Journal of Reproduction and Fertility (1993) **97**, 107–114

Hormonal characteristics of free-ranging female lions (*Panthera leo*) of the Serengeti Plains and Ngorongoro Crater

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Pituitary responses to gonadotrophin-releasing hormone (GnRH) and prolactin and steroid secretory profiles were examined in two populations of adult, female lions in the Serengeti (one outbred in the Serengeti Plains and one inbred in the Ngorongoro Crater) to determine whether reductions in genetic variability adversely affected endocrine function. GnRH-induced gonadotrophin secretion was also examined after adrenocorticotrophic hormone (ACTH) treatment to determine whether acute increases in serum cortisol altered pituitary function. Anaesthetized lions were administered (i) saline i.v. after 10 and 100 min of blood sampling; (ii) saline at 10 min and GnRH (1 $\mu g\ kg^{-1}$ body weight) after 100 min; or (iii) ACTH (3 $\mu g\ kg^{-1}$) at 10 min and GnRH after 100 min of sampling. Basal serum cortisol and basal and GnRH-induced gonadotrophin secretion were similar ($P > 0.05$) between females of the Ngorongoro Crater and Serengeti Plains. After ACTH, serum cortisol increased two- to threefold over baseline values and the response was unaffected ($P > 0.05$) by location. ACTH-induced increases in serum cortisol had no effect on subsequent basal or GnRH-stimulated luteinizing hormone (LH) or follicle-stimulating hormone (FSH) secretion. Overall mean serum progesterone concentrations ranged from 0.2 to 5.4 ng ml^{-1} with the exception of four females (two in the Serengeti and two in the Crater; progesterone range, 18.4–46.5 ng ml^{-1}) that were presumed pregnant (three of these females were observed nursing cubs several weeks later). There were no differences ($P > 0.05$) between Serengeti and Crater lions in mean serum progesterone, oestradiol or prolactin concentrations, and hormone secretion was not influenced ($P > 0.05$) by GnRH or ACTH treatment. Although Ngorongoro Crater lions have decreased genetic variability, the reproductive–endocrine system of females appears functionally normal compared with outbred counterparts living in the Serengeti Plains. Furthermore, the acute rise in serum cortisol after ACTH administration in lions fails to alter subsequent GnRH-induced gonadotrophin release, suggesting that short-term changes in adrenal activity do not markedly affect pituitary responsiveness in this species.

Introduction

Although it is often difficult to determine the influence of degrees of genetic diversity on reproduction in wild populations, the well-studied inbred and outbred lion populations of northern Tanzania offer an unusual opportunity to study the effect of reduced genetic heterozygosity on reproductive performance (Wildt *et al.*, 1987; Brown *et al.*, 1991; Packer *et al.*, 1991). One population resides in the geographically isolated Ngorongoro Crater, an extinct caldera located at the western edge of the Gregory Rift in Tanzania. In 1962, the Crater lions suffered a *Stomoxys* biting fly epizootic (Foosbrooke, 1963) that reduced the population to nine females and one male (Packer *et al.*, 1991). Although an additional seven males apparently immigrated into the Crater in 1964–1965, no further immigrations have since been observed. All members of the current Crater population (about 100 animals) are descended from 15 founders. One consequence of this bottleneck is a measurable reduction in genetic heterozygosity compared with a continuous population of about 3000 outbred free-ranging lions in the adjacent Serengeti Plains (O'Brien *et al.*, 1987; Yuhki and O'Brien, 1990). Comparatively poorer reproductive performance and increased proportions of structural abnormalities of spermatozoa are also characteristic of the Crater population (Wildt *et al.*, 1987; Brown *et al.*, 1991; Packer *et al.*, 1991). Although reductions in genetic diversity are known to adversely affect testis function, immune

Received 10 January 1992.

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responses and offspring survival in many species, including felids (Ralls *et al.*, 1979; O'Brien *et al.*, 1985; Wildt *et al.*, 1987; O'Brien and Evermann, 1988; Marker and O'Brien, 1989), the mechanisms by which compromised genetic variability influence these physiological processes have not been determined.

Our previous studies concentrated on males, and did not examine the potential depressive impact of inbreeding on female reproductive characteristics. Because reproductive success is tightly coupled to endocrine function, the objective of this study was to determine whether pituitary luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin or ovarian (progesterone, oestradiol) hormone secretion differed between inbred Ngorongoro Crater and outbred Serengeti Plains female lions. We also examined adrenal activity in these two populations, to determine whether acute adrenocorticotrophin (ACTH)-induced increases in cortisol secretion affected subsequent basal or gonadotrophin-releasing hormone (GnRH)-induced gonadotrophin secretion.

Materials and Methods

Animals and treatment

Adult, free-ranging female lions were from prides in the Serengeti Plains (mean age ± SEM, 6.5 ± 0.6 years; range 3.8–9 years; $n = 12$) and Ngorongoro Crater (mean age, 6.9 ± 0.9 years; range 3.5–11; $n = 8$) that have been under behavioural observation for about 20 years (Schaller, 1972; Bertram, 1975; Bygott *et al.*, 1979; Packer *et al.*, 1988). All females were examined in October and November (1987) and were identified individually by scars, whisker patterns, natural markings and colorations (Pennycuick and Rudnai, 1970). Females in these prides generally produce their first litter and are considered adult at 3–4 years of age (Packer *et al.*, 1988).

All animals were located by land vehicle and anaesthetized with Telazol (tiletamine hydrochloride plus zolazepam hydrochloride; Warner Lambert, Ann Arbor, MI; 500–600 mg per female) delivered via a projectile dart. Because animals were accustomed to the observation vehicle, they experienced little or no visible excitement either immediately before or after darting. In general, 3–6 females were darted consecutively within 5–10 min which allowed members of a pride to be physically kept together and facilitated blood sampling. A light surgical plane of anaesthesia was maintained with additional Telazol injections (150–350 mg, i.m. or i.v.). A catheter (16-gauge, 5-cm, with obturator; Becton Dickinson, Rutherford, NJ) was placed in a jugular, saphenous, or lateral coccygeal vein, and a blood sample was collected every 5 min for 4 h. The interval between darting and sampling onset averaged 28.5 ± 2.6 min. Adult lions were assigned at random to one of three treatments as follows: (i) saline administered via the cannula after 10 and 100 min of blood sampling (Serengeti Plains, $n = 4$ lions; Ngorongoro Crater, $n = 2$); (ii) saline administered after 10 min and GnRH (1 $\mu g\ kg^{-1}$ body weight; Cystorellin, CEVA Laboratories, Overland Park, KS) administered after 100 min of sampling ($n = 4$, 3, respectively) and (iii) ACTH (3 $\mu g\ kg^{-1}$; Cortrosyn, Organon, Inc., W. Orange, NJ) administered after 10 min and GnRH administered after 100 min ($n = 4$, 3, respectively) (Table 1). The GnRH dose was chosen to ensure a maximal pituitary response and was comparable to that used to examine pituitary function in other felids (Brown *et al.*, 1988, 1989, 1991). Unfortunately, it was not possible to determine the stage of the oestrous cycle at the time of sampling. Animal body weights were estimated from chest girth measurements using a regression equation [$y = 1.8x - 78.2$; where y = body weight (kg) and x = chest girth (cm), U.S. Seal, personal communication]. Body weights were 104.3 ± 3.2 kg for Serengeti Plains females and 127 ± 5.1 kg for Ngorongoro Crater lions. The larger body weight of the Crater lions is probably due to the greater prey availability in that region (Packer *et al.*, 1988). After blood sampling, all animals were monitored during anaesthetic recovery until ataxia was not observed.

Table 1. Summary of lion pride composition and allocation of treatments

Treatment	Lion	Age (years)	Pride
Serengeti Plains			
Saline/Saline	Travesty	9.0	Transect
	L28	4.5	Loliondo
	LLA	8.0	Loliondo
	L25	4.8	Loliondo
Saline/GnRH	Trundel	9.0	Transect
	M24	4.1	Masai
	LLC	8.0	Loliondo
	L23	4.8	Loliondo
ACTH/GnRH	Trifle	9.0	Transect
	M32	3.8	Masai
	LLH	8.0	Loliondo
	L26	4.7	Loliondo
Ngorongoro Crater			
Saline/Saline	LKG	7.0	Lake
	LADU	11.0	Lake
Saline/GnRH	LK3	9.0	Lake
	TKM	7.3	Tokitok
	LK15	3.5	Lake
ACTH/GnRH	LKH	7.3	Lake
	TKR	7.3	Tokitok
	LK18	3.5	Lake

GnRH: gonadotrophin-releasing hormone; ACTH: adrenocorticotrophic hormone.

Radioimmunoassays

All protein hormones were iodinated using low concentrations of chloramine-T (Brown *et al.*, 1991). Serum concentrations of LH and FSH were measured in every serum sample (49 samples per animal) using heterologous double antibody radioimmunoassays previously validated for lion serum (Brown *et al.*, 1991). Canine LH (LER-1685-1), provided by L. E. Reichert Jr, Albany Medical School, NY and ovine FSH (NIH-FSH-S8), provided by the National Hormone and Pituitary Program (NHPP), MD were used as the standard preparations.

Assay sensitivities (defined as 90% of maximum binding) were 0.8 and 12.0 ng ml^{-1} for 100 µl serum, and intra- and interassay coefficients of variation were 5.7 and 10.1%, and 4.8 and 9.3% for the LH and FSH assays, respectively.

Concentration of cortisol was measured in every third serum sample (17 samples per animal) using a double antibody ^{125}I radioimmunoassay kit (New England Nuclear, Boston, MA). The assay was validated for use with lion serum by demonstrating parallelism between dilutions of pooled serum samples and the standard curve. Recovery of 10, 25, 50, 125 and 250 ng cortisol added to lion serum was 11, 30, 55, 140 and 260 ng after subtraction of endogenous hormone ($y = 1.04x + 3.79$; $r = 0.999$). Assay sensitivity was 10 ng ml^{-1}, and the intra- and interassay coefficients of variation were 6.7 and 10.3%, respectively.

Concentration of prolactin was measured in every third serum sample (17 samples per animal) using radioimmunoassay materials supplied by the National Institute of Diabetic and Digestive and Kidney Diseases (NIDDK) and the NHPP, MD. The assay was developed in this laboratory and used an anti-human prolactin antisera (NIDDK-anti-hPRL-3) and ovine prolactin (NIDDK-oPRL-I-2) label and standards. The assay was incubated at room temperature for 3 days in a total volume of 300 µl. Standards (100 µl) or sample (diluted up to 100 µl with radioimmunoassay buffer; 0.01 mol phosphate l^{-1}, 0.5% bovine serum albumin, 2 mmol EDTA l^{-1}, 0.9% NaCl, 0.01% thimerosal, pH 7.4) were incubated with antibody (1:37 500 in 100 µl) for 24 h followed on day 2 by the addition of [^{125}I]-labelled prolactin (20 000 c.p.m. in 100 µl). On day 3, antibody-bound complexes were precipitated by incubation for 1 h with sheep anti-rabbit gamma globulin (1:300 in 1 ml containing 5% polyethylene glycol) and centrifuged for 30 min at 3500 ***g***. The antibody generally bound 20–25% of the iodinated prolactin with 3% nonspecific binding. Serial dilutions of lion serum pools were parallel to the standard curve. Upon addition of 0.039, 0.078, 0.156, 0.313, 0.625 and 1.25 ng prolactin, 0.043, 0.082, 0.148, 0.311, 0.630 and 1.27 ng were recovered after subtraction of endogenous hormone ($y = 1.02x - 0.003$; $r = 0.999$). Assay sensitivity was 0.11 ng ml^{-1}, and the intra- and interassay coefficients of variation were 8.1 and 11.6%, respectively.

Concentration of progesterone was measured in every other serum sample (25 samples per animal) using a double antibody ^{125}I radioimmunoassay kit (ICN, Costa Mesa, CA). Serial dilutions of lion serum pools were parallel to the standard curve. Upon addition of 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 ng progesterone, 0.26, 0.48, 1.14, 2.73, 5.69, 10.97 and 26.99 ng were recovered after subtraction of endogenous hormone ($y = 1.08x + 0.07$; $r = 0.999$). Assay sensitivity was 0.23 ng ml^{-1}, and the intra- and interassay coefficients of variation were 5.6 and 8.9%, respectively.

Concentration of oestradiol was measured in every other serum sample (25 samples per animal) using a double antibody ^{125}I radioimmunoassay kit (ICN). Serial dilutions of lion serum pools were parallel to the standard curve. Addition of 10, 30, 100, 300 and 1000 pg oestradiol resulted in a net recovery of 11, 27, 90, 319 and 985 pg ($y = 0.99x + 1.79$; $r = 0.999$). Assay sensitivity was 5 pg ml^{-1}, and the intra- and interassay coefficients of variation were 8.7 and 11.2%, respectively.

The ^{125}I steroid assay kits described above were developed for use with unextracted human serum. Because analysis of extracted and unextracted lion serum pools yielded similar results ($r > 0.9$), all serum samples were assayed unextracted.

Statistical analysis

Changes in endocrine values over time were analysed using split-plot analyses of variance. Gonadotrophin responses to GnRH and cortisol responses to ACTH were evaluated as peak height, net peak height (greatest post-treatment value minus pretreatment mean), and net area under the response curve. Areas were determined using a planimeter with a 3% coefficient of variation. Basal and pulsatile LH secretions in saline-treated animals were determined by an iterative process described previously (Brown *et al.*, 1988). Differences between basal, peak height and area measurements were determined using Student's *t* tests or Duncan's new multiple range tests. Data are presented as means ± SEM.

Results

Basal concentrations of cortisol in saline-treated lions (mean across 17 samples per female) were similar ($P > 0.05$) between lions of the Serengeti Plains (93.2 ± 14.0 ng ml^{-1}) and Ngorongoro Crater (106.4 ± 28.6 ng ml^{-1}) and were unaffected by GnRH treatment. Cortisol concentrations often doubled over the sampling period (6 of 13 females) within 60–120 min of sampling onset. After ACTH treatment, serum concentrations of cortisol increased approximately threefold over baseline within 45–60 min of injection (Fig. 1). Although cortisol peak height after ACTH was greater ($P < 0.05$) in Ngorongoro Crater (290.1 ± 39.9 ng ml^{-1} at 105 min) than in Serengeti Plains (202.8 ± 18.3 ng ml^{-1} at 105 min) lions, responses were highly variable and neither net peak height (164.2 ± 61.7; 147.9 ± 18.0 ng ml^{-1}, respectively) nor net area under the response curves (3.87 ± 0.32; 4.36 ± 0.69 arbitrary units, respectively) differed ($P > 0.05$) between the two populations.

For saline-treated Serengeti Plains and Ngorongoro Crater lions, respectively, basal serum LH (2.5 ± 0.4; 3.2 ± 0.8 ng ml^{-1}) and FSH (34.7 ± 2.1; 27.9 ± 9.3 ng ml^{-1}) concentrations were similar ($P > 0.05$). Pulses of LH secretion (1–3 pulses in 4 h) were observed in two of four females in the Serengeti Plains and one of two Ngorongoro Crater females (Fig. 2). Pulse amplitudes (above baseline) were 2.2–7.0 ng ml^{-1} and averaged 3.9 ± 0.5 ng ml^{-1}. No FSH pulses were observed (Fig. 2). After GnRH administration, serum LH concentrations peaked within 10–25 min in all animals (Fig. 3). GnRH-induced LH release was similar ($P > 0.05$) between Serengeti Plains and Ngorongoro Crater females, and the response was unaffected ($P > 0.05$) by increases in cortisol secretion after ACTH (Fig. 3b). Pituitary FSH response to GnRH was not influenced ($P > 0.05$) by location or prior ACTH treatment. Overall pre-GnRH FSH concentration averaged 31.7 ± 2.3 ng ml^{-1} and concentrations increased to 45.7 ± 3.6 ng ml^{-1} after GnRH injection.

Basal concentrations of progesterone in serum were unaffected ($P > 0.05$) by GnRH or ACTH treatment (range, 0.2–5.4 ng ml^{-1}). In all but two animals, concentrations fluctuated throughout the sampling period (Fig. 4a, b). These temporal

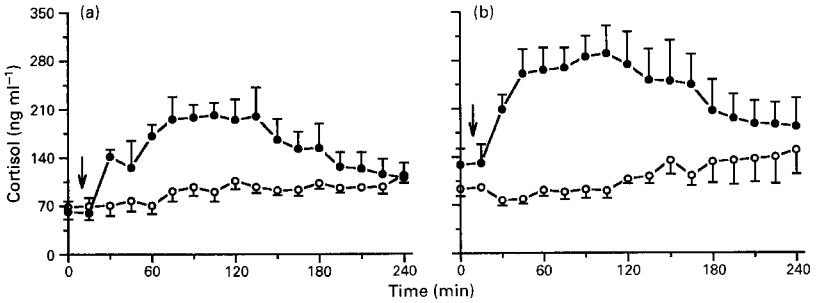

Fig. 1. Mean (± SEM) concentrations of serum cortisol after saline (○) or adrenocorticotrophic hormone (ACTH) (●) injection in Telazol-anaesthetized female lions located in (a) Serengeti Plains and (b) Ngorongoro Crater. Arrows designate time of saline or ACTH injection.

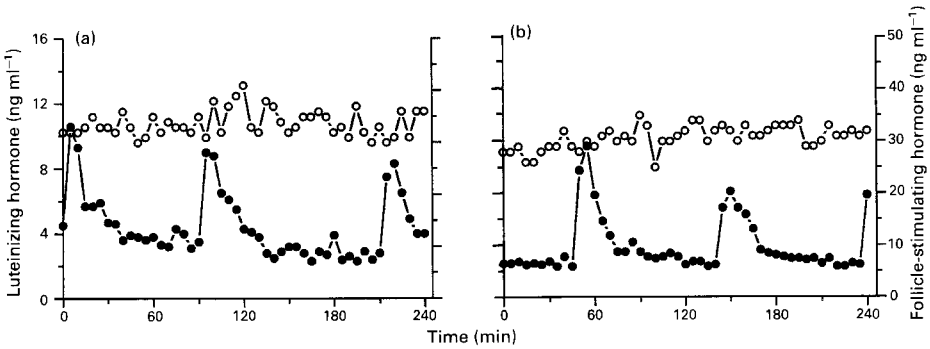

Fig. 2. Representative hormone profiles for serum from individual lions treated with saline from (a) the Serengeti Plains (L28) and (b) the Ngorongoro Crater (LKG) demonstrating pulsatile luteinizing hormone (●), but not follicle-stimulating hormone (○), secretion during Telazol anaesthesia.

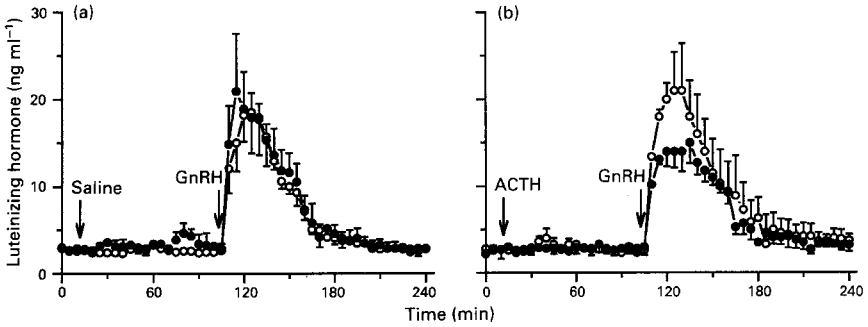

Fig. 3. Mean (± SEM) concentrations of serum luteinizing hormone in Telazol-anaesthetized, female lions living in the Serengeti Plains (○) and Ngorongoro Crater (●) after injection of (a) saline at 10 min and gonadotrophin-releasing hormone (GnRH) at 100 min of blood sampling or (b) adrenocorticotrophic hormone (ACTH) at 10 min and GnRH at 100 min of sampling.

fluctuations, however, could not be related to observed changes in LH, FSH or cortisol secretion. Two females in the Serengeti Plains (M24, M32) and two in the Ngorongoro Crater (LKG, LKH) secreted comparatively higher concentrations (range, 18.4–46.5 ng ml^{-1}) and were presumed pregnant (Fig. 4c, d). Excluding these high progesterone values, average concentrations were similar ($P > 0.05$) between Serengeti Plains (1.1 ± 0.2 ng ml^{-1}) and Ngorongoro Crater (0.9 ± 0.2 ng ml^{-1}) females. Of the lionesses producing high progesterone concentrations, both Serengeti Plains females (4.1 and 3.8 years of age) were pridemates, appeared pregnant on the basis of visual observation on the day of blood sampling and were observed nursing cubs 5 weeks later. In the Crater, both females (7 and 7.3 years of age) were pridemates, but only one of the

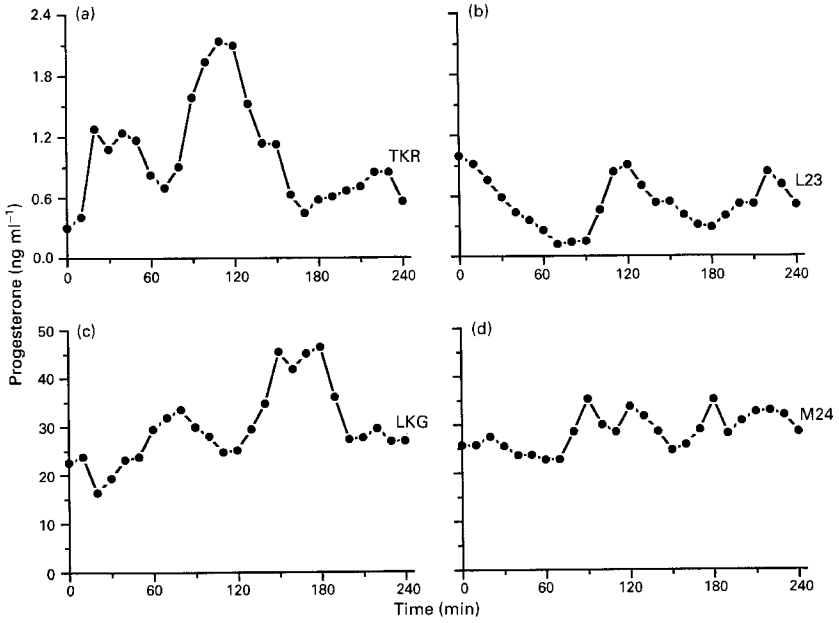

Fig. 4. Concentrations of serum progesterone in (a, b) two nonpregnant (TKR, L23) and (c, d) two pregnant (LKG, M24) adult female lions anaesthetized with Telazol. TKR and LKG are from the Ngorongoro Crater, and L23 and M24 are from the Serengeti Plains.

two appeared visibly pregnant on the day of sampling and was observed with cubs 3 weeks later. The other female was observed only sporadically after blood sampling and was never seen with cubs. This female's pride was taken over by another male coalition shortly after sampling, an event that may have resulted in death of the cubs. Regardless, there was no difference ($P > 0.05$) in serum progesterone concentration between the 'pregnant' females of the Serengeti Plains (24.6 $\pm$ 3.9 ng ml^{-1}) and Ngorongoro Crater (19.5 $\pm$ 6.8 ng ml^{-1}).

Serum oestradiol secretion within individuals was stable over time and was not influenced ($P > 0.05$) by hormonal treatment or pregnancy. Concentrations ranged between 7.9 and 51.4 pg ml^{-1} with overall mean oestradiol concentrations similar ($P > 0.05$) between the Serengeti Plains (25.56 $\pm$ 2.20 pg ml^{-1}) and Ngorongoro Crater (29.10 $\pm$ 1.93 pg ml^{-1}) females.

Concentrations of prolactin in serum were also unaffected ($P > 0.05$) by GnRH or ACTH treatment or pregnancy. Secretion was fairly stable over time with concentrations ranging from 0.5 to 1.7 ng ml^{-1} among all females. Overall mean prolactin concentration in the Serengeti Plains lions (1.02 $\pm$ 0.04 ng ml^{-1}) was similar ($P > 0.05$) to that observed in Ngorongoro Crater females (1.06 $\pm$ 0.03 ng ml^{-1}).

Discussion

As a result of a recent population bottleneck, lions residing in the Ngorongoro Crater demonstrate a diminished level of allozyme heterozygosity and loss in restriction fragment length variation in the class I genes of the major histocompatibility complex compared with free-ranging cohorts in the Serengeti Plains (O'Brien *et al.*, 1987; Yuhki and O'Brien, 1990). Since the mid-1970s, the reproductive performance of Ngorongoro Crater lions has also been steadily declining (Packer *et al.*, 1991). Assessing fertility status of the Serengeti versus Crater lions is complicated by differences in prey biomass (there is greater prey availability in the Crater: Schaller, 1972; Kruuk, 1972) and cub mortality (cub death rate is lower in the Crater: Packer *et al.*, 1988). However, a reduction in the annual reproductive rate of Crater lions (based on per capita yearling production calculated as the number of yearling produced per adult female per year over 2 years) correlates with lowered estimates of average heterozygosity (Packer *et al.*, 1991). Thus, it is possible that reduced genetic variability has a detrimental effect on the reproductive performance of Crater lions.

Reductions in reproductive rate in inbred Crater lions could be due to physiological dysfunctions in the male, female or both, and results suggest that the male may be partially to blame. In previous studies, sperm quality was found to be significantly lower in ejaculates collected from Crater compared with Serengeti males (Wildt *et al.*, 1987; Brown *et al.*, 1991). However, attempts to find an underlying physiological cause for reduced seminal quality in Crater lions have been unsuccessful. Although normal testicular function depends upon appropriate pituitary and gonadal hormone stimulation, endocrine function does not appear compromised in Crater males. Neither basal nor GnRH-induced LH, FSH, or testosterone secretion differs between the two populations (Brown *et al.*, 1991). Similarly, in the present study we could not identify any differences in hormonal secretory patterns between Serengeti and Crater

female lions, suggesting that pituitary, ovarian and adrenal function are also apparently normal in inbred Crater females. However, it is more difficult to conduct studies and interpret data on factors affecting gametogenesis in females than in males.

Temporal patterns of LH and FSH secretion following GnRH were similar to those measured in cheetahs (Wildt *et al.*, 1984), leopards and tigers (Brown *et al.*, 1988, 1989). One noteworthy observation was that the GnRH-induced LH response was similar between the females of this study and male lions challenged with the same dose of GnRH (Brown *et al.*, 1991). In previous studies involving other felid species (cheetah, leopard, tiger), GnRH-induced LH release was always several-fold greater in males than females (Wildt *et al.*, 1984; Brown *et al.*, 1988, 1989). We suggest that this sexual difference in pituitary responsiveness to GnRH may be related to differences in steroid negative feedback sensitivity. The lack of a gender difference in lions is perplexing, but perhaps is somehow related to inherent differences between social (lion) and non-social (cheetah, leopard, tiger) felid characteristics.

Interpretation of endocrine results in any non-domestic species is often difficult because of the potential effects of anaesthesia or immobilization 'stress' on hormonal secretion. In comparison with other felid species, basal cortisol concentrations in lions anaesthetized with Telazol were greater than those reported in cheetahs (Wildt *et al.*, 1988), similar to those in tigers (Wildt *et al.*, 1988; Brown *et al.*, 1988), and less than those in leopards and pumas (Wildt *et al.*, 1988; Brown *et al.*, 1988, 1989). Dissociative anaesthetics such as ketamine HCl and Telazol increase serum cortisol in some (leopards and pumas), but not all (cheetahs, clouded leopards, tigers) felid species (Wildt *et al.*, 1986b, 1988; Brown *et al.*, 1988, 1989). In this study, a gradual increase in cortisol secretion was observed over the sampling period in only about half the lions treated with saline or GnRH. Although a reduction in pituitary responsiveness to GnRH following increased glucocorticoids has been reported in female rats (Baldwin and Sawyer, 1974) and pigs (Pearce *et al.*, 1988), little or no effect is observed in dairy cows (Matteri and Moberg, 1982). Synthetic ACTH was administered 90 min before the GnRH challenge to test whether acute increases in serum cortisol influenced the assessment of pituitary responsiveness. Despite a significant increase in serum cortisol compared with that in non-stimulated animals, subsequent GnRH-induced LH and FSH secretion was unaffected. These data are similar to those reported for male Sri Lankan leopards in which GnRH-induced gonadotrophin secretion 1 h after ACTH was not compromised (Brown *et al.*, 1989). The approximately threefold increase in serum cortisol following ACTH injection in the lion was greater than that measured in the leopard and tiger (Wildt *et al.*, 1988; Brown *et al.*, 1989), but less than that observed in the cheetah and puma (Wildt *et al.*, 1988). Thus, there are wide variations in adrenal activity among felid species and this suggests inherent differences in their physiological responses to stimuli. Yet, an acute stressor, such as an ACTH challenge, failed to alter pituitary responsiveness to GnRH, further suggesting that an adaptive mechanism has evolved in these species to minimize the effects of environmental perturbations on reproductive fitness.

It was possible to identify distinct LH, but not FSH, pulses in some female lions under Telazol anaesthesia. A similar incidence of LH pulsatility has also been measured in male lions (Brown *et al.*, 1991), and in male and female leopards and tigers (Brown *et al.*, 1988, 1989). This is important because some anaesthetics block gonadotrophin pulsatility or reduce the frequency or amplitude of pulses (Peet and Lincoln, 1977; Clark and Doughton, 1983). In domestic cats, circulating concentrations of serum LH are lowered by both pentobarbital and ketamine HCl anaesthesia (Johnson and Gay, 1981). In contrast, GnRH-induced LH secretion does not appear to be affected by a variety of anaesthetic agents (Hobson and Fuller, 1977; Lewis *et al.*, 1985). For these reasons, the most effective and efficient method for assessing pituitary function in the lion, as well as in other species, is to use a GnRH challenge rather than attempting to collect a few blood samples in anticipation of detecting a natural gonadotrophin pulse.

Concentrations of oestradiol and prolactin in serum from female lions were within the ranges reported for other felid species (taking into account differences in prolactin standard potency) (Schmidt *et al.*, 1979, 1983, 1988; Bonney *et al.*, 1981; Banks *et al.*, 1983; Seal *et al.*, 1985). However, there was no difference in concentration for either hormone between pregnant and nonpregnant animals. Schmidt *et al.* (1979) reported no changes in oestradiol secretion in blood samples collected sporadically during pregnancy in a captive lion. Concentrations of oestradiol in serum samples collected weekly throughout pregnancy in a puma were increased for about 1 week during mid-gestation and again 1 week before parturition (Bonney *et al.*, 1981). In domestic cats, serum concentrations of prolactin increase significantly during the last 3 weeks of pregnancy (Banks *et al.*, 1983), while the concentration of oestradiol increases only during the last week (Schmidt *et al.*, 1983). Thus, in our study, the lack of a difference in concentrations of oestradiol and prolactin between pregnant and nonpregnant lions suggests that either the secretory dynamics are not substantially altered during pregnancy, or blood sampling was conducted before normal increases occurred. In contrast, progesterone concentrations were higher in pregnant than in nonpregnant lions, although circulating levels did not approach those reported in captive lions by Schmidt *et al.* (1979). It was surprising that progesterone concentrations fluctuated markedly in nonpregnant lions throughout the sampling period, although at no time did concentrations approach those observed in pregnant females. The source of these progesterone 'pulses' is not known, but they were apparently not gonadotrophin-driven as they did not correspond with LH pulses. It is possible that some of the secreted progesterone was of adrenal origin as has been suggested for tigers (Seal *et al.*, 1985). For this reason, Seal *et al.* (1985) cautioned against using increased serum progesterone as an indicator of pregnancy in that species. We attempted to correlate the progesterone pulses to the time of the ACTH challenge or to specific changes in cortisol secretion, but found no relationships. Thus, more work is needed to determine the physiological significance of these discrete rises and falls in circulating progesterone concentrations in lions. Regardless, these data indicated the importance of multiple blood sampling, even in an acute situation, for accurately assessing hormonal status.

In summary, despite a moderate reduction in genetic diversity, there were no detectable differences in ovarian or pituitary hormone function between adult female lions of the Ngorongoro

Crater and outbred females free-ranging in the adjacent Serengeti Plains. Thus, our results revealed no evidence that lowered reproductive performance in inbred Crater lions is related to an endocrine dysfunction in the female, at least based on the factors examined and the sampling protocol used in this study. Instead, there are several lines of evidence suggesting that the dysfunction may lie with the male. Previous studies determined that the percentage of structurally abnormal spermatozoa in electroejaculates of Crater lions is significantly greater than those of outbred Serengeti animals (Wildt *et al.*, 1987; Brown *et al.*, 1991). Similarly, in the Asiatic lion, clouded leopard, cheetah and domestic cat, a low degree of genetic variability is associated with teratospermic ejaculates (O'Brien *et al.*, 1983, 1985, 1986; Wildt *et al.*, 1983, 1986a, 1987), which ultimately may affect reproductive performance. For example, *in vitro* fertilization (IVF) rates in normospermic domestic cats (Johnston *et al.*, 1991) and tigers (Donoghue *et al.*, 1990) are always considerably higher than rates measured in teratospermic felid species like puma (Miller *et al.*, 1990) and cheetah (Donoghue *et al.*, 1991). Furthermore, recent data suggest that these differences are not due solely to sperm structure. Donoghue *et al.* (1991) recently discovered that the relatively poor IVF rate in cheetahs does not depend upon the source of the oocyte (the female donor), but rather is related to overall sperm motility. Finally, Howard *et al.* (1989) demonstrated that even morphologically normal appearing spermatozoa from teratospermic domestic cats bind and penetrate conspecific oocytes *in vitro* at a reduced rate compared with 'normospermic' males. Comparable studies are now needed to determine how oocyte and sperm viability influence gamete interaction *in vitro* in the lion. From a conservation perspective, these data are important for demonstrating the potential serious impact of population bottlenecks on physiological fitness in wildlife species.

We gratefully acknowledge J. Reeves for the LH antiserum; J. Dias for the FSH antiserum; L. Reichert, Jr for the canine LH and iodination grade LH and FSH; NIDDK for all other hormone preparations; D. Gilbert for help with sample collection and handling and S. Hurlbut, A. Mitchell and M. Nelson for technical assistance. We also are thankful to K. N. Hirji, former Coordinator of the Serengeti Wildlife Research Institute, and the Government of Tanzania for their support. Serum samples were collected in full compliance with specific Federal Fish and Wildlife permits (CITES; Endangered and Threatened Species; Captive Bred) issued to the National Cancer Institute, National Institutes of Health, principal officer, S. J. O'Brien, by the US Fish and Wildlife Services of the Department of Interior. This study was funded, in part, by grants from the National Geographic Society, Friends of the National Zoo and the National Science Foundation (No. 8507087).

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