

## Adrenal–pituitary–gonadal relationships and ejaculate characteristics in captive leopards (*Panthera pardus kotiya*) isolated on the island of Sri Lanka

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**Summary.** In Study 1, semen was collected using a standardized electroejaculation procedure. Males (N = 8) produced ejaculates with a high incidence of sperm abnormalities ( $77 \pm 3.3\%$ ). After electroejaculation under anaesthesia, serum cortisol concentrations increased ( $P < 0.05$ ), while testosterone concentrations decreased ( $P < 0.05$ ) and LH and FSH concentrations were unchanged ( $P > 0.05$ ) over a 2-h bleeding period. In Study 2, male and female leopards were bled at 5-min intervals for 3 h and given (i.v.): (1) saline (N = 2/sex); (2) GnRH (1  $\mu\text{g}/\text{kg}$  body weight) 30 min after the onset of sampling (N = 5/sex); or (3) ACTH (250  $\mu\text{g}$ ) at 30 min followed by GnRH 1 h later (N = 5/sex). Basal concentrations of serum LH, FSH and cortisol were comparable ( $P > 0.05$ ) between male and female leopards. After GnRH, peak LH concentrations were 2-fold greater ( $P < 0.05$ ) in males than females while FSH responses were similar. In males, testosterone concentrations increased 2–3-fold following GnRH. After ACTH, serum cortisol concentrations doubled within 15 min in both sexes. Administration of ACTH 1 h before GnRH did not affect GnRH-induced LH or FSH release ( $P > 0.05$ ); however, testosterone secretion was only 30% of that observed after GnRH alone ( $P < 0.05$ ). We conclude that (1) the high incidence of sperm abnormalities in the leopards of Sri Lanka may be related to parallel findings of genetic homozygosity; and (2) decreases in basal and GnRH-stimulated testosterone secretion were related to increases in serum cortisol after electroejaculation or ACTH and were not associated with changes in pituitary gonadotrophin secretion.

**Keywords:** leopard; spermatozoa; GnRH; ACTH; LH; FSH; cortisol; testosterone

### Introduction

In the Family Felidae, a data base of endocrine and ejaculate norms and their relationship to various environmental and genetic factors is gradually being developed. Of particular interest is the finding of species-specific variations in the incidence of teratospermia as well as differences in circulating concentrations of glucocorticoids (Wildt *et al.*, 1983, 1984b, 1988; Howard *et al.*, 1984a). Among large-sized felids, the anaesthetized North Chinese leopard (*Panthera pardus japonensis*) appears to differ by secreting high concentrations of cortisol in the presence of consistently acute decreases in serum testosterone without concomitant decreases in LH (Wildt *et al.*, 1988). Although we have suspected for years that acute increases in stress-related hormones, such as cortisol, adversely affect gonadal function in wild felids, direct evidence is lacking. The incidence of

pleiomorphic spermatozoa is also high in this subspecies, although this finding has not been directly associated with either hyperadrenal activity or a compromised genotype.

There are at least 27 recognized subspecies of leopard distributed throughout Africa and Asia (Ellerman & Morrison-Scott, 1951; Smithers, 1971). Although the leopard exists in a wide range of habitats and occupies a broad niche compared to other felids, the species is considered endangered in Asia (Seidensticker *et al.*, 1989). One subspecies, *P. p. kotiya*, found on the island of Sri Lanka is 1 of 3 world-wide extant island populations (Deraniyagala, 1949, 1956). The National Zoological Gardens of Sri Lanka at Dehiwala currently maintains about 40 wild-caught and captive-born individuals under similar environmental and managerial conditions. This situation offered a unique opportunity to study ejaculate characteristics and the relationship of adrenal activity to pituitary and gonadal function in a presumably homogeneous population of an endangered species. A simultaneous survey of the genetic diversity of these leopards (utilizing electrophoretic analyses of blood isoenzymes) allowed a comparison of seminal-endocrine characteristics with genetic variability, similar to previous efforts in the cheetah (*Acinonyx jubatus*) (O'Brien *et al.*, 1985) and geographically-isolated African (*P. leo leo*) and Asian lion (*P. l. persica*) (O'Brien *et al.*, 1987; Wildt *et al.*, 1987). The specific objectives were 2-fold: (1) to determine ejaculate characteristics including the incidence of teratospermia; and (2) on the assumption that acute stressors may affect pituitary or gonadal function, exogenous GnRH and adrenocorticotrophin (ACTH) were used to investigate the impact of adrenal-stimulated corticosteroid production on gonadotrophin release in males and females and testosterone secretion in males.

### Materials and Methods

*Animal maintenance and anaesthesia.* Male and female leopards, maintained in indoor-outdoor enclosures were studied in February and March of 1987. It is not known whether the leopards of Sri Lanka undergo seasonal reproduction. In Africa and India, leopards are known to mate throughout the year while in Manchuria and eastern Siberia a prominent mating season has been observed in January and February (Guggisberg, 1975).

For semen collection and blood sampling, animals were anaesthetized with a combination of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil; Laboratoire Virbec, Nice, France; 4–6 mg/kg, i.m.) delivered via blow dart. Once tractable, each animal was immediately transported ~0.25 km to the veterinary facility and weighed. Immobilization was maintained with supplemental Zoletil injections (50–100 mg i.m.).

*Study 1.* Eight males which had sired at least 1 litter (proven males) were used for analysis of seminal-endocrine characteristics. Four males were wild caught, 2 were captive born and 2 were of unknown origin. Males ranged in age from 3 to 10 years (average  $8.3 \pm 1.8$  years) and their mean body weight was  $63.1 \pm 2.6$  kg. Three males were housed as a group, 3 were paired with a female and 2 were housed singly.

Semen was collected by a standardized electroejaculation technique (Wildt *et al.*, 1983, 1986b, 1988; Howard *et al.*, 1986) using an electrostimulator (P-T Electronics, Boring, OR, USA) and rectal probe. The regimented sequence consisted of a total of 80 incremental stimuli (3 sec on, 3 sec off) given in 3 series. Series I and II consisted of 30 stimuli each, divided into 3 sets of 10 stimuli at 5, 6 and 7 V (Series I) and 6, 7 and 8 V (Series II). Then 20 stimuli were administered in Series III, with 10 stimuli each at 7 and 8 V. Semen from each series was evaluated qualitatively for percentage motility and progressive status (speed of forward progression based on a scale of 0 = no movement, to 5 = rapid forward movement) (Howard *et al.*, 1984b). Gross morphological assessments were made by fixing a 100  $\mu$ l semen sample from each collection series in 500  $\mu$ l glutaraldehyde (1% v/v in saline) and later evaluating 300 spermatozoa/ejaculate using phase contrast microscopy ( $\times 1000$  under oil) (Pursel & Johnson, 1974; Howard *et al.*, 1986). For each male, there were no differences ( $P > 0.05$ ) in ejaculate characteristics among the 3 collection series; therefore, semen data within males were averaged for presentation.

Blood samples were obtained by saphenous venepuncture immediately before the onset of electroejaculation, after each electroejaculation series and at 15, 30, 45, 60, 75 and 90 min after the last electrical stimulus (10 samples/male). All samples were maintained at 5°C for ~1 h before centrifugation. Recovered sera were stored at -20°C until analysed.

*Study 2.* Twelve female leopards and 12 male leopards not utilized in Study 1 were used to study the effects of ACTH and GnRH on pituitary-gonadal responses. With the exception of 2 males and 1 female, all animals were captive born. Of the males, 5 were maintained as singletons, 2 were housed together and 5 were paired with a female. Three females were maintained as singletons, 3 were housed together and 6 were paired with a male. All animals in Studies 1 and 2 had visual, auditory and/or olfactory contact with conspecifics of both sexes. Males in this study were not breeders (unproven males) and only one female had given birth to a single litter. Body weight for males averaged  $54 \pm 2.5$  kg and age ranged from 3 to 10 years (average  $6.1 \pm 1$  years). Mean female body weight was  $36.8 \pm 1.5$  kg and age ranged from 3 to 10 years (average  $5.5 \pm 1.3$  years).

Immediately after induction of anaesthesia and transport to the veterinary facilities, a 16-gauge, 5-cm indwelling cannula (Becton Dickinson, Rutherford, NJ, USA) was placed into a saphenous or lateral coccygeal vein. Blood sampling was initiated within 30 min after the initial injection of anaesthetic.

Animals were assigned at random and treated (i.v.) with: (1) saline (0.154 M-NaCl, pH 6.9; 2 ml) administered after 30 min of sampling (2 males, 2 females); (2) GnRH (1 µg/kg body weight; Gonadorelin, Abbott Labs, Chicago, IL, USA) delivered via the cannula after 30 min of sampling (GnRH; 5 males, 5 females); or (3) ACTH (250 µg; Cortrosyn, Organon, Inc., W. Orange, NJ, USA) administered at 30 min, followed 1 h later by GnRH (ACTH/GnRH; 5 males, 5 females). Blood samples (3 ml) were collected at 5 min intervals for 3 h and sera recovered and stored as described for Study 1. To confirm that males used in Study 2 were spermatogenic, a single electroejaculate was collected immediately after the last blood sample. The technique utilized was the same as that described for Series 1 in Study 1.

**Radioimmunoassays.** Serum LH and FSH concentrations were quantified using specific radioimmunoassays previously validated for leopard serum (Brown *et al.*, 1988), using dog LH (LER-1685-1) and sheep FSH (NIH-FSH-S8) as the reference standards. Assay sensitivities (defined as 95% of maximum binding) were 0.7 and 10 ng/ml for 100 µl serum; inter- and intra-assay coefficients of variation for 100 µl of a serum pool (N = 10) were 8.1 and 6.5, and 8.5 and 5.9% for LH and FSH, respectively. The 20, 50 and 80% binding values of the dog LH standard curve were 40.5, 4.9 and 1.1 ng/ml, and of the sheep FSH standard curve were 164, 51 and 15 ng/ml, respectively.

Testosterone was measured in unextracted serum using a double-antibody <sup>125</sup>I radioimmunoassay kit (Radio Systems Laboratories, Carson, CA, USA). The primary antiserum (rabbit anti-testosterone-19-carboxymethyl-ethanol-bovine serum albumin) had the following cross-reactivities (determined as the cross-reactivity at 50% displacement compared to testosterone): 100% with testosterone; 10.3% with 5α-dihydrotestosterone; <1% with 5β-dihydrotestosterone, androstenedione, androsterone, epiandrosterone, oestrone, oestradiol-17β, oestriol, progesterone and corticosterone. The assay was validated for leopard serum by demonstrating recovery of mass. Upon addition of 0.125, 0.25, 0.5, 1.25, 2.5 and 5 ng testosterone to 50 µl leopard serum, 0.11, 0.24, 0.49, 1.18, 2.45 and 4.97 ng were recovered, respectively, after subtraction of endogenous hormone ( $y = -0.024 + 0.996x$ ;  $r = 0.999$ ). Inhibition curves for pooled sera and the testosterone standard were parallel. Assay sensitivity was 0.05 ng/ml for 50 µl serum and the inter- and intra-assay coefficients of variation for 50 µl samples of a serum pool were 5.5 and 4.6%, respectively. The 20, 50 and 80% binding values of the testosterone standard curve were 7.9, 0.9 and 0.2 ng/ml, respectively.

Cortisol was measured in unextracted serum using a double antibody <sup>125</sup>I radioimmunoassay kit (New England Nuclear, N. Billerica, MA, USA). The antiserum complex (rabbit cortisol antibody prereacted with rabbit gamma-globulin antiserum) had the following cross-reactivities: 100% with cortisol; 62.8% with prednisolone; 14.7% with corticosterone; 4.3% with 11-deoxycortisol; 3.5% with 17α-hydroxyprogesterone; 1.9% with 11-deoxycorticosterone; 1.6% with cortisone; 1.6% with progesterone; <1% with testosterone, dihydrotestosterone, oestradiol-17β and aldosterone. Because of high cortisol concentrations, sera were diluted 1:1 with PBS buffer (0.01 M-NaPO<sub>4</sub>, 0.154 M-NaCl, pH 7.2) before analysis. The assay was validated for use with leopard serum. Recovery estimates after subtraction of endogenous hormone from the analysis of 10, 25, 50 and 125 ng cortisol in 10 µl serum were 8, 29, 58 and 143 ng, respectively, ( $y = -1.45 + 1.16x$ ;  $r = 0.996$ ). Inhibition curves for serum pools were parallel to the standard curve. Sensitivity was 7.5 ng/ml for 10 µl serum and inter- and intra-assay coefficients of variation were 8.8 and 5.8% for 10 µl samples of a serum pool, respectively. The 20, 50 and 80% binding values of the cortisol standard curve were 492, 107 and 29 ng/ml, respectively.

The <sup>125</sup>I steroid RIA kits described above were developed for use with unextracted human serum. Analysis of extracted and unextracted leopard serum pools ( $n = 10$ ) yielded similar results. Therefore, all serum samples were assayed unextracted.

**Data analysis.** Changes in endocrine values over time were analysed using split-plot analyses of variance. Gonadotrophin responses to GnRH, testosterone responses to LH and cortisol responses to ACTH were evaluated as peak height, net peak height (greatest post-treatment value minus mean pretreatment level) and net area under the response curves. Net area after hormone treatment, expressed as cm<sup>2</sup>/90 min (LH, FSH and testosterone) or cm<sup>2</sup>/150 min (cortisol), was determined using a planimeter with a 3% coefficient of variation (Brown *et al.*, 1988). Differences in endocrine data between sexes or GnRH vs ACTH/GnRH treatments were determined by Student's *t* tests. Data are presented as means ± s.e.m.

Basal and pulsatile serum hormone patterns were evaluated as described previously (Brown *et al.*, 1988). Briefly, basal hormone concentrations were determined by an iterative process in which high values (those exceeding the mean plus 2 s.d.) were excluded. A pulse was significant if: (1) the amplitude was at least 2 standard deviations greater than the mean concentration; (2) the peak occurred within 2 samples of a previous nadir; and (3) the peak was followed by at least 2 successive values that were declining or represented baseline values. Pulse amplitude was defined as the highest point associated with the peak minus the mean basal concentration.

## Results

### Ejaculate characteristics

Among all Study 1 and 2 males, sperm concentration, % motility and progressive status ratings were: 0.3–74.3 × 10<sup>6</sup> spermatozoa/ml; 20–85%; and 2–4.5, respectively. Ejaculates from all males were characterized by a high percentage of abnormal spermatozoa (range, 45.6–93.5%). The major

sperm defect was a tightly coiled flagellum, although spermatozoa with an abnormal acrosome, bent midpiece, bent flagellum or cytoplasmic droplet were also prevalent (Table 1).

**Table 1.** Ejaculate traits of captive male leopards (*Panthera pardus kotiya*) in Studies 1 and 2

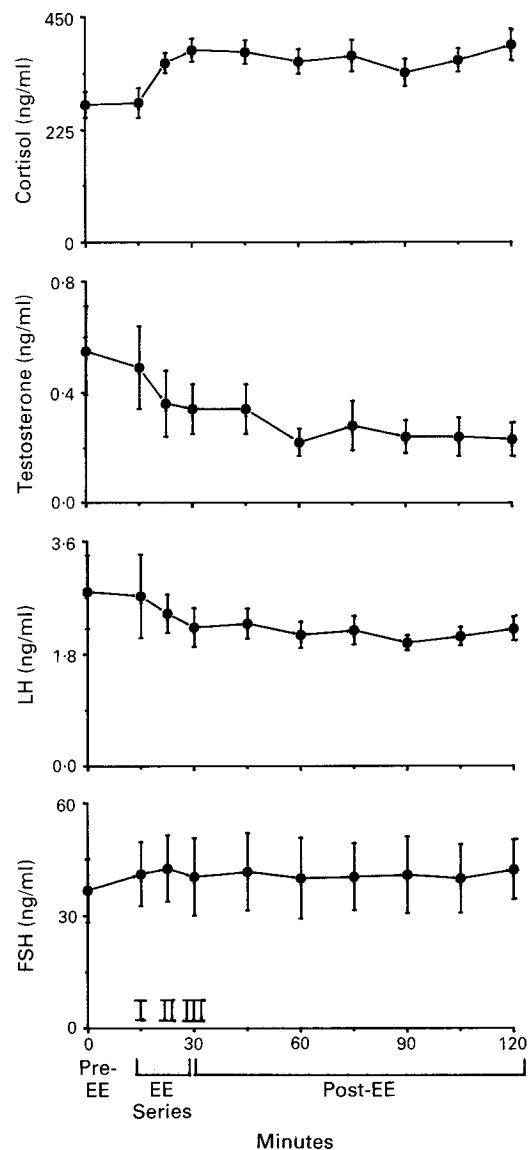
	Study 1 (N = 8)	Study 2 (N = 12)
Sperm concentration/ml ( $\times 10^{-6}$ )	13.1 $\pm$ 5.5	11.4 $\pm$ 3.8
Motility (%)	55.0 $\pm$ 5.2	54.0 $\pm$ 2.5
Progressive status (0-5)	3.3 $\pm$ 0.2	3.1 $\pm$ 0.2
Normal spermatozoa (%)	22.8 $\pm$ 5.8	19.9 $\pm$ 2.5
Abnormality type (%)		
Microcephalic	0.4 $\pm$ 0.2	0.1 $\pm$ 0.1
Bicephalic	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2
Abnormal acrosome	12.7 $\pm$ 1.8	12.2 $\pm$ 3.0
Loose head	0.9 $\pm$ 0.7	0.9 $\pm$ 0.9
Bent neck	1.8 $\pm$ 0.4	3.3 $\pm$ 0.7
Abnormal midpiece	2.0 $\pm$ 0.4	4.1 $\pm$ 1.2
Bent midpiece	7.2 $\pm$ 1.2	7.1 $\pm$ 1.3
Bent midpiece with cytoplasmic droplet	10.8 $\pm$ 1.8	8.0 $\pm$ 1.3
Bent flagellum	5.4 $\pm$ 0.9	5.9 $\pm$ 0.7
Coiled flagellum	22.4 $\pm$ 5.3	19.5 $\pm$ 4.9
Proximal cytoplasmic droplet	7.3 $\pm$ 1.2	7.9 $\pm$ 1.9
Distal cytoplasmic droplet	5.9 $\pm$ 1.3	10.8 $\pm$ 3.1

Values are mean  $\pm$  s.e.m.

#### Endocrine characteristics

Mean serum cortisol concentrations in Study 1 males increased ( $P < 0.05$ ) 40% after the first electroejaculation series and were sustained at this level throughout the bleeding interval (Fig. 1). Serum testosterone concentrations decreased over time ( $P < 0.05$ ) from  $0.55 \pm 0.16$  ng/ml before electroejaculation to  $0.23 \pm 0.06$  ng/ml in the final blood sample (Fig. 1). In contrast, neither serum LH nor FSH concentrations were altered ( $P > 0.05$ ). In Study 2, mean serum cortisol concentrations were not different ( $P > 0.05$ ) between saline- and GnRH-treated leopards and so the results were pooled for presentation (Fig. 2a). Cortisol concentrations increased gradually (as much as 25%) in about two-thirds of the saline- and GnRH-treated animals, usually within 70–120 min of blood sampling onset (Fig. 2a). After ACTH, serum cortisol doubled within 10–15 min of injection and remained elevated for the sampling duration (Fig. 2b). There were no differences ( $P > 0.05$ ) in ACTH-induced cortisol responses between males and females.

Basal concentrations of LH ( $1.9 \pm 0.2$ ;  $1.8 \pm 0.1$  ng/ml) and FSH ( $28.7 \pm 7.8$ ;  $27.5 \pm 8.6$  ng/ml) were similar ( $P > 0.05$ ) between saline-treated male and female leopards, respectively. Pulses of LH (1–3 pulses/180 min) were detected in all control animals. Pulse amplitudes ranged from 1.0 to 1.8 ng/ml and averaged  $1.3 \pm 0.1$  ng/ml. Pulses of FSH, based on previously described criteria, were not observed. After GnRH, serum LH concentrations peaked within 10–20 min, with maximal LH values being 2-fold greater ( $P < 0.05$ ) in males than females (Fig. 3a). Serum FSH concentrations doubled in all animals within 15–30 min after GnRH and responses were similar ( $P > 0.05$ ) between sexes. In males, increases in serum testosterone occurred within 15–30 min of the GnRH injection and values remained elevated 2.5-fold over baseline concentrations throughout the bleeding period (Fig. 3a). Administration of ACTH 1 h before GnRH did not affect ( $P > 0.05$ ) pituitary release of LH or FSH, while testosterone secretion was less ( $P < 0.05$ ) compared to that

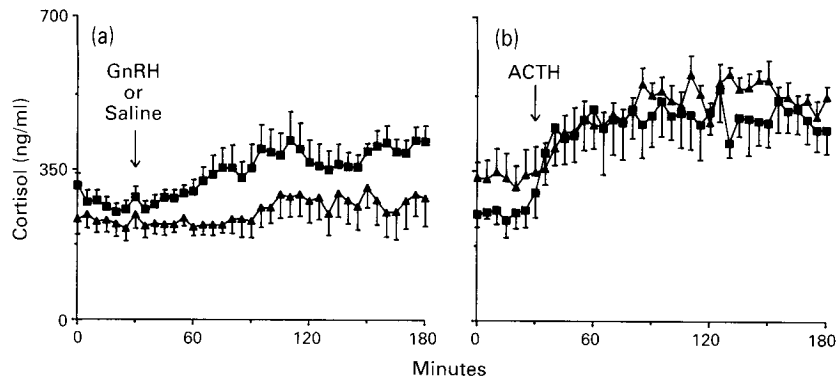


**Fig. 1.** Mean  $\pm$  s.e.m. serum cortisol, testosterone, LH and FSH concentrations in Sri Lankan leopards ( $N = 8$ ) subjected to a standardized electroejaculation regimen. Pre-EE = after anaesthesia, before electroejaculation; I, II and III = end of electroejaculation Series I, II and III, respectively. Post-EE = 15-min samples collected 15–90 min after electroejaculation.

observed after GnRH along (Fig. 3a, b). Testosterone peak height ( $1.80 \pm 0.14$  vs  $0.81 \pm 0.26$  ng/ml), net peak height ( $1.07 \pm 0.27$  vs  $0.35 \pm 0.05$  ng/ml) and net area under the curve ( $9.95 \pm 2.21$  vs  $2.92 \pm 0.64$  cm<sup>2</sup>/90 min) were 2- to 3-fold more ( $P < 0.05$ ) in GnRH-treated males than in those treated with ACTH + GnRH.

#### Discussion

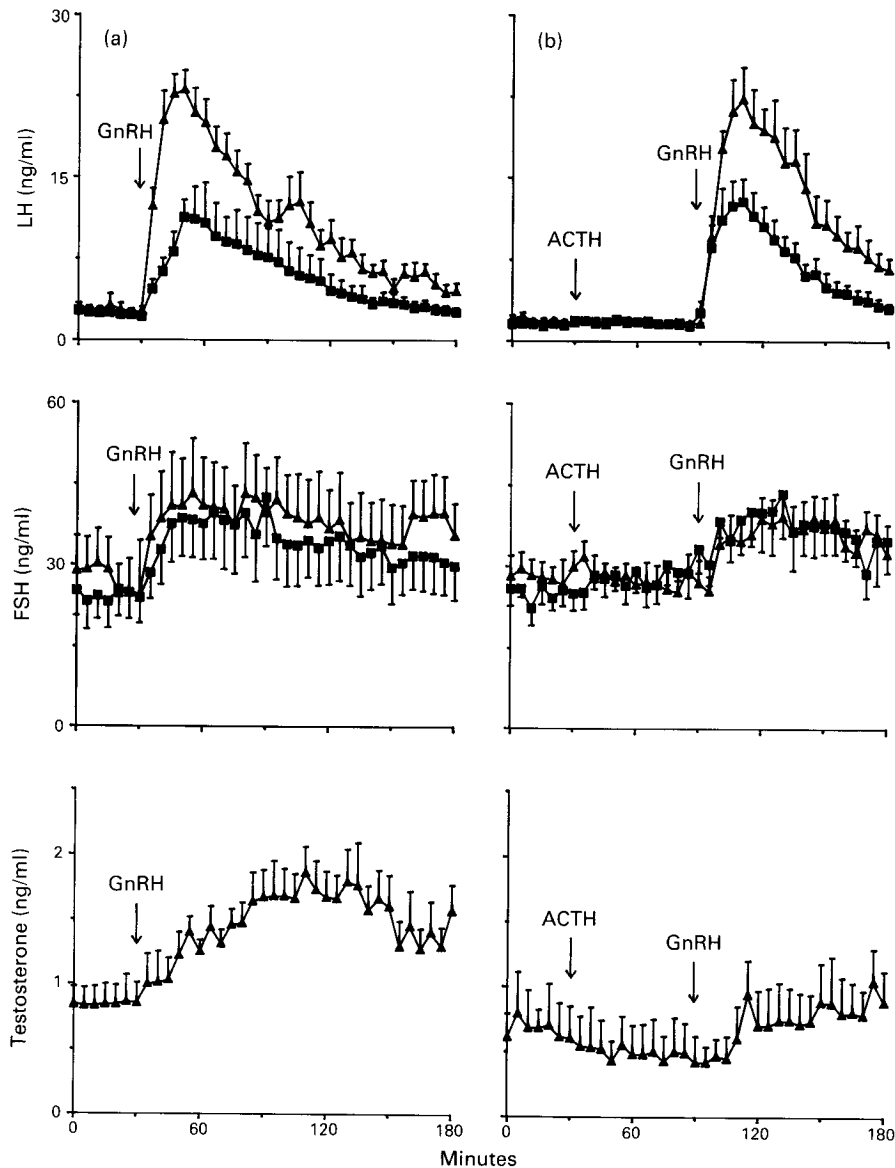
The high serum cortisol concentrations obtained in this study were similar to those reported for the North Chinese leopard (Brown *et al.*, 1988; Wildt *et al.*, 1988), but were several-fold higher than



**Fig. 2.** Mean  $\pm$  s.e.m. serum cortisol concentrations after injection of (a) saline (2 animals/sex) or GnRH (5/sex), or (b) ACTH (5/sex) in male ( $\blacktriangle$ ) and female ( $\blacksquare$ ) leopards.

those found in other felids (Carter *et al.*, 1984; Wildt *et al.*, 1984b, 1988). In anaesthetized North Chinese leopards and pumas (*Felis concolor*), serum cortisol increases over time; however, concomitant decreases in serum testosterone are consistently observed only in the leopard (Wildt *et al.*, 1988). In the present study, serum cortisol elevations during electroejaculation were associated temporally with acute decreases in circulating testosterone. Furthermore, ACTH-induced elevations in serum cortisol had no influence on GnRH-stimulated LH or FSH release in either sex; however, testosterone secretion in response to GnRH-induced LH release in males was significantly attenuated. These observations confirm an earlier suggestion that the influence of increased adrenal activity on pituitary-gonadal function among felids is species-specific (Wildt *et al.*, 1988), and suggests that the leopard may be particularly sensitive to the influence of increased adrenal activity. The effect of increased serum cortisol concentrations on LH secretion in carnivores (including felids) is poorly understood. Reduced pituitary responsiveness to exogenous GnRH has been demonstrated after an elevation of corticosteroids in female rats (Baldwin & Sawyer, 1974), rams (Matteri *et al.*, 1984), gilts (Pearce *et al.*, 1988) and boars (Liptrap & Raeside, 1975). In contrast, Liptrap & Raeside (1983) found that cortisol infusion increased pituitary response to GnRH in adult boars. Still others report no effect of elevated corticosteroids on either basal or GnRH-stimulated LH release (Barnes *et al.*, 1983). Studies in primates have demonstrated that exogenous ACTH or glucocorticoids can markedly alter testosterone release without affecting basal or GnRH-stimulated gonadotrophin secretion (Doerr & Pirke, 1976; Sapolsky, 1985). In rats, decreases in testosterone secretion have been related to a direct inhibitory effect of glucocorticoids on the testis since both dexamethasone and corticosterone reduce the concentrations of Leydig cell LH receptors (Saez *et al.*, 1977; Bambino & Hsueh, 1981). In agreement with these reports, the present study is the first to demonstrate that suppressed testicular function occurs after acute elevations of serum cortisol in a wild species of felid, without any observed perturbations in circulating LH or FSH.

Basal and pulsatile LH and FSH secretion in Sri Lankan leopards was similar to that reported for North Chinese leopards (Brown *et al.*, 1988). Basal testosterone concentrations in Sri Lankan leopards were less than values reported for the domestic cat (*F. catus*), clouded leopard (*Neofelis nebulosa*), tiger (*P. tigris*) and puma (Goodrowe *et al.*, 1985; Wildt *et al.*, 1986b, 1988; Brown *et al.*, 1988), but were similar to the lower values reported for the cheetah and North Chinese leopard (Wildt *et al.*, 1984a, b, 1988; Brown *et al.*, 1988). In this study, male and female leopards responded to GnRH with increases in serum LH and FSH that were nearly identical in magnitude to those described for North Chinese leopards (Brown *et al.*, 1988). The LH response to GnRH was greater in males than females, although no gender differences in GnRH-stimulated FSH secretion were observed. Sex differences in GnRH-stimulated LH secretion have also been



**Fig. 3.** Mean  $\pm$  s.e.m. serum LH, FSH and testosterone concentrations after (a) GnRH (5 animals/sex), or (b) ACTH followed 1 h later by GnRH (5/sex) in male ( $\blacktriangle$ ) and female ( $\blacksquare$ ) leopards.

demonstrated in the cheetah, tiger and North Chinese leopard (Wildt *et al.*, 1984a; Brown *et al.*, 1988).

The high incidence of teratospermia observed in the leopards of Sri Lanka is a common finding among many felid species. High percentages of structurally abnormal spermatozoa have also been reported in the electroejaculates of the North Chinese leopard, puma, cheetah, clouded leopard, tiger and some populations of lions (range 38–80%) (Wildt *et al.*, 1986b, 1987, 1988). In contrast, using the same electroejaculation procedure, males of other species such as the domestic cat, leopard cat (*Prionailurus bengalensis*), or known outbred lion populations produce ejaculates

containing fewer than 30% abnormal spermatozoa (Wildt *et al.*, 1983, 1987; Howard *et al.*, 1984a). Although the specific causes of teratospermia in felids are unknown, there is increasing evidence that genetic variation and hormonal status influence ejaculate characteristics. Two felid species known to have survived severe population bottlenecks and/or geographical isolation (cheetah and lion) exhibit low genetic diversity, poor ejaculate quality and depressed basal testosterone secretion (Wildt *et al.*, 1983, 1984b, 1987; O'Brien *et al.*, 1985, 1987). Androgens are essential for most stages of spermatogenesis (Mann & Lutwak-Mann, 1981); however, it is not known whether circulating testosterone concentrations in some felid species with compromised sperm morphology are sub-optimal for sustaining normal spermatogenic function. The observation of depressed testosterone values in some species or populations, despite similar immunoreactive concentrations of the controlling hormone LH (Goodrowe *et al.*, 1985; Wildt *et al.*, 1986a, 1987, 1988; Brown *et al.*, 1988), suggests that compromised genotype probably affects the gonad and not the pituitary. However, alterations in bioactivity of the secreted gonadotrophins may also be involved.

A parallel study of genetic diversity in the leopards of Sri Lanka ( $N = 33$ ) revealed a low frequency of polymorphism ( $P = 4\%$ ) and average heterozygosity ( $\bar{H} = 0.014$ ) (based on a survey of 50 alloenzyme loci: Miththapala *et al.*, 1989). The extent of genetic monomorphism in this population was not as marked as that observed in the cheetah and Asian lion ( $P = 0\%$ ;  $\bar{H} = 0$ ), but was comparable to that of a lion population geographically isolated within a volcanic caldera in the Serengeti ecosystem ( $P = 4\%$ ;  $\bar{H} = 0.015$ ) and greater than that for the clouded leopard ( $P = 6\%$ ;  $\bar{H} = 0.023$ ), tiger ( $P = 10\%$ ;  $\bar{H} = 0.035$ ), free-ranging African lion in the Serengeti ( $P = 11\%$ ;  $\bar{H} = 0.038$ ) (Newman *et al.*, 1985; O'Brien *et al.*, 1987) and domestic cat ( $P = 21\%$ ;  $\bar{H} = 0.082$ ) (O'Brien, 1980). Today, human encroachment and the destruction of wildlife habitats, including those in Sri Lanka, increase the incidence of population fragmentation and isolation, thereby decreasing the ability of these species to remain genetically diverse. The present data support previous assertions that isolated populations are susceptible to genetically derived alterations in normal physiological function.

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