Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal steroids

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Faecal oestradiol and progestogen metabolite excretion was monitored in adult, female cheetahs (*Acinonyx jubatus*) ($n = 26$) for 1–24 months. Increased faecal oestradiol excretion was associated with mating or equine chorionic gonadotrophin (eCG) administration for artificial insemination, whereas increased progestogen metabolites were observed during natural and human chorionic gonadotrophin (hCG)-induced pregnant and nonpregnant luteal phases. On the basis of oestradiol excretory patterns, duration of the oestrous cycle (mean ± SEM) was 13.6 ± 1.2 days with high oestradiol concentrations lasting for 4.1 ± 0.8 days. In non-gonadotrophin-treated cheetahs, 75% showed evidence of oestrous cyclicity; however, none evaluated for 1 year or longer were continuously cyclic. Rather, cyclicity was interrupted by periods of anoestrus, often exceeding several months in duration. These inactive ovarian periods were unrelated to season and were not synchronous among females. Mean duration of gestation (breeding to parturition) was 94.2 ± 0.5 days, whereas duration of faecal progestogen metabolite excretion during the nonpregnant luteal phase was 51.2 ± 3.5 days. On the basis of progestogen metabolite evaluations, spontaneous ovulation (non-mating induced) occurred only once in two females (2 of 184 oestrous cycles; 1.1%). Peak eCG-stimulated, preovulatory oestradiol concentrations were similar to those associated with natural oestrus, whereas progestogen metabolite profiles after hCG resembled those during pregnant and nonpregnant luteal phases after natural mating. In summary, results confirm that the cheetah is polyoestrus and ovulation is almost always induced. However, new evidence suggests that many females inexplicably experience periods of anoestrus unrelated to season, while 25% of the cheetahs examined expressed no ovarian activity during the study period.

Introduction

It is estimated that fewer than 15 000 wild cheetahs (*Acinonyx jubatus*) remain in southern and eastern Africa, and their continued existence is threatened by many factors, including predation and competition by other carnivores, especially lions and hyenas, and extermination by humans (Laurenson *et al.*, 1992; Marker-Kraus and Grisham, 1993; Caro, 1994). Cheetahs in captivity and in the wild also suffer from a lack of genetic diversity which may negatively impact reproductive function and affect long-term survival (O'Brien *et al.*, 1983, 1985). Even so, the reproductive rate of free-ranging cheetahs appears to be relatively high with perhaps 80% of adults producing offspring during their lifetime (Laurenson *et al.*, 1992). In contrast, the species has proven difficult to breed in captivity despite considerable effort (Guggisburg, 1975). Only about one-third

of zoo-maintained cheetahs have ever reproduced and infant mortality, usually related to maternal neglect, averages 30–40% (Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993).

Possible causes of poor fertility in captivity were determined by conducting a reproductive survey of North American cheetahs (sanctioned by the Cheetah Species Survival Plan) between January 1990 and June 1991 (Wildt *et al.*, 1993). In general, reproductive tract anatomy and pituitary function were normal in most adult females irrespective of breeding success. Furthermore, although male cheetahs naturally produce a high proportion of malformed spermatozoa (Wildt *et al.*, 1983, 1987), there were no differences in seminal quality between proven and unproven breeders (Wildt *et al.*, 1993). In contrast, > 50% of females appeared acyclic on the basis of laparoscopic observations of inactive ovaries combined with parallel, one-time measurements of baseline circulating ovarian steroids. This survey was the first organized and

comprehensive attempt (60 males:68 females at 18 institutions) to identify possible biological causes of poor reproduction in cheetahs and suggested that poor fecundity in captivity may reflect suboptimal husbandry and management conditions rather than a fundamental loss in reproductive fitness. Longitudinal studies now are needed to evaluate more fully the dynamics of reproductive steroid secretion in this species.

In the present study, non-invasive faecal steroid monitoring was used to evaluate reproductive events in cheetahs to confirm and explain the apparent lack of ovarian cyclicity. In addition, new data were generated on the 'normality' of ovarian responses to exogenous hormonal ovulation induction and artificial insemination protocols by comparing faecal steroid profiles in pregnant versus nonpregnant animals after natural mating.

Materials and Methods

Animals and faecal sample collection

Study animals included adult, female cheetahs maintained at: the Phoenix Zoo, Phoenix, AZ ($n = 4$; 5.8 ± 3.4 years of age, range = 3–10 years); the Metro Toronto Zoo, Toronto ($n = 3$; 5.7 ± 1.7 years of age, range = 3.5–9 years); the White Oak Conservation Center, Yulee, FL ($n = 8$; 6.4 ± 1.2 years of age, range = 2.5–12 years); the Sacramento Zoo, Sacramento, CA ($n = 2$; both 2.5 years of age); and Wildlife Safari, Winston, OR ($n = 6$; 5.5 ± 1.7 years of age, range = 3–13 years). Five animals at the White Oak Conservation Center and the two Sacramento Zoo cheetahs were monitored on two separate occasions. Three additional females maintained at the Caldwell Zoo, Tyler, TX (8.3 ± 2.2 years of age, range = 4–11 years) were subjected to ovulation induction for laparoscopic artificial insemination (see below) twice, at an interval of eight months. Faecal samples were collected 3–7 times a week from all cheetahs for periods of 1–24 months and were stored frozen ($-20°C$) in 50 ml conical polypropylene vials until processed.

Cheetah management differed markedly among institutions making it impossible to correlate husbandry practices with specific biological events. Social groups and caging situations also varied throughout the year even within institutions. However, there was consistency in that all animals were exposed to natural fluctuations in photoperiod and each institution had a least one male housed within olfactory proximity to females. In general, females were housed with other females (at least occasionally) and, with the exception of one cheetah at the Wildlife Safari and two at the White Oak Conservation Center, all had been exposed to males for breeding (although not necessarily during the study period). Breeding strategies varied: some females were introduced to a male on a single day when she appeared in oestrus (affective behaviour, rolling, calling or lordodic posturing), whereas others were housed with a male for various time periods (days or weeks). Cheetahs at the Sacramento Zoo, Phoenix Zoo and White Oak Conservation Center were fed Nebraska Canine Diet (North Platte, NE), supplemented weekly with bones, chicken carcasses or horse ribs. Cheetahs at the Wildlife Safari were fed carcass meat only (horse, cow, deer, chicken, turkey) supplemented with calcium and vitamins. Animals at the Metro

Toronto Zoo were fed ground horsemeat supplemented with minerals/vitamins and whole carcasses (rabbit, guinea pig).

Semen collection, induction of ovulation and artificial insemination

Electroejaculates for artificial insemination were collected from two males at the Caldwell Zoo (6 and 7 years of age). Semen was collected under ketamine HCl ($15–20$ mg kg^{-1}, i.m.; Vetalar$^{\text{R}}$; Parke-Davis, Morris Plains, NJ) anaesthesia administered via a projectile dart as described by Howard *et al.* (1992). In brief, an AC sine-wave electroejaculator with rectal probe was used in a regimented sequence consisting of 80 incremental electrical stimuli (3–7 volts) given in an on–off pattern in three series over about 20 min (Wildt *et al.*, 1983, 1987, 1993). Total ejaculate volume, sperm cell concentration and progressive motility of spermatozoa were determined as described by Wildt *et al.* (1983, 1987, 1993), and Howard *et al.* (1992). Each ejaculate was diluted (1:1) with Ham's F10 medium (Irvine Scientific, Santa Ana, CA) containing 5% (v/v) heat-inactivated fetal calf serum (Irvine Scientific), centrifuged (at 300 *g* for 10 min); the supernatant was discarded and the sperm pellet resuspended gently in 250–300 µl of fresh Ham's F10 medium (Howard *et al.*, 1992).

Cheetahs designated for artificial insemination were induced to ovulate using a gonadotrophin regimen established by Howard *et al.* (1992). In brief, equine chorionic gonadotrophin (eCG; 200 iu; Sigma Chemical Co., St Louis, MO) and human chorionic gonadotrophin (hCG; 100 iu; Sigma Chemical Co.) were injected i.m., 80 h apart to stimulate follicular development and ovulation, respectively. Intrauterine insemination was performed laparoscopically about 45 h after hCG injection using a method similar to that described by Howard *et al.* (1992). Anaesthesia was induced with ketamine HCl (5–10 mg kg^{-1}, i.m.) and xylazine (0.5–2 mg kg^{-1}, i.m; Rompun$^{\text{R}}$, Miles Laboratory, Inc., Shawnee Mission, KS) administered via a projectile dart. Surgical anaesthesia was maintained with isoflurane gas–oxygen administered via intubation. Each cheetah was placed in a supine head-down position, a pneumoperitoneum produced and a 10 mm laparoscope (Olympus Corporation, Lake Success, NJ) inserted at the midline. An accessory grasping forcep was used to stabilize the uterine horn and an 18-gauge catheter (Sovereign$^{\text{R}}$, Sherwood Medical, St Louis, MO) was inserted transabdominally into each uterine horn as a conduit for sterile polyethylene tubing (PE-10; Intramedic$^{\text{R}}$, Clay Adams Parsippany, NJ) containing about 10×10^6 motile spermatozoa in Ham's F10 medium. The PE tubing was placed into the uterine lumen beyond the tip of the catheter and the diluted spermatozoa (125–150 µl per horn) were expelled.

Faecal steroid analysis

Faecal oestradiol and progestogen metabolites were extracted from samples as described by Brown *et al.* (1994, 1995). Briefly, samples were lyophilized, pulverized and about 0.2 g well-mixed powder boiled in 5 ml aqueous ethanol 90% (v/v) for 20 min. After centrifuging at 500 *g* for 10 min, supernatant was recovered and the pellet resuspended in 5 ml 90% ethanol, vortexed for 1 min and re-centrifuged. Both

ethanol supernatants were combined, dried completely and then redissolved in 1 ml methanol. Extractants were vortexed (1 min), placed in an ultrasonic cleaner for 30 s and re-vortexed (15 s). Samples were diluted (1:40 for oestradiol; 1:800–1:80 000 for progestogens) in PBS (0.01 mol PO_4 l^{-1}, 0.14 mol NaCl l^{-1}, 0.5% (w/v) BSA, 0.01% (w/v) NaN_3) before analysis. Recovery of [^{3}H]oestradiol and [^{14}C]progesterone (New England Nuclear, Wilmington, DE) added to faecal samples before extraction exceeded 90%.

Faecal oestradiol and progestogen metabolites were quantified using radioimmunoassays validated for cheetahs as described by Brown *et al.* (1994). The oestradiol radio-immunoassay relied upon an antibody provided by S. Wasser (Center for Wildlife Conservation, Seattle, WA) (Risler *et al.*, 1987), a ^{3}H-labelled oestradiol tracer (New England Nuclear) and oestradiol standards. This assay specifically quantified faecal oestradiol, with minimal crossreactivity ($\leq 2\%$) with other faecal oestrogen metabolites (oestradiol sulfate and oestrone). The progesterone radioimmunoassay relied upon a monoclonal progesterone antibody produced against 4-pregnen-11-ol-3,20-dione hemisuccinate:BSA (331; provided by J. Roser, University of California, Davis, CA), an ^{125}I-labelled progesterone tracer (ICN Biomedical, Inc., Costa Mesa, CA) and progesterone standards. The assay specifically quantified the major conjugated progestogen metabolite(s) and several free pregnanolone epimers (Brown *et al.*, 1994). Assay sensitivities, based on 90% of maximum binding, were 5 pg per tube and 7.5 pg per tube for the oestradiol and progesterone assays, respectively. Intra- and interassay coefficients of variation were < 10% for both assays. All faecal data are expressed as g^{-1} dry mass.

Statistical analyses

Significant increases in faecal oestradiol concentrations were determined by an iterative process in which high values were excluded if they exceeded the mean + 1.5 SD. Baseline values were those remaining after all high values had been excluded. The duration of the oestrous cycle was calculated as the number of days between peaks in oestradiol concentrations (presumed to be associated with oestrus) for periods not exceeding 30 days (that is, > twice the estimated oestrous cycle duration: Eaton and Craig, 1973; Bertschinger *et al.*, 1984; Asa *et al.*, 1992). Interoestradiol peak intervals > 30 days were considered as anoestrous periods. The number of days on which oestradiol was raised above baseline (indicative of oestrus) was calculated only during periods when faecal samples were collected for a minimum of five times per week. Data from females monitored < 60 days and during pregnant and nonpregnant luteal phases were not included in oestrous cycle calculations. In females subjected to induction of ovulation and artificial insemination, baseline oestradiol concentrations were calculated from all samples before induction of ovulation. The beginning of the oestradiol surge was determined by a value that exceeded preceding values by 50%. Basal progestogen metabolite concentrations were calculated from values preceding preovulatory oestradiol surges. Postovulatory increases in progestogen metabolite excretion were considered significant if values exceeded the mean ± 2 SD of the preceding

values and remained high for at least 1 week. Mean progestogen metabolite concentrations during pregnant and non-pregnant luteal phases contained values from the time of observed mating or artificial insemination to parturition or the sustained return of progestogen metabolite excretion to baseline values. Weekly or three times weekly means were calculated for each individual female and then averaged to provide the respective group means. Differences in preovulatory peak oestradiol concentrations or mean progestogen metabolite concentrations between pregnant and nonpregnant luteal phases, or gonadotrophin-treated versus naturally mated females, were determined using Student's *t* tests. Data are presented as means ± SEM.

Results

General observations

On the basis of 184 cycles from 18 individuals, oestrous cycle duration was 13.6 ± 1.2 days (range, 5–30 days) with increases in oestradiol concentrations lasting 4.1 ± 0.8 days (range, 1–14 days; *n* = 132 cycles). When partitioned by duration, the percentages of oestrous cycles ≤ 7, 8–13, 14–19 and ≥ 20 days in length were 20, 28, 35 and 17%, respectively. There was considerable variation in the duration of the oestrous cycle, both within and among individuals. For females evaluated for ≥ 1 year, overall mean duration of the oestrous cycle (average of all oestrous cycles for each individual) ranged from 10.4 ± 1.0 to 19.0 ± 2.2 days. Even within a female, oestrous cycle duration typically spanned the entire range from < 7 to > 20 days.

Baseline oestradiol concentrations generally ranged from 25–60 ng g^{-1} dry faecal mass with peak concentrations ranging from 100 to 750 ng g^{-1}. There were no differences ($P > 0.05$) in peak preovulatory oestradiol concentrations between animals that conceived (284.3 ± 45.5 ng g^{-1}; *n* = 5) and those that mated but did not conceive (314.8 ± 41.9 ng g^{-1}; *n* = 8) (Fig. 1). Similarly, there were no differences ($P > 0.05$) in preovulatory oestradiol concentrations between naturally-mated and eCG-treated (281.0 ± 39.6 ng g^{-1}; *n* = 6) females (Fig. 1). Peak oestradiol concentrations in nonmated females averaged 302.1 ± 12.3 ng g^{-1}. Faecal oestradiol excretion during pregnancy tended to remain at baseline values until several weeks before parturition, when concentrations increased up to tenfold and then declined after parturition (Fig. 1). In contrast, mean oestradiol concentrations during the nonpregnant luteal phase generally remained at baseline values, although random peaks occasionally occurred.

Average baseline faecal progestogen metabolite concentrations among individuals ranged from 0.7 to 6.0 µg g^{-1}. Faecal progestogens in ovulating females increased within 1–10 days of the oestradiol surge. In pregnant females, concentrations remained 100- to 400-fold greater than baseline throughout gestation, rarely decreasing to less than 20-fold over baseline until near parturition (Fig. 1). There were no differences ($P > 0.05$) in overall mean progestogen metabolite concentrations between pregnant (202.9 ± 15.3 µg g^{-1}) and nonpregnant (240.6 ± 26.4 µg g^{-1}) cheetahs during the period

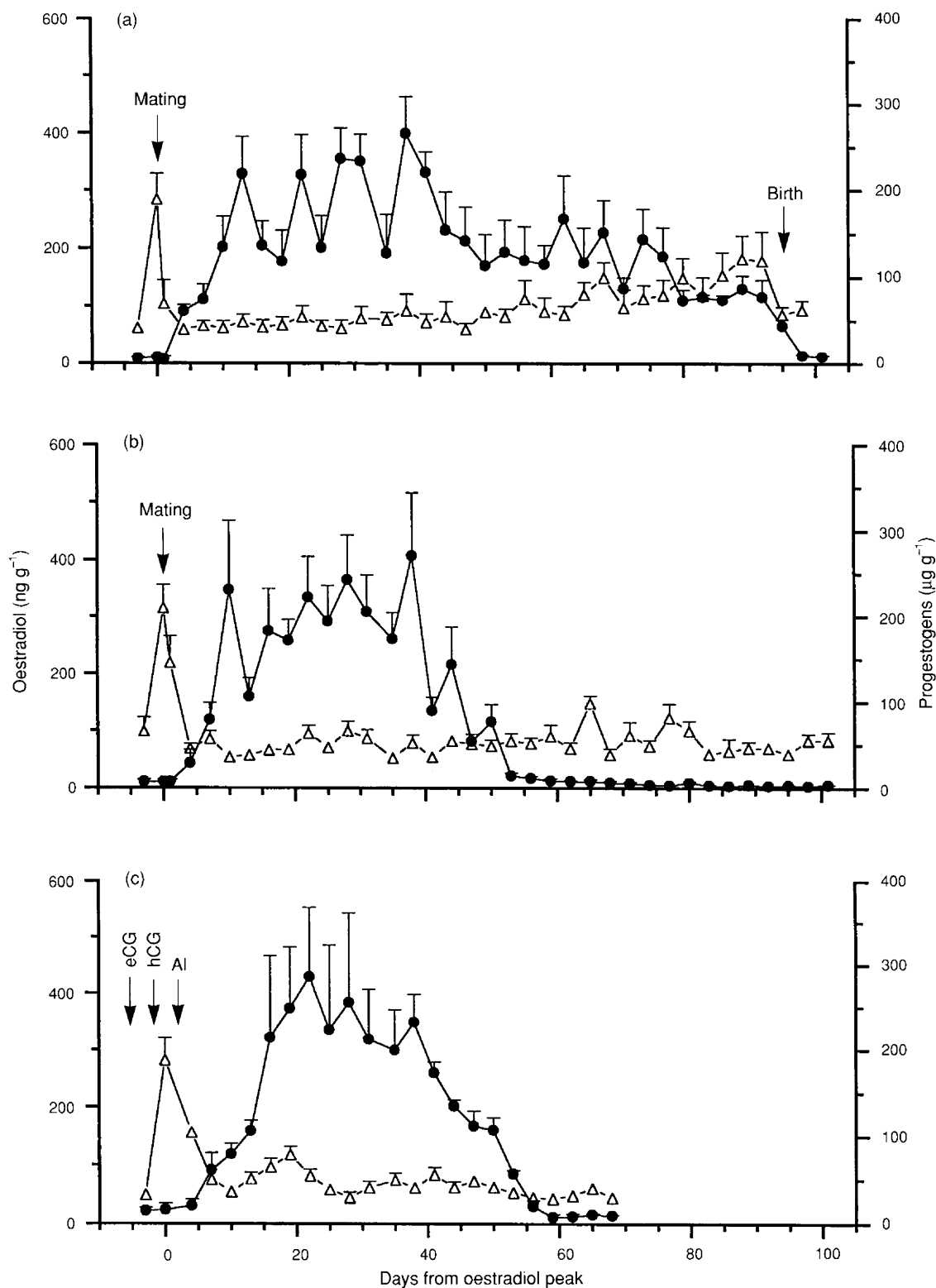**Fig. 1.** Mean (± SEM) faecal oestradiol (△) and progestogen (●) metabolite concentrations in (a) pregnant (*n* = 5) and (b) nonpregnant (*n* = 8) cheetahs after natural mating and in (c) nonpregnant cheetahs after induction of ovulation by gonadotrophin and artificial insemination (AI) (*n* = 6). Data are aligned to the oestradiol peak (day 0).

of increased excretion or between the nonpregnant luteal phases of gonadotrophin-stimulated (247.1 ± 29.9 μg g^{-1}) versus naturally-mated individuals (Fig. 1). Mean duration of

gestation (from the day of observed mating or preovulatory oestradiol surge to birth) was 94.2 ± 0.5 days (range, 93–96 days), whereas the duration of the nonpregnant luteal phase
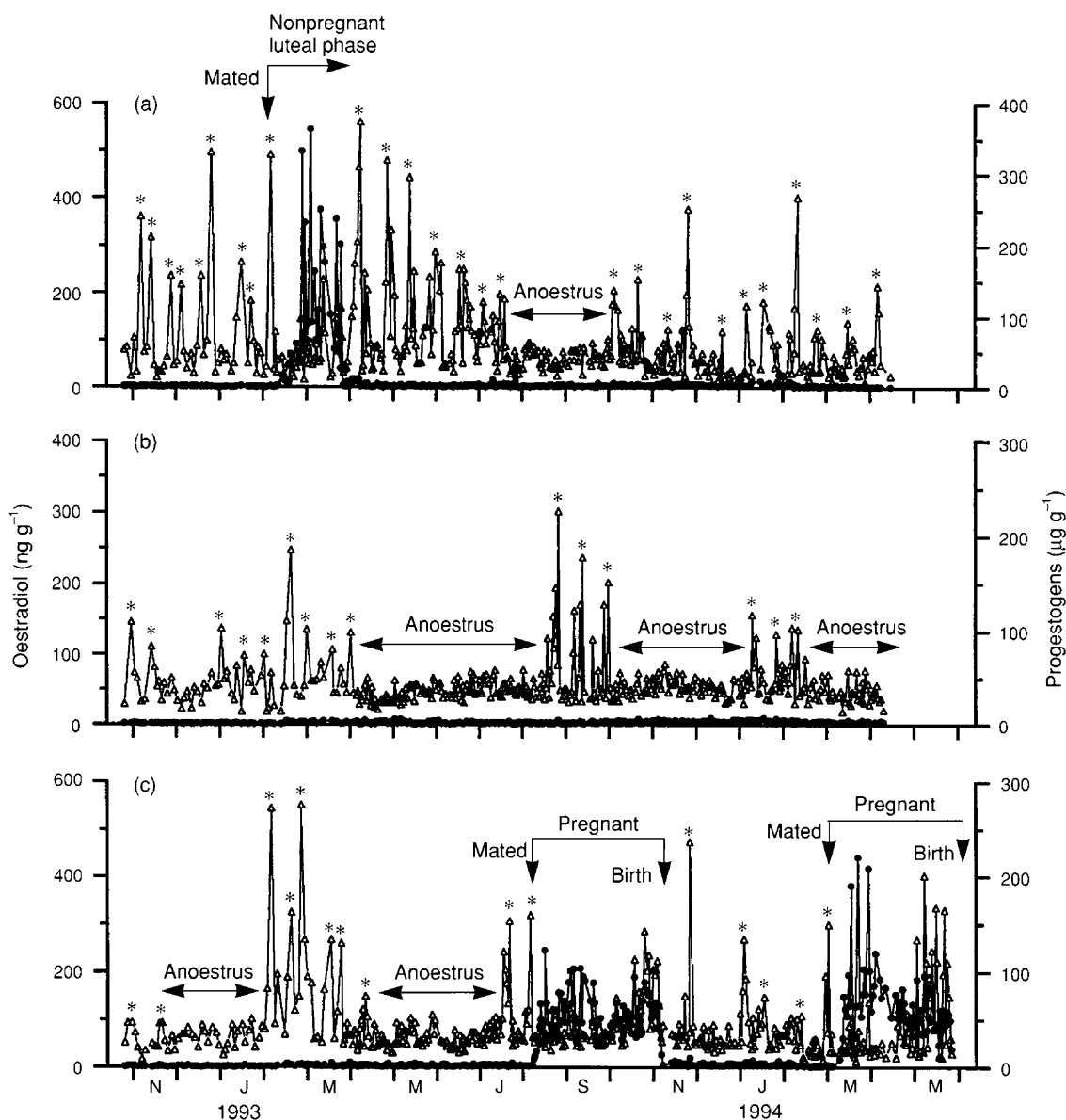
Fig. 2. Representative individual longitudinal profiles of faecal oestradiol (△) and progestogen (●) metabolite concentrations in female cheetah at the Phoenix Zoo. Asterisks denote peaks in oestradiol excretion significantly above the baseline.

was about half ($P < 0.05$) that of pregnancy (51.2 ± 3.5 days; range, 38–59 days).

Longitudinal endocrine evaluations

Eighteen of 24 cheetahs (75%) monitored for 60 days or more exhibited some evidence of oestrous cyclicity on the basis of regular fluctuations in oestradiol excretion. In addition, all individuals monitored ≥ 1 year ($n = 7$) expressed cyclic activity, although none were continuously cyclic (Figs 2 and 3). Instead, follicular activity was interrupted by anoestrous periods, 2–5 months in duration, that were neither synchronous among females within facilities nor associated with season or other obvious environmental fac-

tors. In females identified as acyclic, faecal monitoring had been conducted for 90 days or fewer. Two cheetahs (depicted in Fig. 2b,c) tended to express cyclic activity when the third female (Fig. 2a) was reproductively inactive (during a nonpregnant luteal phase or anoestrus). The cheetah in Fig. 2a was older (10 years) and was cyclic about 80% of the time compared with the two younger sibling females (3 years; Fig. 2b,c), each of which was cyclic about 40% of the time. Similarly, the cheetahs depicted in Fig. 3 also displayed periods of anoestrus that were not synchronous. In the female depicted in Fig. 2c, two pregnancies occurred within one year. At the end of the first pregnancy, a single cub was born and removed for hand-rearing which resulted in a resumption of ovarian cyclicity within one week.
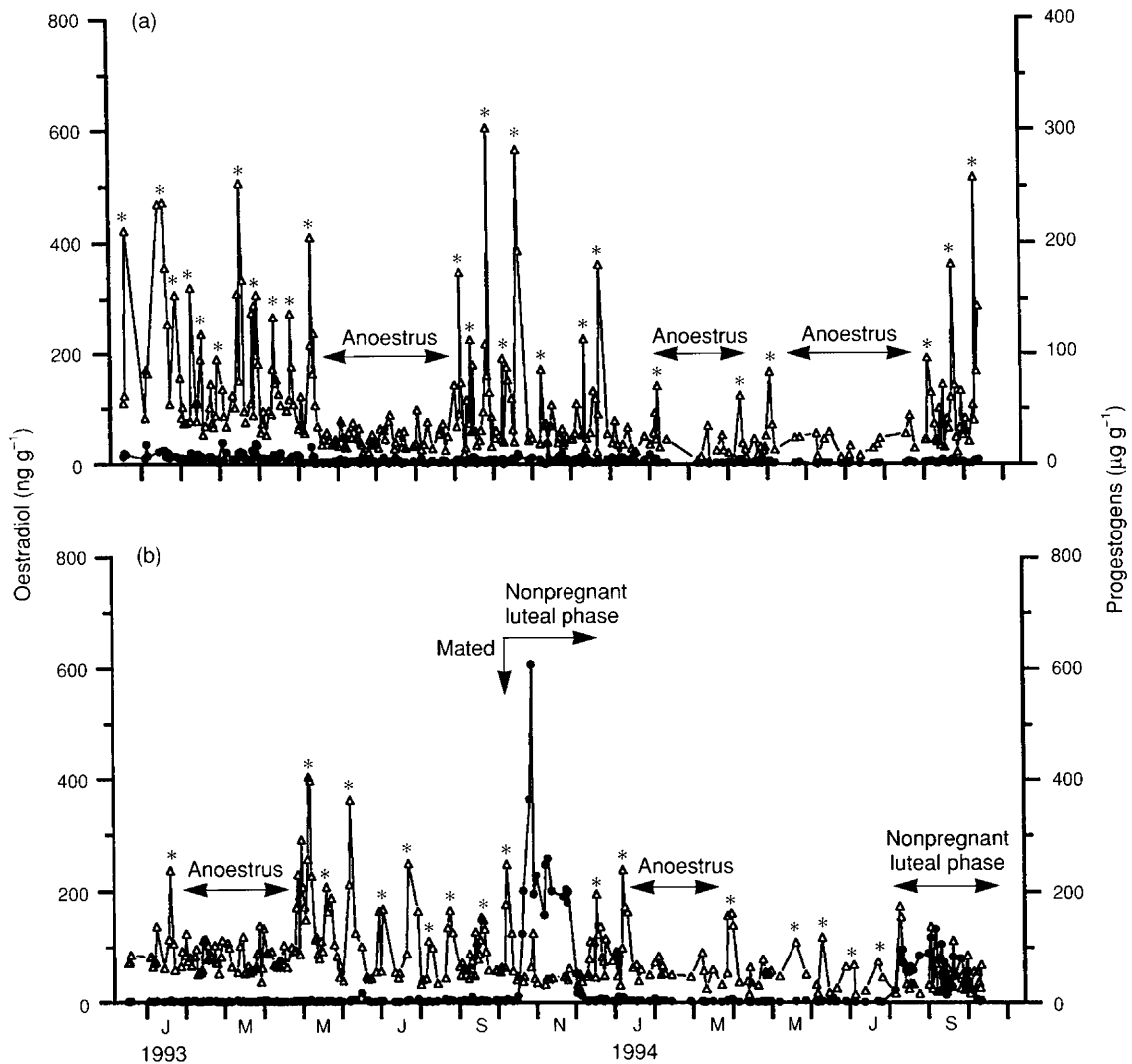
Fig. 3. Representative individual longitudinal profiles of faecal oestradiol (△) and progestogen (●) metabolite concentrations in female cheetah at the Metro Toronto Zoo. Asterisks denote peaks in oestradiol excretion significantly above the baseline.

In almost all cases, episodic increases in oestradiol excretion (presumed indicative of oestrus) occurred without a subsequent rise in progestogen metabolite excretion in non-mated females (even those housed with other females), indicating a lack of spontaneous ovulation. However, two females were exceptions, exhibiting significant increases in faecal progestogen metabolite concentrations after an oestradiol surge in the absence of physical contact with a male. Progestogen excretory patterns in these two females were similar to those observed after induced ovulations, although overall concentrations tended to be lower (Fig. 4). In one case, increased progestogen excretion was observed within days after the female was relocated to a new enclosure and a male was moved into the adjacent pen on the same day (Fig. 4a). In the other case, the female was translocated and a male introduced into her enclosure one week later. The male showed interest in the female (calling, approaches to female), but was removed from the enclosure before mounting occurred (Fig. 4b).

Endocrine patterns after ovulation induction and artificial insemination

In general, oestradiol concentrations increased four- to tenfold after eCG injection (Figs 1 and 5). In the female becoming pregnant after gonadotrophin therapy and artificial insemination, increased progestogen metabolite excretion was sustained throughout the 94 day gestation, although concentrations fluctuated markedly throughout the luteal period (Fig. 5a). In one female that failed to conceive, similar increases in progestogen metabolite concentrations were apparent for 57 days after the gonadotrophin-induced oestradiol surge (Fig. 5b). The individual shown in Fig. 5c had only two distinct follicles > 2 mm in diameter and no corpora lutea at the time of laparoscopy and, based on a lack of increased progestogen metabolite excretion, ovulation never occurred in response to hCG. A similar anovulatory profile was observed in this female in the subsequent procedure for induction of ovulation. The steroid excretory
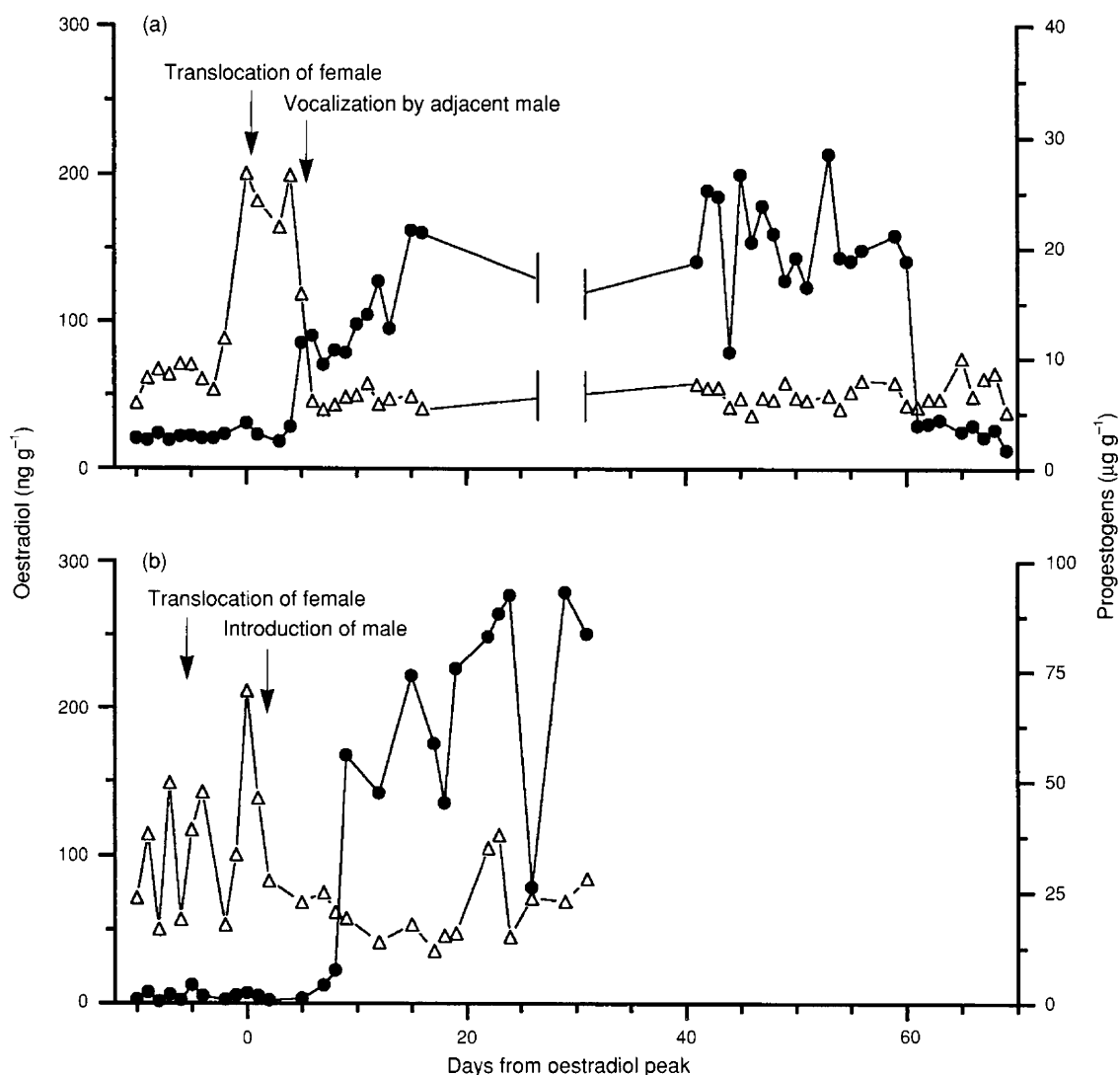
Fig. 4. Individual profiles of faecal oestradiol (△) and progestogen (●) metabolite concentrations in cheetah females at (a) the White Oak Conservation Center and (b) Wildlife Safari exhibiting spontaneous ovulation (without mounting or intromission by a male). The female in (a) was relocated to a new enclosure and a male moved into the adjacent enclosure on the same day. Five days later the male showed interest in the female by vocalizing a 'stutter call'. In (b), the female was translocated and a male introduced into her enclosure 1 week later. The male showed interest in the female (calling, approaches to female), but was removed from the enclosure before mounting occurred.

profiles of the remaining individual not conceiving after insemination were similar to that depicted in Fig. 5b.

Discussion

Several studies have used faecal oestradiol and progestogen metabolite analyses to examine ovarian activity in cheetahs; however, endocrine assessments were of short duration (< 90 days) (Brown *et al.*, 1994; Czekala *et al.*, 1994) or based on only a few animals ($n = 5$, Brown *et al.*, 1994; $n = 7$, Czekala *et al.*, 1994; $n = 2$, Graham *et al.*, 1995). The present study evaluated endocrine patterns for extended periods (up to 24 months) in 26 individuals ($n = 36$ total observations, since ten females were evaluated twice) to examine seasonality,

ovulatory mechanisms (spontaneous versus induced) and steroid profiles during pregnancy versus 'pseudopregnancy' (the nonpregnant luteal phase) more comprehensively. This information was then used to evaluate possible causes of poor reproductive performance in captive cheetahs.

In general, the 13.6 day oestrous cycle determined in this study by faecal oestradiol analysis was consistent with the 12–14 day cycle proposed by Eaton and Craig (1973), Bertschinger *et al.* (1984) and Asa *et al.* (1992), based on behavioural observations, plasma oestradiol concentrations and vaginal cytology, respectively. Together these data confirm that the cheetah is unique among the 'great' cats in exhibiting a shorter cycle on average than the approximately 20–30 day cycle reported for large felids (lion, *Panthera leo*, Schmidt *et al.*, 1979; puma, *Felis concolor*, Bonney *et al.*, 1981; tiger, *Panthera*
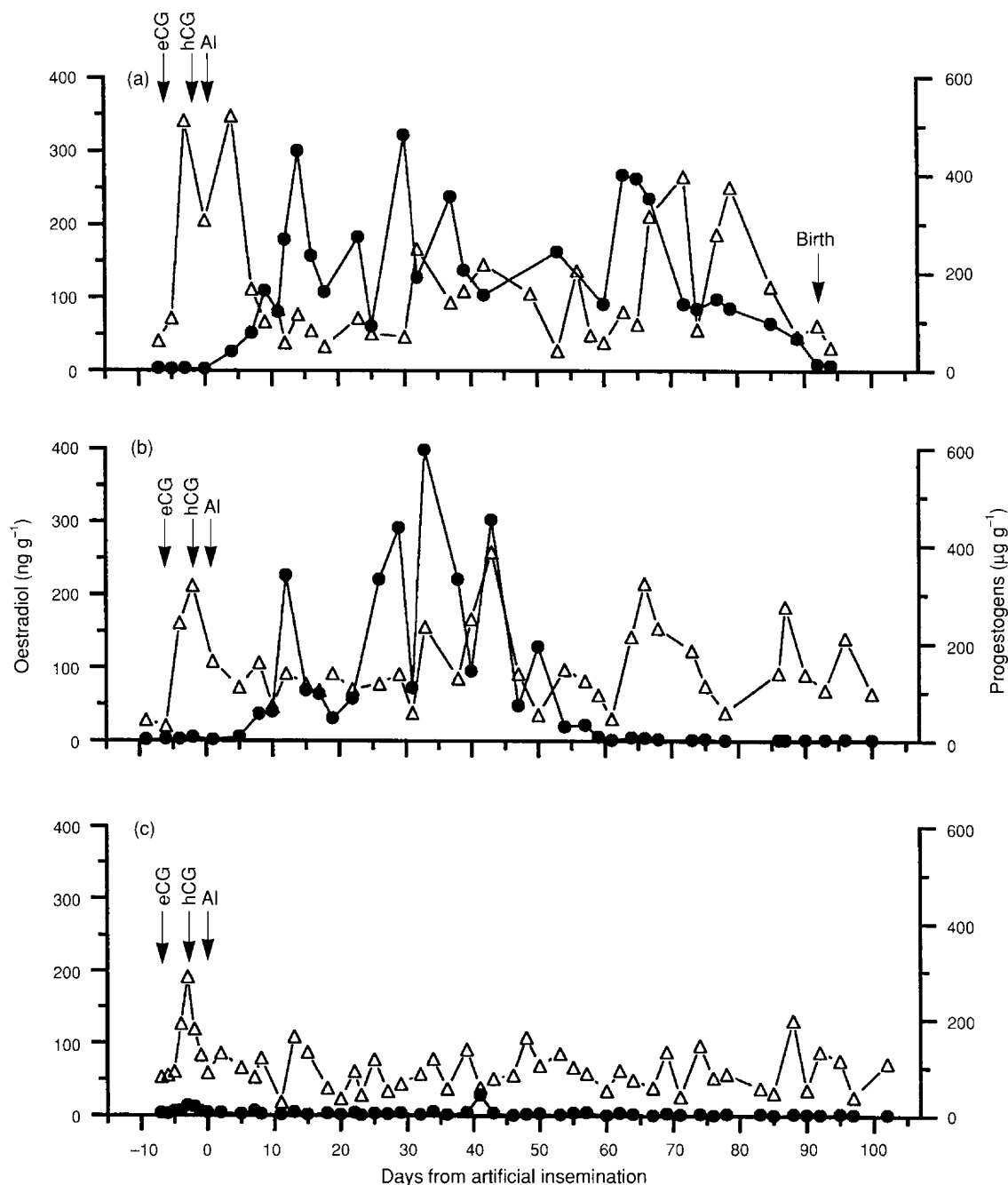
Fig. 5. Individual profiles of faecal oestradiol (△) and progestogen (●) metabolite concentrations in (a) pregnant, (b) nonpregnant and (c) anovulatory female cheetah subjected to ovulation induction and artificial insemination at the Caldwell Zoo. Females were injected i.m. with 200 iu eCG followed 80 h later by 100 iu hCG and artificial insemination (AI) 46–48 h after hCG.

tigris, Seal *et al.*, 1985; leopard, *Panthera pardus*, Schmidt *et al.*, 1988; snow leopard, *Panthera uncia*, Schmidt *et al.*, 1993; clouded leopard, *Neofelis nebulosa*, Brown *et al.*, 1995). However even with daily faecal collection, there was considerable variation within and among individuals in oestrous cycle dynamics. Such variability in follicular steroid and behavioural cycle activity is common within species and members of the Felidae (Eaton and Craig, 1973; Kleiman, 1974; Schmidt *et al.*, 1979, 1988, 1993; Bonney *et al.*, 1981; Seal *et al.*, 1985; Yamada

and Durrant, 1989; Asa *et al.*, 1993; Brown *et al.*, 1995; Graham *et al.*, 1995) and, in part, may be related to the induced ovulatory characteristics of these species. Compared with spontaneous ovulators, the signalling of oestrus onset and its termination in felids appears to be less finely regulated, making cyclicity more variable.

During pregnancy, progestogen metabolite concentrations increased several hundred-fold above baseline values, peaked about mid-term and then gradually declined until after

parturition, in a similar way to circulating blood progesterone concentrations in domestic cats (Schmidt *et al.*, 1983). In mated females that failed to conceive, the duration of the nonpregnant luteal phase was about half that of pregnancy. Except for duration of excretion, however, there were no obvious qualitative or quantitative differences in progestogen metabolite profiles between pregnant and nonpregnant cheetahs, which is typical of observations in other felids (domestic cat, Shille and Stabenfeldt, 1979; Wildt *et al.*, 1981; lion, Schmidt *et al.*, 1979; Graham *et al.*, 1995; puma, Bonney *et al.*, 1981; leopard, Schmidt *et al.*, 1988; snow leopard, Schmidt *et al.*, 1993; clouded leopard, Brown *et al.*, 1995). Two of 26 cheetahs in the study population showed evidence of spontaneous (nonmating-induced) ovulations on the basis of increased faecal progestogens after an increase in oestradiol. During the reproductive survey, Wildt *et al.* (1993) observed no active luteal tissue on the ovaries of nonpregnant or nonlactating females and concluded that cheetahs were induced ovulators. However, distinct luteal scars were found on the ovaries of 12% of cheetahs that had never produced young, although mating histories were unknown. In other studies, neither Czekala *et al.* (1994) nor Bertschinger *et al.* (1984) found evidence of nonmating-induced ovulations, whereas Asa *et al.* (1992) did quantify a sustained increase in serum concentrations of progesterone in a singleton cheetah. Comparatively, no 'spontaneous' ovulations have been reported in pumas (Bonney *et al.*, 1981), tigers (Seal *et al.*, 1985) or snow leopards (Schmidt *et al.*, 1993), whereas occasional non-mating-induced ovulations have been observed in lions (Schramm *et al.*, 1994) and clouded leopards (Brown *et al.*, 1995; Howard *et al.*, in press) maintained in female groups or as singletons, and in leopard females housed together, but not alone (Schmidt *et al.*, 1988). Taken together, these data demonstrate that, while usually ovulating only after copulation, ovulation in some individual felids can be triggered occasionally by physical or psychosocial stimuli unrelated to mating. Furthermore, although the incidence of spontaneous ovulations may vary among species, for cheetahs it appears to be extremely low.

In the survey of Wildt *et al.* (1993), females demonstrated minimal, if any, ovarian activity at the time of examination. Although about 67% of the surveyed population had at least one ovarian follicle ≥ 2 mm in diameter, only 23% had ovaries containing follicles considered mature (≥ 4 mm). Most of these surveyed cheetahs also had low serum oestradiol concentrations, further suggesting they were reproductively inactive (Wildt *et al.*. 1993). However, this single-point-in-time survey was not designed to evaluate ovarian dynamics. By monitoring longitudinal ovarian steroid excretion, our study found that, over prolonged periods, 75% of the cheetahs did exhibit some follicular activity. In addition, all females examined for a year or more demonstrated waves of follicular activity for as little as 25% and up to 80% of the time. None of this reproductive activity appeared to be seasonally mediated. It now remains to be determined what mediates this discontinuous ovarian cyclicity in cheetahs and how (or if) it affects overall reproductive performance. Several physiological causes were eliminated by the survey of Wildt *et al.* (1993), which reported no differences in reproductive tract anatomy, pituitary function or gonadal activity between proven and unproven breeders. It also is known that cheetahs exhibit a high degree of genetic

monomorphism (O'Brien *et al.*, 1983, 1985) and that loss of heterozygosity reduces overall reproductive fitness in other species (Ralls *et al.*, 1979; Ralls and Ballou, 1983). This reduced genetic diversity probably accounts for the extremely high percentage of morphologically abnormal spermatozoa noted in cheetah electroejaculates (Wildt *et al.*, 1983, 1987; Wildt, 1994). However, poor ejaculate quality and low genetic diversity are observed in both captive cheetahs and in successfully reproducing free-living cheetahs (O'Brien *et al.*, 1983, 1985; Wildt *et al.*, 1987). Thus, genetic factors alone are not likely to be the major contributors to poor fertility within the captive population. Rather, behavioural problems may be an underlying cause of poor reproductive success in cheetahs (Laurenson *et al.*, 1992; Caro, 1993, 1994). Breeding success is associated with widely varying husbandry practices and no single method has proven successful across institutions (Caro, 1993). Furthermore, the fact that behavioural signs often are difficult to interpret in cheetahs reinforces the need for integrated studies correlating behavioural observations with actual endocrine events. The unexpected finding that animals within the same institution often alternated periods of oestrous cyclicity leads to speculation that reproductive suppression may be occurring among some cheetah females housed together or in close proximity. Although not documented within the Felidae (most of which are solitary), reproductive suppression of subordinates occurs in many social species, including callitrichid primates (Abbott, 1984; Epple and Katz, 1984; French *et al.*, 1984), naked mole rats (*Heterocephalus glaber*, Faulkes *et al.*, 1990), dwarf mongooses (*Helogale parvula*, Creel *et al.*, 1992) and African wild dogs (*Lycaon pictus*, Frame *et al.*, 1979; Fuller *et al.*, 1992). In the wild, female cheetahs tend to travel alone, whereas males live in stable coalitions of 2–3 animals (Laurenson *et al.*, 1992; Caro, 1993, 1994). Keeping males and females together continually in captivity or the absence of male coalitions may be detrimental to promoting natural courtship behaviour in both sexes. This finding warrants further investigation using more controlled experimental procedures.

It is clear that any meaningful evaluation of reproductive status in individual female cheetahs requires long-term evaluation of ovarian activity and emphasizes the power of noninvasive faecal steroid monitoring for assessing reproductive activity. From a practical perspective, faecal steroid analyses will provide critical information needed for making appropriate captive management decisions, especially on how environmental changes or husbandry practices affect reproductive activity. These assays are also an important adjunct tool for assessing ovarian responses to gonadotrophin therapy, allowing for subtle improvements that eventually should permit assisted reproduction to be even more useful for maintaining genetic diversity within small populations.

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