been linked to lowered progestagen concentrations, periods of anestrus, silent heats, and subsequently reduced fertility [26,29]. The period of study took place during a drought where rainfall in both 2007 (405.4 mm) and 2008 (333 mm) fell below the average 601 mm reported for the study area [30]. The suboptimal conditions arising from the poor rainfall during the study period likely resulted in nutritional stress in the Thornybush elephant population which in turn could explain the low frequency of cyclic patterns in their progestagen concentrations. Overall average monthly progestagen concentrations closely followed rainfall patterns (Fig. 2) and further verified past studies that indicated availability of water, food, and body condition influence reproduction [26,29].

Our knowledge regarding reproductive endocrine function in free-ranging elephants is limited and information gleaned from captive populations cannot be transferred one-on-one without limitations due to inconsistencies in the environmental and social set-up. Therefore, further investigation of cycling patterns in both free-ranging untreated and treated populations of African elephant is necessary to evaluate the effect of pZP on the reproductive cycle. Our results demonstrate that in the 2 yr after the commencement of pZP treatment at TPNR, estrous cycles are present amongst nearly half of treated individuals, indicating ovarian functionality. Previous studies already documented alternation between cyclic and noncyclic periods as well as erratic progestagen secretion in untreated African elephant populations [27,55]. Furthermore, most data on wild populations have been unable to determine continuous cyclicity status, as in most cases, the subjects already are or become pregnant during the course of the study [26–28]. Interpreting causes for irregular or noncyclic patterns of progestagen secretion in African elephants is fraught with complexity given a vast variety of potential influences, such as an individual's social status, body condition, climatic, and seasonal influences [26,29,55]. Regarding the potential influence of the pZP treatment, episodic ovulatory failure amongst pZP-treated feral horses has also been reported but could not be irrefutably linked to pZP treatment alone and estrous cycle characteristics were consistent with untreated mares [56]. Additionally, seasonal and social influences on hormone activity also complicate analysis as both factors have an impact on body condition thus affecting reproductive fitness [29,55]. Thus, it is difficult to conclude the effect of pZP treatment on elephant reproductive function as the exact effects of each of the aforementioned factors on reproduction in untreated populations is not yet fully understood.

In conclusion, the absence of an indefinite period of anestrus within the study population is encouraging as it demonstrates that pZP treatment unlikely interferes with follicular development and ovulation in the African elephant. Nonetheless, it is recommended that some cows be left off treatment periodically to prevent population crash and a skew in age groups. Future study should be geared toward monitoring pZP-treated females alongside a comparable untreated, free-ranging, control group and for a longer duration (we suggest a minimum study period of 2 yr) to minimize external influences on reproduction as well as ascertain the long-term effects of pZP vaccination on free-ranging African elephant populations.

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