Analysis of ovarian and adrenal activity in Namibian cheetahs

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Captive breeding of cheetahs (*Acinonyx jubatus*) has had limited success because a high percentage of captive females exhibit a lack of ovarian activity. This study examined concentrations of ovarian and adrenal hormones in wild-caught cheetahs (*n* = 3) housed in large outdoor enclosures on private game ranches in Namibia. Cheetahs were monitored for a 16-month period to investigate the effect of season on ovarian and adrenal function. Secretory profiles of oestradiol, progestagen, and cortisol metabolites were quantified non-invasively using faecal steroid analysis. All three cheetahs exhibited ovarian activity; however, none cycled continuously. Periods of anoestrus occurred during overlapping periods between August and December 1994, but not during the same time period in 1995. Mean duration of the oestrous cycle, oestrus period and baseline concentrations of reproductive hormones were consistent with those observed in other captive cheetah populations. Concentrations of faecal corticoids were lower than those from captive cheetahs in North America. There was no correlation between adrenal activity and ovarian function. Spontaneous ovulation was documented in one cheetah. These findings support those of earlier studies that even under natural and, therefore, presumably ideal environmental conditions, reproductive activity in captive cheetahs is not continuous.

INTRODUCTION

The cheetah (*Acinonyx jubatus*) is an endangered species whose free-ranging population (approximately 12 000) has its largest concentration in Namibia (approximately 2500) (Marker 1998). From 1975–1987 the wild population in Namibia was halved due to conflicts with farmers (Morsbach 1987). Rapidly declining numbers of wild cheetahs have promoted captive breeding programs and the management of remaining wild populations considered important for the survival of this species.

Studies of wild female cheetahs have shown that more than 90% reproduce (Laurenson *et al.* 1992) whereas less than 20% of captive cheetahs have produced offspring (Marker & O'Brien 1989). Female reproductive studies are difficult in most wildlife species because of the complexity of ovarian activity and function. The intractable nature of wild cats prevents long-term studies of hormone concentrations by blood analysis.

Cats void their urine by spraying which limits the feasibility of non-invasively measuring hormone concentrations in urine. As a consequence, faecal steroid analysis is now widely used to monitor reproductive status in a variety of mammalian species, including the cheetah (Brown *et al.* 1996; Czekala *et al.* 1991).

Behavioural studies on wild cheetahs suggest that females may be seasonally polyoestrus (Laurenson *et al.* 1992). Summaries of captive birth dates have described a November–February and June/July seasonality at some southern African facilities (Bertschinger *et al.* 1984; Bertschinger *et al.* 1998; Brand 1980) while others in South Africa have documented births throughout the year (Manton 1975). Previous research using faecal steroid analysis to study ovarian activity in female cheetahs housed in North America identified sporadic periods of anoestrus which did not correlate with season. It has been suggested that this reproductive inactivity may be due to elevations in cortisol (Jurke *et al.* 1997). Disruption of the neuroendocrine regulation of follicular development, ovulation, embryo implantation and fetal development by glucocorticoids has been documented (Coubrough 1985; Moberg 1991; Rivier & Rivest 1991; Rivier *et al.* 1986). This study characterized long-term endocrine

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profiles in captive Namibian cheetahs. Access to cheetahs in Namibia allowed investigating the possibility of reproductive seasonality in animals that experience the same photoperiod and season as free-ranging cheetahs. By measuring concentrations of faecal cortisol metabolites, this study also examined the effects of adrenal activity on reproductive cyclicity in cheetahs that have not been exposed to environmental perturbations, other than captivity. These baseline data are important for improving our understanding of basic reproductive physiology of the cheetah, which could potentially lead to better management and breeding strategies for cheetahs.

MATERIALS AND METHODS

Animals

Wild-caught female cheetahs (n = 3) held captive in private facilities in north-central Namibia were used in this study. All three cheetahs had been wild-caught as young cubs (approximately one month of age) and were hand-reared. One cheetah (International Cheetah Studbook (SB) No. 2673) was housed singly; however, there was a single male housed in an adjacent but separate enclosure. In addition, a group of three males were housed within visual range of this female from 19 May – 20 August 1994, and a single male also was housed within visual range from 16 –21 July 1994. The enclosure containing the female was two acres of natural habitat enclosed with chain-link fencing and contained a man-made shelter. The other two females (Nos 2590 and 2589) were unrelated, but housed together in a one-acre enclosure. These two females were the only cheetahs at this facility. Neither facility housed other species of large carnivores. The ages of Nos 2673, 2589 and 2590 were three, six, and six years, respectively, at the beginning of the study, and all were nulliparous. Cheetahs were fed diets of wild game meat, partial carcasses, whole carcasses from small mammals and guinea fowl. Water was available *ad libitum.* Faecal samples were collected daily, as available, from No. 2673 (n = 193) between 18 April 1994 and 20 March 1995 and from No. 2589 (n = 303) and No. 2590 (n = 293) between 6 July 1994 and 21 October 1995. Individual faecal samples from the enclosure containing the two females were visually identified to the individuals as these females defecated soon after being released from a night holding pen.

Faeces were mixed and a representative ~20 g sample was collected into a plastic vial and frozen (–20°C) until transport to a laboratory in the United States (U.S.A.). The faecal samples were preserved during transport by adding 5 ml of absolute ethanol to each vial to completely cover the sample (Terio *et al.* 2002). Vials were tightly capped, covered with parafilm® and transported at room temperature. On arrival at the laboratory, ethanol was evaporated in a fume hood and the samples refrozen.

Faecal steroid extraction and analysis

Faecal samples were dried, pulverized and the steroids extracted as previously described (Brown *et al.* 1996; Brown *et al.* 1994; Graham & Brown 1996). Briefly, ~0.20 g of dried faecal sample was boiled in 5 ml of 90% ethanol for 20 min. After centrifugation (500 g, 15 min), the supernatant was recovered and the pellet resuspended in an additional 5 ml of 90% ethanol, vortexed for 1 min and recentrifuged. The ethanol supernatants were combined, dried under air, and resuspended in 1 ml of methanol before diluting (1:40 oestradiol; 1:800–1:80 000 progesterone, and 1:10 cortisol) in phosphate buffer (0.01 M PO_4, 0.14 M NaCl, 0.01% $NaNH_3$, pH 7.4) for radioimmunoassay (RIA). Extraction efficiency of exogenous steroid was >90%.

Faecal oestradiol and progesterone concentrations were quantified using radioimmunoassay protocols previously validated for cheetahs (Brown *et al.* 1994; Brown *et al.* 1996). A ^{3}H-oestradiol single-antibody assay was used with a sensitivity of 2 pg/ml. Faecal progesterone concentrations were quantified using a ^{125}I-progesterone, double-antibody assay with a sensitivity of 3 pg/ml (progesterone monoclonal antibody 331, courtesy of J. Roser, Davis, CA, U.S.A.). Faecal glucocorticoid (corticoid) metabolite concentrations were quantified using a commercial ^{125}I–corticosterone RIA (ICN Biomedicals, Costa Mesa, CA, U.S.A.) validated for use in cheetahs (Terio *et al.* 1999). Sensitivity of the corticosterone RIA is 12.5 ng/ml. All samples were assayed in duplicate. Intra- and interassay coefficients of variation were <12%. All data are expressed on a per gram dry mass basis.

Data analysis

Peak oestradiol concentrations were determined by an iterative process in which high estradiol concentrations (indicative of behavioural oestrus) were excluded if they exceeded the mean
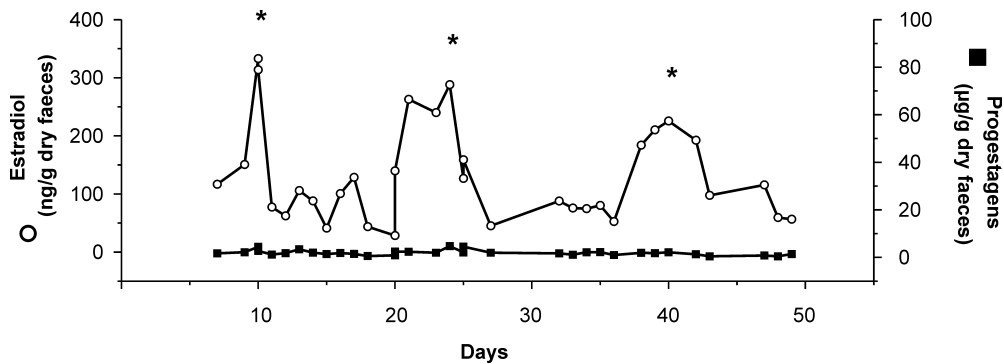
Fig. 1. Representative oestradiol and progestagen profile from a non-pregnant female cheetah. Asterisks denote peak oestradiol concentrations indicative of oestrus.

oestradiol concentration plus two standard deviations. Baseline values were those data points remaining after all of the high values had been excluded. Peak corticoid concentrations were also determined using the same iterative process. To ensure accurate calculation of oestrus length, the number of days oestradiol was elevated above baseline was calculated only for periods when baseline faecal samples were available for the days before and following the period of oestradiol elevation. Interoestrus periods were calculated as the number of days between oestradiol peaks that did not exceed 30 days (twice the previously estimated interoestrous interval: Asa *et al.* 1992; Bertschinger *et al.* 1984; Eaton 1973). Periods between oestradiol peaks exceeding 30 days were considered periods of anoestrus. Data are presented as mean ± S.E. The relationship between oestradiol and corticoid concentrations was analysed using a one-way analysis of covariance with estrogen as the dependent variable, individual cheetah as separate groups, and cortisol concentration as the covariate.

RESULTS

Figure 1 shows a representative faecal oestradiol and progestagen profile for an individual cheetah during a period of ovarian activity. Oestrous cyclicity, as demonstrated by successive oestradiol peaks, occurs while progestagen metabolite concentrations are at baseline levels. Data on individual oestrous cycle lengths are summarized in Table 1. On the basis of oestradiol secretory patterns, interoestrus interval was 13.9 ± 0.7 days (range of 6–27 days, *n* = 55) with elevated oestradiol (oestrus) lasting 4.0 ± 0.3 days. The percentage of interoestrous intervals <7 days was 6.5% and >20 days was 12.9%.

All three cheetah exhibited evidence of ovarian activity based on regular fluctuations in oestradiol excretion, although none of the females monitored exhibited continuous follicular activity (Figs 2, 3, 4). The periods of cyclicity were interrupted by a single period of anoestrous in each cheetah which varied in duration among individuals (33, 69, 136 days). In 1994, all anoestrous periods occurred between 1 August and 21 December; however, anoestrus was not observed from August to October 1995.

Oestradiol surges were not accompanied by a subsequent rise in faecal progestagen metabolites except in one case where progestagens were elevated for 27 days after a significant increase in oestradiol (Fig. 4). This event is considered a spontaneous ovulation because the female was housed singly. While there were other males

**Table 1**. Mean ± S.E. and range of oestrous and interoestrous durations.

Cheetah SB No.	*n*	Oestrous duration Mean ± S.E. (days)	Range (days)	Interoestrous duration Mean ± S.E. (days)	Range (days)
2589	24	4.00 ± 0.43	1–7	14.90 ± 1.18	7–27
2590	23	3.87 ± 0.40	1–7	11.58 ± 0.75	6–19
2673	8	4.42 ± 0.97	1–9	16.89 ± 1.41	12–23
Total	55	4.00 ± 0.28	1–9	13.90 ± 0.69	6–27
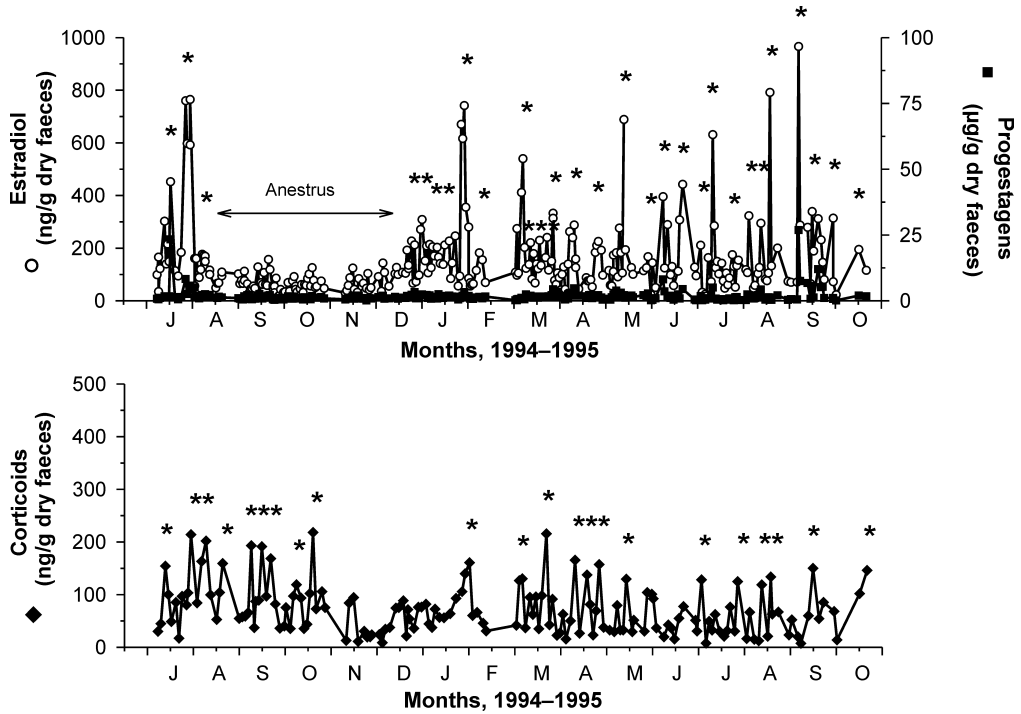
Fig. 2. Long-term profile of oestradiol, progestagen and corticoid metabolites from a female cheetah (SB No. 2589). Asterisks denote peak concentrations of oestradiol (indicative of oestrus) and peak corticoid concentrations. A period of anoestrus occurred between August and December, lasting 136 days.
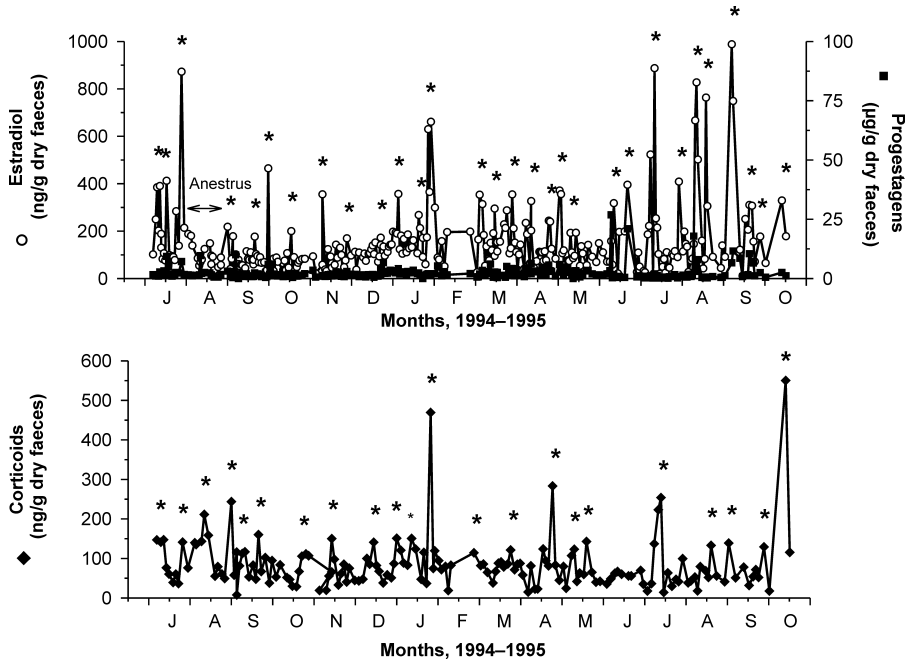
Fig. 3. Long-term profile of oestradiol, progestagen and corticoid metabolites from a female cheetah (SB No. 2590). Asterisks denote peak concentrations of oestradiol (indicative of oestrus) and peak corticoid concentrations. A period of anoestrus occurred between August and the beginning of September, lasting 33 days.
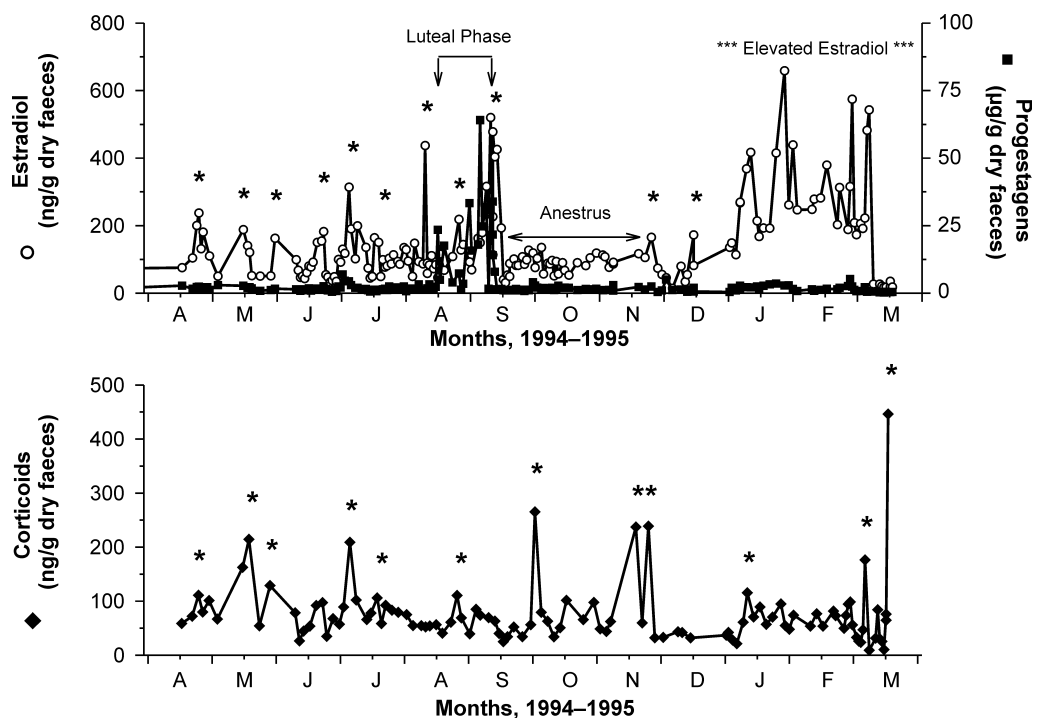
Fig. 4. Long-term profile of oestradiol, progestagen and corticoid metabolites from a female cheetah (SB No. 2673). Asterisks denote peak concentrations of oestradiol (indicative of oestrus) and peak corticoid concentrations. A period of anoestrus occurred between September and November, lasting 69 days.

housed at the facility, the female had no physical contact with a male in the period preceding the progestagen elevation. During the study (January–March 1995), the same female exhibited a period of oestradiol elevation above baseline (Fig. 4). Throughout this period there were regular fluctuations in oestradiol concentration mimicking periodic follicular activity. The oestradiol concentration then dropped rapidly to below baseline on 11 March 1995 and corticoid concentrations increased dramatically on 20 March 1995. Progestagen concentrations remained constant throughout this period. This cheetah died on 22 March 1995 of lymphosarcoma associated with feline leukemia virus (FeLV).

Figures 2 and 3 show the hormone profiles of two females living at the same facility that were in direct physical contact. The cheetahs showed concurrent oestrous cycles 53% of the time and same day peak oestradiol concentrations were observed in 10% of the total cycle periods.

Baseline hormone concentrations for individual female cheetahs are given in Table 2. Corticoid concentrations fluctuated around a mean value with random elevations above baseline which

were unrelated to concentrations of oestradiol metabolites ($P = 0.1033$).

DISCUSSION

All three cheetahs exhibited ovarian activity during the sample collection period. These hormone profiles are similar both qualitatively and quantitatively to those previously reported in captive cheetahs in North America (Brown *et al.* 1996). The mean interoestrous interval of 13.9 days is similar to the 13-day length reported by Brown *et al.* (1996) and consistent with the 12–14-day interval based on behavioural observations and vaginal cytology (Asa *et al.* 1992; Bertschinger *et al.* 1984; Eaton *et al.* 1973). The four-day duration of oestrus is also consistent with that previously reported (Brown *et al.* 1996). A large amount of variability in oestrous and interoestrous lengths was observed between and within individual cheetahs. Variability in oestrus, the oestrus cycle and interoestrus intervals has been reported in several species of the Felidae and this variability is speculated to be characteristic of induced ovulation. It has been hypothesized that the signaling of onset and termination of oestrus may be less

Table 2. Mean ± S.E. and range of baseline faecal steroid concentrations.

Cheetah SB No.	Oestradiol (ng/g) Mean ± S.E. (range)	Progestagens (ug/g) Mean ± S.E. (range)	Corticoids (ng/g) Mean ± S.E. (range)
2589	86.67 ± 2.52 (11.31–158.72)	1.32 ± 0.04 (0.14–2.46)	58.47 ± 2.08 (7.70–104.03)
2590	84.64 ± 2.50 (17.97–155.69)	1.30 ± 0.04 (0.12–2.58)	54.59 ± 2.47 (7.60–111.48)
2673	81.97 ± 3.13 (4.38–152.55)	1.43 ± 0.05 (0.25–2.55)	58.81 ± 2.31 (8.99–102.06)

important in species exhibiting induced ovulation (Brown *et al.* 1996).

A single spontaneous or non-coital induced ovulation was observed in a singly-housed cheetah (Fig. 4). The faecal progestagen concentrations fluctuated during this luteal phase, but remained elevated above baseline for 27 days. Although the length of this spontaneous luteal phase was shorter than those reported previously (Asa *et al.* 1992; Brown *et al.* 1996), the overall concentrations of progestagen metabolites were similar (Brown *et al.* 1996). This female was in auditory and presumably olfactory range of male cheetahs housed at the same facility. It is possible that these non-physical stimuli influenced ovulation (Hinds & Smith 1982; Fadem 1985). This is not the first evidence of spontaneous ovulation in non-domestic felids. Studies of reproductive hormones in non-mated lions, clouded leopards, and leopards have demonstrated sustained elevation of progesterone concentrations (Schramm *et al.* 1994; Brown *et al.* 1995; Howard *et al.* 1997; Schmidt *et al.* 1988). Comparatively, the incidence of spontaneous ovulations in the cheetah appears to be very low.

All three cheetahs exhibited periods of anoestrus during overlapping time periods. By contrast, captive cheetahs housed in North American facilities showed periods of anoestrus that were neither synchronous nor seasonal (Brown *et al.* 1996). While the overlapping periods of anoestrus could be coincidental, it is possible that some entrained, endogenous circannual rhythm in cheetahs remains functional even after animals have been brought into captivity. This rhythm might require appropriate cues that are absent in the North American environment. The cheetahs in this study were anoestrus between August and December. This time period corresponds with the end of the winter dry season and ends immediately before

the summer rainy season in Namibia. Although births can occur throughout the year in Namibia, data from 53 wild-born litters showed birth peaks during the early wet season (October/November) and the primary wet season (February/March) (Marker-Kraus *et al.* 1996). If birth peaks do occur they are likely to coincide with increased prey availability (*i.e.* the birth of antelope fawns). The captive cheetahs in this study were fed regularly and thus not affected by a change in prey availability. They were, however, exposed to all other seasonal changes such as temperature and rainfall. Interestingly, while the overall rainfall for Namibia was significantly below average in 1994, there was a marked increase in rainfall totals during the 1995 rainy season (Halpert *et al.* 1996). The improved environmental conditions in 1995 may provide a possible explanation for the absence of anoestrus in the two surviving females during the second year of the study.

Seasonal birth peaks during the wet season have also been observed in East Africa (Laurenson *et al.* 1992). A captive institution in South Africa has reported seasonal peaks in matings and subsequently births during the wet season; however, these data may be biased by management preferences (Bertschinger *et al.* 1994; Bertschinger *et al.* 1998). Further research is needed to determine if the seasonal trend seen in these data can be reproduced.

Alternatively, anoestrous periods may not be caused by any specific environmental factors but, moreover, may be related to how cheetahs are housed in captivity. In the wild, female cheetahs are generally solitary, but many facilities maintain cheetahs in groups of 2–3 individuals because they rarely display overt intraspecific aggression. At two different institutions, ovarian cyclicity (based on faecal oestrogen patterns) alternated among females housed together (Brown *et al.*

1996). In another study, ovarian cyclicity was reduced in some pairs of cheetahs, with the behaviourally subordinate female showing the most suppression (Wielebnowski & Brown 1998). In the present study, there was no indication of ovarian suppression between group-housed Namibian cheetahs, as both cycled or were in anoestrus concurrently. This finding may be due to the females being socially 'bonded', similar to domestic cats (MacDonald *et al.* 1987). These females displayed numerous behaviours including mutual grooming, playing and sleeping together. Wielebnowski *et al.* (1998) also noted continuous ovarian cyclicity (on the basis of faecal estradiol patterns) in a pair of behaviourally-compatible cheetahs, further supporting the idea that the manifestation of reproductive suppression may be socially-mediated.

Periods of anoestrus were not found to be associated with preceding elevations in corticoid metabolites. Acyclic females have been found to have higher overall baseline corticoid concentrations than cycling females, but the relationship between corticoid concentrations and periods of anoestrus was not evaluated (Jurke *et al.* 1997).

The cause of sustained oestradiol elevation observed in one cheetah before death is not known (Fig. 4). There was no known involvement of either the adrenal glands or the ovaries with the lymphosarcoma (Marker-Kraus *et al.* 1997), although the elevation could be due to increased oestrogen production associated with the disease process, or to the secretion of another steroid that cross-reacts with the antibody. The marked elevation in corticoid concentrations during the week prior to death was not unexpected.

In conclusion, faecal steroid analysis is a powerful tool enabling long-term endocrine monitoring of intractable species. This study provides valuable preliminary data on the basic reproductive physiology of cheetahs living in their natural habitat. Application of the knowledge gained through long-term monitoring, as described herein, to studies of captive and free-ranging populations will assist in the improvement of captive breeding programs as well as management of wild populations.

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