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

Journal of Reproduction and Fertility, 1996 (in press)

J. L. Brown[1], D. E. Wildt[1], N. Wielebnowski[2], K. L. Goodrowe[3], L. H. Graham[1], S. Wells[4] and J. G. Howard[1]

[1]Conservation and Research Center, National Zoological Park, Smithsonian Institution, Front Royal, VA 22630, USA; [2]Department of Wildlife and Fisheries Biology, University of California, Davis, CA 95616, USA; and [3]Metro Toronto Zoo, Scarborough, M1B 5K7, Ontario, Canada; and [4]Department of Mammals, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA

Summary

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 elevated progestogen metabolites were observed during natural and human chorionic gonadotrophin (hCG)-induced pregnant and non-pregnant luteal phases. On the basis of oestradiol excretory patterns, duration of the oestrous cycle (mean ± SEM) was 13.6 ± 1.2 days with elevated oestradiol lasting 4.1 ± 0.8 days. In non-gonadotrophin-treated cheetahs, 75% showed evidence of oestrous cyclicity; however, none evaluated at least 1 year cycled continuously. 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 gestation length (breeding to parturition) was 94.2 ± 0.5 days, whereas duration of faecal progestogen metabolite excretion during the non-pregnant luteal phase was 51.2 ± 3.5 days. On the basis of progestogen metabolite evaluations, spontaneous ovulation (i.e., non-mating induced) occurred only once in two females (2/184 oestrous cycles; 1.1%). Peak eCG-stimulated, pre-ovulatory oestradiol concentrations were similar to those associated with natural oestrus, whereas progestogen metabolite profiles after hCG resembled those during pregnant and non-pregnant luteal phases after natural mating. In sum, results confirm that the cheetah is polyoestrus and ovulation almost always is 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 a host of factors, including predation and competition by other carnivores, especially lions and hyenas, and extermination by humans (Caro, 1994; Laurenson *et al.*, 1992; Marker-Kraus and Grisham, 1993). 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 propagate in captivity despite centuries of 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).

To determine possible causes of poor fertility in captivity, a reproductive survey of North American cheetahs (sanctioned by the Cheetah Species Survival Plan) was conducted between January 1990 and June 1991 (Wildt *et al.*, 1993). In general, the majority of adult females had normal reproductive tract anatomy and pituitary function irrespective of breeding success. Further, 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 the cheetah 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 more fully evaluate 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 the cheetah to confirm and explain the apparent lack of ovarian cyclicity. New data also 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 non-pregnant 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, Canada ($n = 3$; 5.7 ± 1.7 years of age, range = 3.5-9 years); White Oak Conservation Center, Yulee, FL ($n = 8$; 6.4 ± 1.2 years, 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, range = 3-13 years). Five animals at the White Oak Conservation Center and the two Sacramento Zoo cheetahs were monitored on two separate occassions. Three additional females maintained at the Caldwell Zoo, Tyler, TX (8.3 ± 2.2 years, range = 4-11 years) were subjected to ovulation induction for laparoscopic artificial insemination (see below) twice, eight months apart. Faecal samples were collected 3-7 times weekly 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 at 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 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 with some females being 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 varied 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, ovulation induction 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®, Parke-Davis, Morris Plains, NJ) anaesthesia administered via a projectile dart as described previously (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 3 series over ~20 min (Wildt *et al.*, 1983, 1987, 1993). Total ejaculate volume, sperm cell concentration and spermatozoal progressive motility were determined as described previously (Wildt *et al.*, 1983, 1987, 1993; Howard *et al.*, 1992). Each ejaculate was diluted (1:1) with Ham's F10 medium (Irvine Scientific, Santa Ana, CA) containing 5% heat-inactivated fetal calf serum (Irvine Scientific), centrifuged (300 **g**, 10 min), the supernatant 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 previously established gonadotrophin regimen (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 ~45 h after hCG injection similar to that described previously (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®, 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®, Sherwood Medical, St. Louis, MO) was inserted transabdominally into each uterine horn as a conduit for sterile polyethylene tubing (PE-10; Intramedic®, Clay Adams, Parsippany, NJ) containing ~10 x 10^6 motile sperm in Ham's F10 medium. The PE tubing was placed into the uterine lumen beyond the tip of the catheter and the diluted sperm (125-150 µl/horn) expelled.

Faecal steroid analysis

Faecal oestradiol and progestogen metabolites were extracted from samples as described previously (Brown *et al.*, 1994, 1995). Briefly, samples were lyophilized, pulverized and ~0.2 g of well-mixed powder boiled in 5 ml of aqueous ethanol (90% ethanol) for 20 min. After centrifuging at 500 ***g*** for 10 min, supernatant was recovered and the pellet resuspended in 5 ml of 90% ethanol, vortexed for 1 min and re-centrifuged. Both ethanol supernatants were combined, dried completely and then redissolved in 1 ml of methanol. Extractants were vortexed (1 min), placed in an ultrasonic cleaner for 30 sec and re-vortexed (15 sec). Samples were diluted (1:40 for oestradiol; 1:800-1:80 000 for progestogens) in phosphate buffered saline (0.01 mol l^{-1} PO_4, 0.14 mol l^{-1} NaCl, 0.5% BSA, 0.01% NaN_3) before analysis. Recovery of ^{3}H-oestradiol and ^{14}C-progesterone (New England Nuclear, Wilmington, DE) added to fecal samples before extraction exceeded 90%.

Faecal oestradiol and progestogen metabolites were quantified using radioimmunoassays validated for the cheetah as previously described (Brown *et al.*, 1994). The oestradiol radioimmunoassay relied upon an antibody provided by Dr. Samuel Wasser (Center for Wildlife Conservation, Seattle, WA) (Risler *et al.*, 1987), an ^{3}H-labeled oestradiol tracer (New England Nuclear) and oestradiol standards. This assay specifically quantified faecal oestradiol, with minimal crossreactivity (≤ 2%) with other faecal oestrogen metabolites (i.e., 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 Dr. Jan Roser, University of California, Davis, CA), an ^{125}I-labeled 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 tube^{-1} and 7.5 pg tube^{-1} for the oestradiol and progesterone assays, respectively. Intra- and interassay coefficients of variation were <10% for both assays. All faecal data are expressed on a per g dry weight basis.

Data analysis

Significant increases in faecal oestradiol concentrations were determined by an iterative process in which high values were excluded if they exceeded the mean plus 1.5 standard deviations. The highest concentration within a group of elevated samples was considered the peak. Baseline values were those remaining after all high values had been excluded. Oestrous cycle length was calculated as the number of days between oestradiol peaks (presumed to be associated with oestrus) for periods not exceeding 30 days (i.e., > twice the estimated oestrous cycle length: Eaton and Craig, 1973; Bertschinger *et al.*, 1984; Asa *et al.*, 1992). Inter-oestradiol peak intervals >30 days were considered anoestrus periods. The number of days oestradiol was elevated above baseline (indicative of oestrus) was only calculated during periods when faecal samples were collected a minimum of five times per week. Data from females monitored <60 days and during pregnant and non-pregnant luteal phases were not included in oestrous cycle calculations. In females subjected to ovulation induction and artificial insemination, baseline oestradiol concentrations were calculated from all samples before ovulation induction. The beginning of the oestradiol surge was determined by a value which exceeded preceding values by 50%. Basal progestogen metabolite concentrations were calculated from values preceding pre-ovulatory oestradiol surges. Post-ovulatory increases in progestogen metabolite excretion were considered significant if values exceeded the mean plus 2 standard deviations of the preceding values and remained elevated 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. Weekly or three times weekly means were calculated for each individual female and then averaged to provide the respective group means. Differences in pre-ovulatory peak oestradiol concentrations or mean progestogen metabolite concentrations between pregnant and non-pregnant 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 length was 13.6 ± 1.2 days (range, 5-30 days) with elevations in oestradiol 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 oestrous cycle length, both within and among individuals. For females evaluated for ≥ 1 year, overall mean oestrous cycle length (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 length typically spanned the entire range from <7 to >20 days.

Baseline oestradiol concentrations generally ranged from 25-60 ng g^{-1} dry fecal weight with peak concentrations ranging from 100-750 ng g^{-1}. There were no differences ($P > 0.05$) in peak pre-ovulatory oestradiol concentrations between animals that conceived (314.8 ± 41.9 ng g^{-1}; $n = 5$) and those that mated, but did not conceive (284.3 ± 45.5 ng g^{-1}; $n = 8$) (Fig. 1). Similarly, there were no differences ($P > 0.05$) in pre-ovulatory oestradiol concentrations between naturally-mated (314.8 ± 41.9 ng g^{-1}) and eCG-treated (281.0 ± 39.6 ng g^{-1}; $n = 6$) females (Fig. 1). Peak oestradiol concentrations in non-mated females averaged 302.1 ± 12.3 ng g^{-1} ($n = 171$). Faecal oestradiol excretion during pregnancy tended to remain at baseline until several weeks before parturition when concentrations increased up to 10-fold and then declined after parturition (Fig. 1). In contrast, mean oestradiol concentrations during the non-pregnant luteal phase generally remained baseline, although random spikes occasionally occurred.

Average baseline faecal progestogen metabolite concentrations among individuals ranged from 0.7-6.0 μg g^{-1}. Faecal progestogens in ovulating females increased within 1-10 days of the oestradiol surge. In pregnant females, concentrations remained elevated 100 to 400-fold over baseline throughout gestation, rarely decreasing to less than 20-fold over baseline until near or within days after 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 non-pregnant (240.6 ± 26.4 μg g^{-1}) cheetahs during the period of elevated excretion or between the non-pregnant luteal phases of gonadotrophin-stimulated (247.1 ± 29.9 μg g^{-1}) versus naturally-mated individuals (Fig. 1). Mean gestation length (from 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 non-pregnant luteal phase 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. Additionally, all individuals monitored at least one year ($n = 7$) expressed cyclic activity, although none cycled continuously (Figs 2 and 3). Instead, follicular activity was interrupted by anoestrus periods (2-5 months in duration) that were neither synchronous among females within facilities nor associated with season or other obvious environmental factors. In females identified as acyclic, faecal monitoring had been conducted for 90 days or less. Interestingly, two of the cheetahs depicted in Fig. 2 (panels b,c) tended to express cyclic activity when the third female (panel a) was reproductively inactive (during a non-pregnant luteal phase or anoestrus). The cheetah in panel (a) was older (10 years) and cycled ~80% of the time compared to the two younger sibling females (3 years, Fig. 2b,c), each of which cycled ~40% of the time. Similarly, the cheetahs depicted in Fig. 3 also displayed periods of anoestrus which 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.

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 re-located 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 4- to 10-fold 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 period, although concentrations fluctuated markedly throughout the luteal period (Fig. 5a). In one female failing 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 Figure 5c had only 2 distinct follicles > 2 mm in diameter and no corpora lutea at the time of laparoscopy and, based on a lack of elevated progestogen metabolite excretion, ovulation never occurred in response to hCG. A similar anovulatory profile was observed in this female in the subsequent ovulation induction procedure. The steroid excretory profiles of the remaining individuals not conceiving after insemination were similar to that depicted in Fig. 5b.

Discussion

Several recent reports have utilized faecal oestradiol and progestogen metabolite analyses to examine ovarian activity in the cheetah; 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 10 females were evaluated twice) to more comprehensively examine seasonality, ovulatory mechanisms (spontaneous versus induced) and steroid profiles during pregnancy versus 'pseudopregnancy' (e.g., the non-pregnant luteal phase). This information was then used to try and evaluate possible causes of poor reproductive performance in captive cheetahs.

In general, the 13.6-day estrous 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.* (1993) 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 ~20-30-day cycle reported for large-sized felid species (tiger, *Panthera tigris*, Seal *et al.*, 1985; leopard, *Panthera pardus*, Schmidt *et al.*, 1988; snow leopard, *Panthera uncia*, Schmidt *et al.*, 1993; puma, *Felis concolor*, Bonney *et al.*, 1981; lion, *Panthera leo*, Schmidt *et al.*, 1979; 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 individuals in the Felidae family (Eaton and Craig, 1973; Kleiman, 1974; Schmidt *et al.*, 1979; Bonney *et al.*, 1981; Seal *et al.*, 1985; Schmidt *et al.*, 1988; Yamada and Durrant, 1989; Asa *et al.*, 1993; Schmidt *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 to spontaneous ovulators, the signaling 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, peaked about mid-term and then gradually declined until after parturition, similar to circulating blood progesterone concentrations in the domestic cat (Schmidt *et al.*, 1983). In mated females that failed to conceive, the length of the non-pregnant 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 non-pregnant cheetahs, which is typical of that observed in other felid species (domestic cat, Shille *et al.*, 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). Interestingly, 2 of 26 cheetahs in the study population showed evidence of spontaneous (i.e., non-mating-induced) ovulations on the basis of elevated 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 the species was an induced ovulator. 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 non-mating-induced ovulations, whereas Asa *et al.* (1992) did quantify a sustained increase in serum progesterone concentrations 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 and/or psychosocial stimuli unrelated to mating. Further, 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 ~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 time periods 75% of the cheetahs did exhibit some follicular activity. Additionally, all females examined a year or more demonstrated waves of follicular activity 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 the cheetah and how (or if) it impacts 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 (Wildt *et al.*, 1993). 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 sperm noted in cheetah electroejaculates (Wildt *et al.*, 1983, 1987; 1994). However, poor ejaculate quality and low genetic diversity are observed in both captive cheetahs and their successfully reproducing free-living counterparts (O'Brien *et al.*, 1983; 1985; Wildt *et al.*, 1987). Thus, genetic factors alone likely are not the major contributors to poor fertility within the captive population. Rather, behavioral problems may be an underlying cause of poor reproductive success in the cheetah (Laurenson *et al.*, 1992; Caro, 1993, 1994). Frustratingly, 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 the cheetah strongly 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 taxa (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), the naked mole rat (*Heterocephalus glaber*, Faulkes *et al.*, 1990), dwarf mongoose (*Helogale parvula*, Creel *et al.*, 1992) and African wild dog (*Lycaon pictus*, Frame *et al.*, 1979; Fuller *et al.*, 1992). In the wild, cheetah females generally travel alone whereas males live in stable coalitions of 2-3 animals (Caro, 1993, 1994; Laurenson *et al.*, 1992). Keeping males and females together continually in captivity and/or the absence of male coalitions may be detrimental to promoting natural courtship behavior in both sexes. Thus, this finding warrants further investigation using more controlled experimental procedures.

It now 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 non-invasive 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. Finally, these assays are an important adjunct tool for assessing ovarian responses to gonadotrophin therapy, allowing for subtle improvements that should eventually permit assisted reproduction to be even more useful for maintaining genetic diversity within small populations.

The authors thank Reg Hoyt and Terri Volk of the Phoenix Zoo, Cathey Marsh and Mike Tucker of the Caldwell Zoo, Jane Hausjergen, Kevin Van der Molen and Toni Vargas of the Sacramento Zoo, Jan Fleming, Karin Ziegler and Liz Ferguson of the White Oak Conservation Center, Terry Peterson, Shimon Russell and Gretchen Ziegler of the Wildlife Safari, and Astrid Bellem, Heather Tomaso, Wayne Rowntree and Ken Grieg of the Metro Toronto Zoo for logistical

support and sample collection. We also are grateful to Sylvie Beekman, Karen Terio and Anneke Moresco for technical assistance. This study was supported by the Friends of the National Zoo, the New Opportunities in Animal Health Sciences Center, the Conservation Endowment Fund/Ralston Purina Big Cat Survival Fund of the American Zoo and Aquarium Association and the Scholarly Studies Program of the Smithsonian Institution.

References

Abbott DH (1984) Behavioural and physiological suppression of fertility in female marmoset monkeys *American Journal of Primatology* **6** 169-186

Asa CS, Junge RE, Bircher SJ, Noble GA, Sarri KJ and Plotka ED (1992) Assessing reproductive cycles and pregnancy in cheetahs (*Acinonyx jubatus*) by vaginal cytology *Zoo Biology* **11** 139-151

Bertschinger HJ, Meltzer DG, van Dijk A, Coubrough RI, Soley JT and Collett FA (1984) Cheetah life-line *Nuclear Active* **30** 2-7

Bonney RC, Moore HDM and Jones DM (1981) Plasma concentrations of oestradiol-17β and progesterone, and laparoscopic observations of the ovary in the puma (*Felis concolor*) during oestrus, pseudopregnancy and pregnancy *Journal of Reproduction and Fertility* **63** 523-531

Brown JL, Wasser SK, Wildt DE and Graham LH (1994) Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured non-invasively in feces *Biology of Reproduction* **51** 776-786

Brown JL, Wildt DE, Graham LH, Byers AP, Collins L, Barrett S and Howard JG (1995) Natural versus chorionic gonadotropin-induced ovarian responses in the clouded leopard (*Neofelis nebulosa*) assessed by fecal steroid analysis *Biology of Reproduction* **53** 93-102

Caro TM (1993) Behavioral solutions to breeding cheetahs in captivity: Insights from the wild *Zoo Biology* **12** 19-30

Caro TM (1994) In *Cheetahs of the Serengeti Plains: Group living in an asocial species* Ed GB Schaller. University of Chicago Press, Chicago, Illinois

Creel SR, Creel NM, Wildt DE and Monfort SL (1992) Behavioural and endocrine mechanisms of reproductive suppression in Serengeti dwarf mongooses *Animal Behaviour* **43** 231-245

Czekala NM, Durrant BS, Callison L, Williams M and Millard S (1994) Fecal steroid hormone analysis as an indicator of reproductive function in the cheetah *Zoo Biology* **13** 119-128

Eaton RL and Craig SJ (1973) Captive management and mating behavior of the cheetah. In *The World's Cats I: Ecology and Conservation* pp 217-254 Ed. RL Eaton. World Wildlife Safari, Winston, Oregon

Epple G and Katz Y (1984) Social influences on estrogen excretion and ovarian cyclicity in saddle-back tamarins *American Journal of Primatology* **6** 215-227

Faulkes CG, Abbott DH and Jarvis JUM (1990) Social suppression of ovarian cyclicity in captive and wild colonies of naked mole rats, *Heterocephalus glaber Journal of Reproduction and Fertility* **88** 559-568

Frame LH, Malcolm JR, Frame GW and van Lawick H (1979) Social organization of African wild dogs (*Lycaon pictus*) on the Serengeti Plains, Tanzania, 1967-1978. *Z Tierpsychology* **50**:225-249

French JA, Abbott DH, Cheffler G, Robinson J and Goy RW (1984) Cyclic excretion of urinary oestrogens in female tamarins (*Sanguinus oedipus*) *Journal of Reproduction and Fertility* **68** 177-184

Fuller TK, Kat PW, Bulger JB, Maddock AH, Ginsberg JR, Burrows R, McNutt JW and Mills DR (1992) Population dynamics of African wild dogs. In *Wildlife 2001: Populations* Eds DR McCullough and RH Barret. Elsevier Applied Science, New York

Graham LH, Goodrowe KL, Raeside JI and Liptrap RM (1995) Non-invasive monitoring of ovarian function in several felid species by measurement of fecal estradiol-17β and progestins *Zoo Biology* **14**:223-237

Guggisburg CAW (1975) In *Wild Cats of the World* Ed CAW Guggisburg. Taplinger, New York

Howard JG, Donohgue AM, Barone MA, Goodrowe KL, Blumer ES, Snodgrass K, Starnes D, Tucher M, Bush M and Wildt DE (1992) Successful induction of ovarian activity and laparoscopic intrauterine artificial insemination in the cheetah (*Acinonyx jubatus*) *Journal of Zoo and Wildlife Medicine* **23** 288-300

Howard JG, Byers AP, Brown JL, Barrett SJ, Evans MZ, Schwartz RJ and Wildt DE Successful ovulation induction and laparoscopic intrauterine artificial insemination in the clouded leopard (Neofelis nebulosa) *Zoo Biology* (in press)

Kleiman DG (1974) The estrus cycle in the tiger (*Panthera tigris*). In *The World's Cats, Vol. 2. Biology, Behavior and Management of Reproduction* pp 60-75 Ed RL Eaton. Feline Research Group, Seattle, Washington

Laurenson MK, Caro T and Borner M (1992) Female cheetah reproduction *National Geographic Research and Exploration* **8** 64-75

Marker L and O'Brien SJ (1989) Captive breeding of the cheetah (*Acinonyx jubatus*) in North American zoos (1871-1986) *Zoo Biology* **8** 3-16

Marker-Kraus L and Grisham J (1993) Captive breeding of cheetahs in North American zoos (1987-1991) *Zoo Biology* **12** 5-18

O'Brien SJ, Wildt DE, Goldman CR, Merril CR and Bush M (1983) The cheetah is depauperate in genetic variation *Science* **221** 459-462

O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, Meltzer D, Colly L, Bush M, Evermann JR and Wildt DE (1985) Genetic basis for species vulnerability in the cheetah *Science* **227** 1428-1434

Ralls K and Ballou J (1983) Extinction: Lessons from zoos. In *Genetics and Conservation: A reference for managing Wild Animal and Plant Populations* pp 35-56 Ed CM Schonewald-Cox, SM Chambers, B MacBryde and L Thomas. Benjamin-Cummings, Menlo Park, California

Ralls K, Brugger K and Ballou J (1979) Inbreeding and juvenile mortality in small populations of ungulates. *Science* **206** 1101-1103

Risler L, Wasser SK and Sackett GP (1987) Measurement of excreted steroids in *Macaca nemestrina American Journal of Primatology* **12** 91-100

Schmidt AM, Nadal LA, Schmidt MJ and Beamer NB (1979) Serum concentrations of oestradiol and progesterone during the normal oestrous cycle and early pregnancy in the lion *(Panthera leo) Journal of Reproduction and Fertility* **57** 267-272

Schmidt AM, Hess DL, Schmidt MJ, Smith RC and Lewis CR (1988) Serum concentrations of oestradiol and progesterone, and sexual behavior during the normal oestrous cycle in the leopard (*Panthera pardus*) *Journal of Reproduction and Fertility* **82** 43-49

Schmidt AM, Hess DL, Schmidt MJ and Lewis CR (1993) Serum concentrations of oestradiol and progesterone and frequency of sexual behaviour during the normal oestrous cycle in the snow leopard (*Panthera uncia*) *Journal of Reproduction and Fertility* **98** 91-95

Schmidt PM, Chakraborty PK and Wildt DE (1983) Ovarian activity, circulating hormones and sexual behavior in the cat. I. Relationships during the pregnancy, parturition, lactation and the postpartum estrus *Biology of Reproduction* **28** 657-671

Schramm RD, Briggs MB and Reeves JJ (1994) Spontaneous and induced ovulation in the lion (*Panthera leo*) *Zoo Biology* **13** 301-307

Seal US, Plotka ED, Smith JD, Wright FH, Reindl N, Taylor RS and Seal MF (1985) Immunoreactive luteinizing hormone, estradiol, progesterone, testosterone and androstenedione levels during the breeding season and anestrus in Siberian tigers *Biology of Reproduction* **32** 361-368

Shille VM and Stabenfeldt GH (1979) Luteal function in the domestic cat during pseudopregnancy and after treatment with prostaglandin $F_{2\alpha}$ *Biology of Reproduction* **21** 1217-1223

Shille VM, Haggerty MA, Shackleton C and Lasley BL (1990) Metabolites of estradiol in serum, bile, intestine and feces of the domestic cat (*Felis catus*) *Theriogenology* **3** 779-794

Wildt DE, Chan SYW, Seager SWJ and Chakraborty PK (1981) Ovarian activity, circulating hormones and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating *Biology of Reproduction* **25** 15-28

Wildt DE, Bush M, Howard JG, O'Brien SJ, Meltzer D, van Dyk, Ebedes H and Brand DJ (1983) Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat *Biology of Reproduction* **29** 1019-1025

Wildt DE, O'Brien SJ, Howard JG, Caro TM, Roelke ME, Brown JL and Bush M (1987) Similarity in ejaculate-endocrine characteristics in captive versus free-ranging cheetahs of two subspecies *Biology of Reproduction* **36** 351-360

Wildt DE, Brown JL, Bush M, Barone MA, Cooper KA, Grisham J and Howard JG (1993) Reproductive status of cheetahs (*Acinonyx jubatus*) in North American Zoos: The benefits of physiological surveys for strategic planning *Zoo Biology* **12** 45-80

Wildt DE (1994) Endangered species spermatozoa: diversity research and conservation. In *Function of Somatic cells in the Testes* pp 1-24 Ed A Bartke. Springer Verlag, New York

Yamada JK and Durrant BS (1989) Reproductive parameters of clouded leopards (*Neofelis nebulosa*) *Zoo Biology* **8** 223-231

Fig. 1 Mean (± SEM) fecal oestradiol and progestogen metabolite concentrations in (a) pregnant ($n = 5$) and (b) non-pregnant ($n = 6$) cheetahs after natural mating and in (c) non-pregnant cheetahs after gonadotrophin ovulation induction and artificial insemination ($n = 5$). Data are aligned to the oestradiol peak (Day 0).

Fig. 2. Representative individual longitudinal profiles of fecal oestradiol and progestogen metabolite concentrations in cheetah females at the Phoenix Zoo. Asterisks denote significant peaks in oestradiol excretion above baseline.

Fig. 3. Representative individual longitudinal profiles of fecal oestradiol and progestogen metabolite concentrations in cheetah females at the Metro Toronto Zoo. Asterisks denote significant peaks in oestradiol excretion above baseline.

Fig. 4. Individual profiles of fecal oestradiol and progestogen metabolite concentrations in cheetah females at the White Oak Conservation Center (a) and Wildlife Safari (b) exhibiting spontaneous ovulation (i.e., without mounting or intromission by a male). The female in panel (a) was re-located 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 panel (b), 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. 5. Individual profiles of fecal oestradiol and progestogen metabolite concentrations in (a) pregnant, (b) non-pregnant and (c) anovulatory cheetah females 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 46-48 h post-hCG. All data are aligned to the oestradiol peak (Day 0).

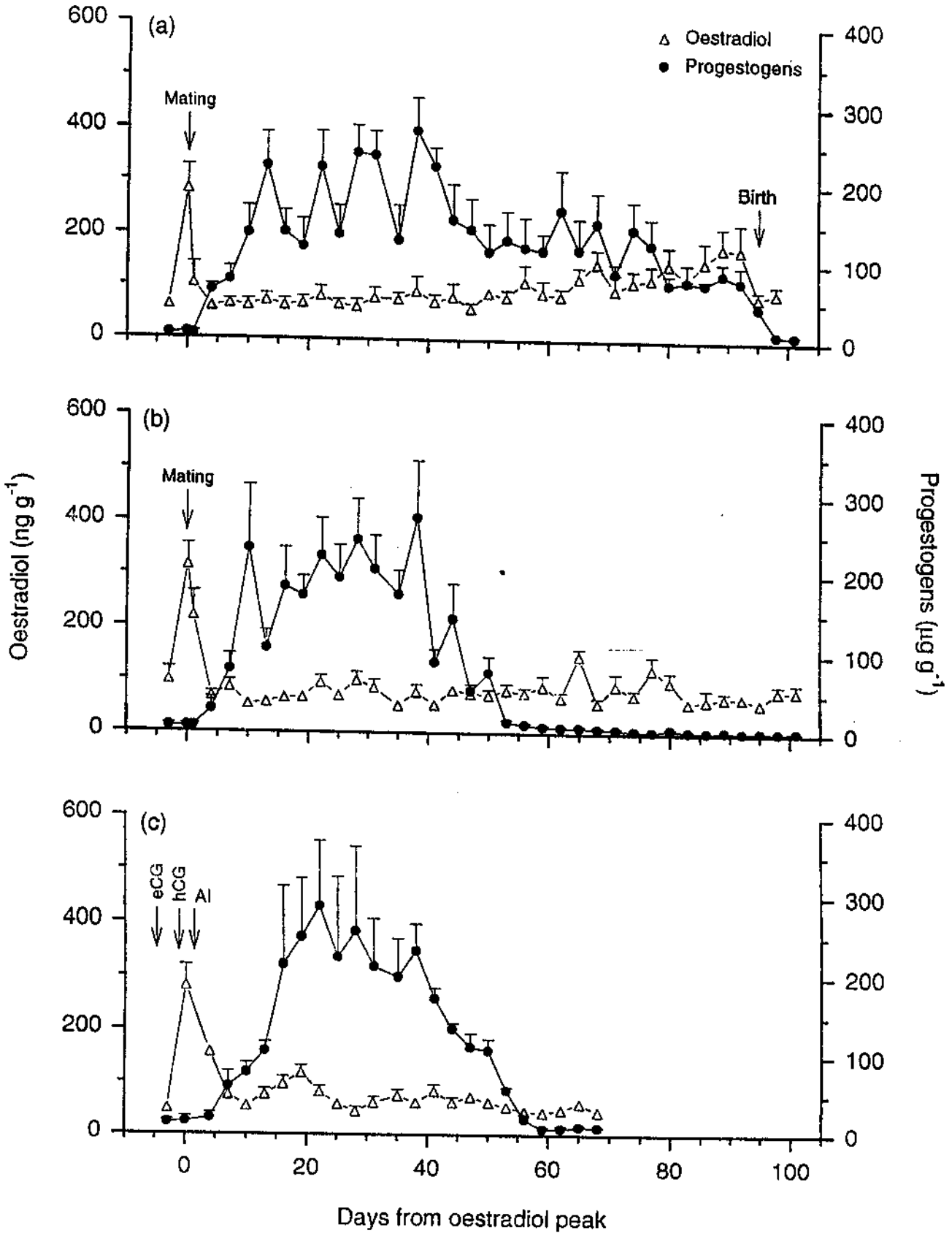
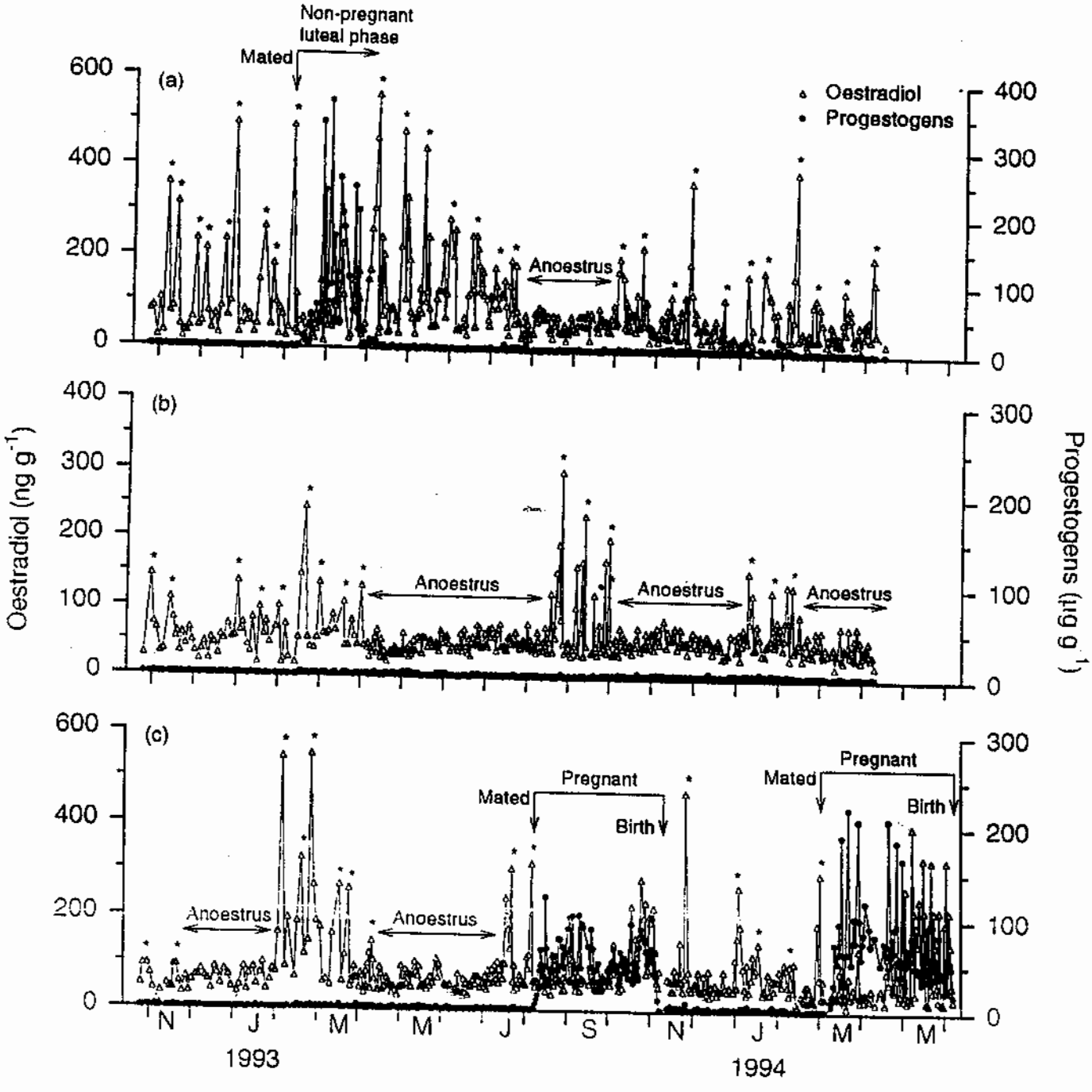
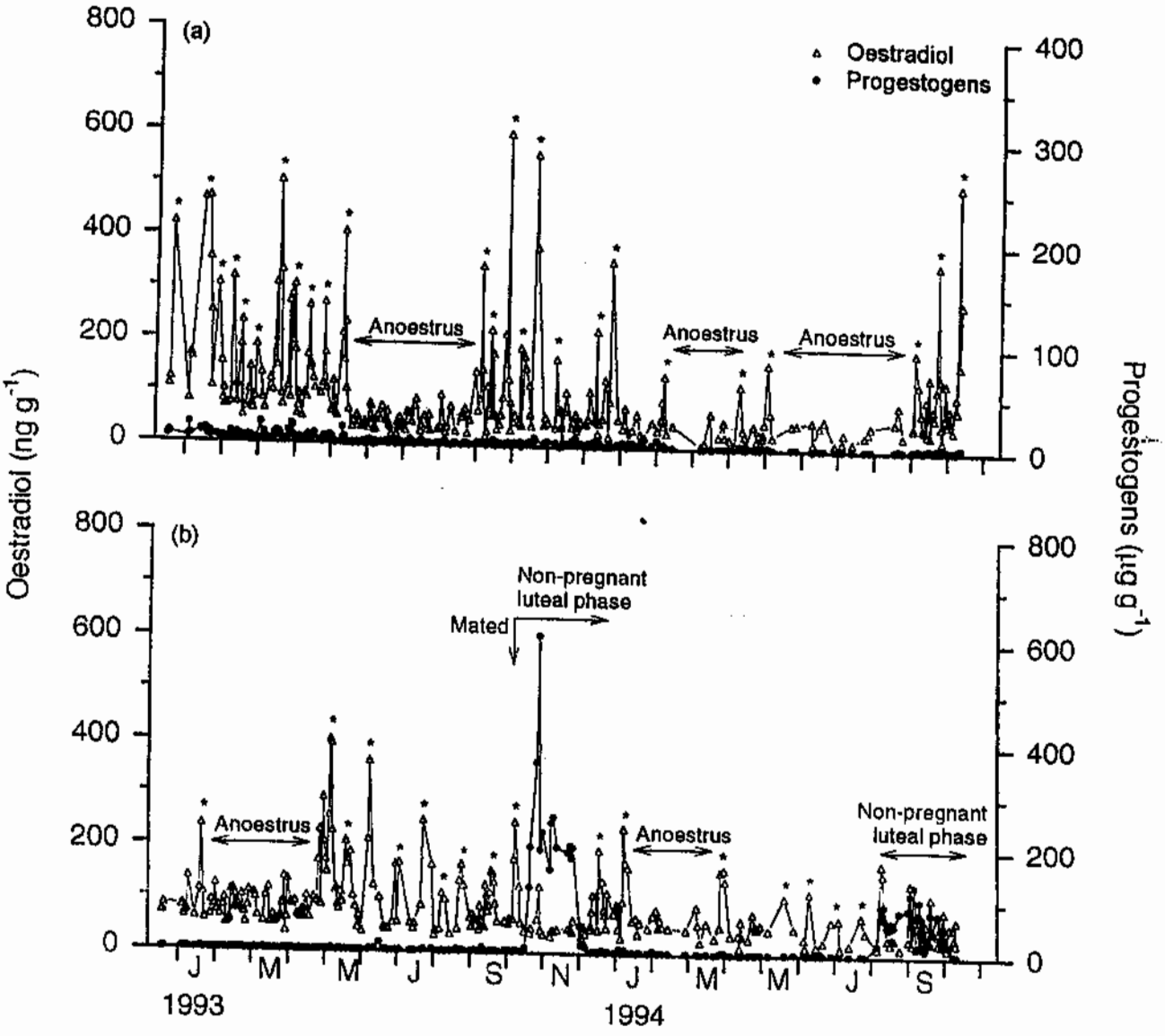

(a)
Oestradiol
Progestogens
Mating
Birth
(b)
Mating
(c)
eCG
hCG
AI
Oestradiol (ng g-1)
Progestogens (µg g-1)
Days from oestradiol peak
0
20
40
60
80
100
200
400
600
100
300

Fig. 1


Non-pregnant
luteal phase
Mated
(a)
Oestradiol
Progestogens
Anoestrus
(b)
Anoestrus
Anoestrus
Anoestrus
(c)
Mated
Pregnant
Birth
Mated
Pregnant
Birth
Anoestrus
Anoestrus
N
J
M
M
J
S
N
J
M
M
1993
1994
Oestradiol (ng g-1)
Progestogens (µg g-1)


(a)
Oestradiol
Progestogens
Anoestrus
Anoestrus
Anoestrus
(b)
Non-pregnant
luteal phase
Mated
Anoestrus
Anoestrus
Non-pregnant
luteal phase
Oestradiol (ng g-1)
Progestogens (µg g-1)
J
M
M
J
S
N
J
M
M
J
S
1993
1994

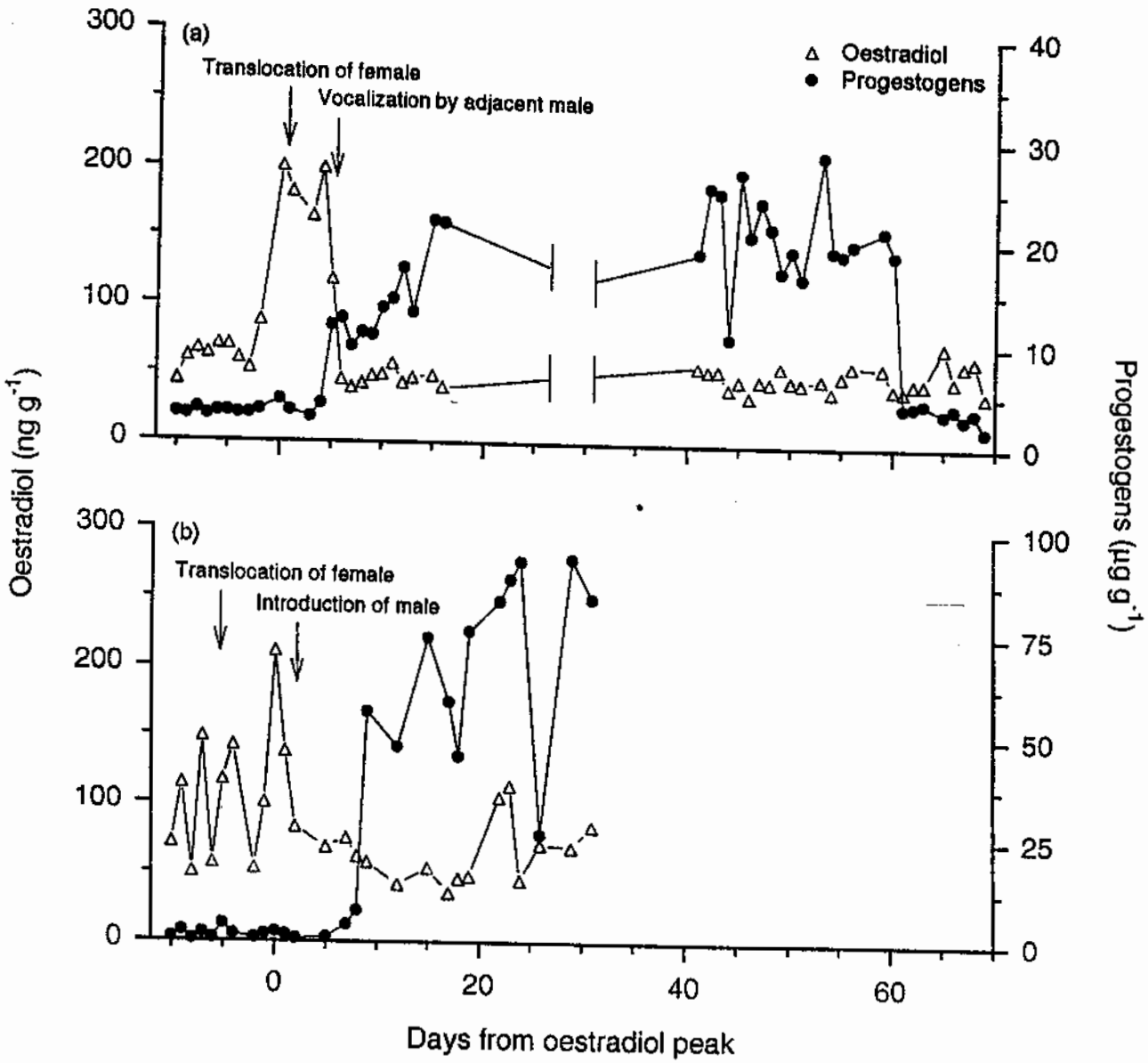
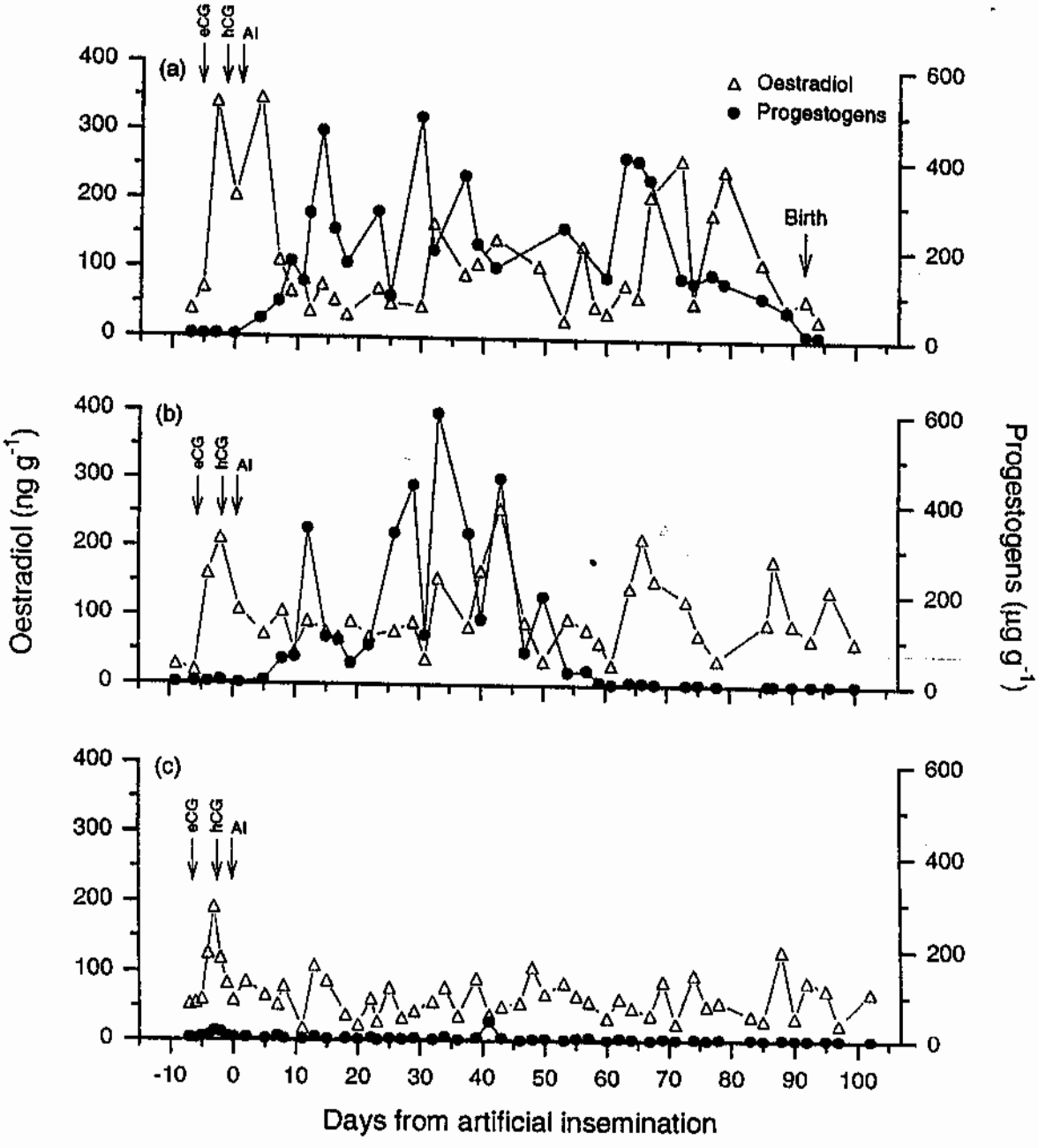

(a)
Translocation of female
Vocalization by adjacent male
Oestradiol
Progestogens
(b)
Translocation of female
Introduction of male
Oestradiol (ng g-1)
Progestogens (µg g-1)
Days from oestradiol peak
300
200
100
0
40
30
20
10
0
75
50
25
0
20
40
60

Fig. 4


(a)
(b)
(c)
eCG
hCG
AI
Oestradiol
Progestogens
Birth
Oestradiol (ng g-1)
Progestogens (µg g-1)
Days from artificial insemination
0
100
200
300
400
600
-10
0
10
20
30
40
50
60
70
80
90
100