Seasonal patterns of basal and GnRH-induced LH, FSH and testosterone secretion in Eld's deer stags (*Cervus eldi thamin*)

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Plasma LH, FSH and testosterone were measured in blood samples collected via remote catheterization from six adult Eld's deer stags every 10 min for 8 h before and 2 h after GnRH ($1 \mu\text{g kg}^{-1}$, i.v.) administration. Blood samples were collected within 2 weeks of the summer solstice (21 June), autumn equinox (22 September), winter solstice (21 December) and spring equinox (20 March). Marked seasonal variations in basal LH, FSH and testosterone concentrations were observed. From autumn, well-defined LH pulses were temporally associated with small, but detectable pulses in testosterone. During the winter transition into the breeding season, episodic LH pulses were also temporally associated with corresponding testosterone surges that lasted 2–3 h. High amplitude, low frequency testosterone surges were also observed during the spring, but often in the absence of detectable LH pulses. Basal LH and testosterone concentrations decreased during the summer and, although LH pulses were detected, associated testosterone pulses were absent. Only 37% of LH pulses occurred coincidentally with FSH pulses, and FSH pulses were generally less prominent. The increases in LH and FSH above basal concentrations after GnRH treatment were significant ($P < 0.05$) for all seasons. Increases in testosterone after GnRH treatment were greatest during the winter and spring, but testosterone also increased to a lesser extent during the autumn ($P < 0.05$). In contrast, testosterone concentrations were not different before and after GnRH treatment during summer. The net LH increase after GnRH treatment was similar ($P > 0.05$) for all seasons, whereas the proportional increase in LH was greatest ($P < 0.05$) during the summer and autumn when basal concentrations were lowest. Although the net FSH increase after GnRH treatment ranged only from 20 to 40 ng ml⁻¹ during all seasons, the proportional rise in FSH after GnRH treatment tended to be highest during the summer and autumn when basal concentrations were lowest. Basal testosterone secretion and the net increase in testosterone after GnRH treatments were greatest during the spring rut ($P < 0.05$). Thus, in Eld's deer, it appears that pituitary gonadotrophs seasonally adjust their basal secretory 'set point', but the capacity to respond above basal concentrations is constrained to a maximum net increase in both LH and FSH, regardless of season. Because both basal LH and the net releasable pool of LH remain relatively constant, increased testosterone secretion after GnRH treatment probably represents increased testicular sensitivity to LH.

Introduction

The Burmese brow-antlered deer, or Eld's deer (*Cervus eldi thamin*), is sparsely distributed from eastern India to Indochina and southern China (3°–25°N latitude). Eld's deer represent a striking example of a subtropical species that does not exhibit a latitudinal shift in the timing or duration of annual reproductive rhythms, expressing pronounced seasonality in its native habitat as well as at northern, temperate latitudes (38°N) (Monfort *et al.*, 1993). Unlike most temperate cervid

species, the circannual hypothalamic–pituitary–gonadal cycle of the Eld's deer stag begins with pituitary activation during autumn and winter, and peak testis size, testosterone secretion, rutting behaviour and maximal sperm production occurring during winter and spring, as daylengths increase (Monfort *et al.*, 1990; 1993).

The seasonal cycle of antler growth, body weight, neck and chest girth in Eld's deer (Monfort *et al.*, 1993) is qualitatively similar to that of red deer (*Cervus elaphus*, Suttie *et al.*, 1984), chital (*Axis axis*, Loudon and Curlewis, 1988) and fallow deer (*Dama dama*, Asher *et al.*, 1989). However, for the Eld's deer, these rhythms are shifted 6 months out-of-phase relative to

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most temperate cervids. Despite the seasonal phase difference, the interactive dynamics of the pituitary–gonadal axis in Eld's deer (Monfort *et al.*, 1993) is remarkably similar to red deer stags (Lincoln and Kay, 1979) and fallow deer bucks (Asher *et al.*, 1989). Antler growth begins before LH and FSH increase and continues while LH and FSH concentrations are high and testosterone and prolactin are low (Monfort *et al.*, 1993). As in other deer studied, antler mineralization, velvet stripping and rut occur when testosterone is high, whereas casting occurs after testosterone has declined (Suttie *et al.*, 1984). Peak FSH secretion in Eld's deer occurs 3 months later than the LH peak, closely paralleling the cycle of testosterone secretion and sperm production (Monfort *et al.*, 1993). Overall, these data are similar to observations in white-tailed deer (*Odocoileus virginianus*, Mirarchi *et al.*, 1978; Bubenik *et al.*, 1982), roe deer (*Capreolus capreolus*, Sempère and Lacroix, 1982) and red deer (Suttie *et al.*, 1984) and can be interpreted to suggest that LH plays a critical role in initiating testicular steroidogenic activity. In contrast, FSH and testosterone appear to be important for supporting later spermatogenesis, during the peak of the circannual testicular cycle.

The short-term secretory dynamics of LH and testosterone secretion have been well-characterized only in red deer (Lincoln and Kay, 1979; Suttie *et al.*, 1984; Fennessy *et al.*, 1988) and fallow deer (Asher *et al.*, 1989; Asher and Peterson, 1991), whereas hour-to-hour basal FSH secretion patterns have not been described for any cervid. Pituitary or gonadal responsiveness to an exogenous GnRH challenge has been studied in white-tailed deer (Plotka *et al.*, 1984; Bubenik *et al.*, 1987), red deer (Lincoln and Kay, 1979; Suttie *et al.*, 1984; Fennessy *et al.*, 1988) and roe deer (*Cervus roosevelti*, van Mourik *et al.*, 1986), and only one report (white-tailed deer, Bubenik *et al.*, 1987) has described seasonal pituitary FSH responsiveness to an exogenous GnRH challenge. Thus, the existing database describing seasonal changes in the secretory dynamics of the reproductive axis of male deer has been almost exclusively derived from studies of three temperate species. There is no information describing the hour-to-hour secretory changes in gonadotrophins and testosterone in any tropical cervid species.

The Eld's deer represents a unique opportunity to study the interrelationships between the hypothalamic–pituitary–gonadal axis in a subtropical, endangered species that expresses a reproductive rhythm 6 months out-of-phase with temperate cervid species. The present study used a remote catheterization technique that permitted repeated collection of blood samples from unanaesthetized, unrestrained stags. Our objectives were to (i) investigate the temporal secretory dynamics of basal LH, FSH and testosterone, (ii) compare GnRH-induced pituitary and gonadal responsiveness during the summer, autumn, winter and spring and (iii) relate our findings to the existing database for temperate cervid species.

Materials and Methods

Animals and treatments

Six adult Eld's deer stags (2–8 years of age; 70–120 kg body weight) maintained at the Smithsonian Institution's Conservation and Research Center, Front Royal, VA (38°N) were used in the

study. Males were housed individually in indoor stalls (3.4 m × 4.6 m with skylights) connected to outdoor enclosures (3.6 m × 36.6 m) and exposed to natural fluctuations in photoperiod. Individuals were maintained within visual proximity to other males and within auditory and olfactory proximity to conspecific adult males and females.

Collection of blood samples

Blood samples were collected every 10 min for 8 h before and 2 h after GnRH (Cystorelin: Abbott Laboratories, Chicago, IL; 1 µg kg⁻¹, i.v.) administered within 2 weeks of the summer solstice (21 June), autumn equinox (22 September), winter solstice (21 December) and spring equinox (20 March). On the afternoon before the day of collection of serial blood samples, each stag was sedated using xylazine (Rompun: Mobay Corp., Shawnee, KA; 0.3–0.5 mg kg⁻¹, i.m.), and dual i.v. jugular catheters (16 gauge × 22.9 cm, Centralsil: Baxter Healthcare Corp., Deerfield, IL) were inserted, sutured into place and connected to a 76 cm extension tube (Abbott Hospitals, Inc., Chicago IL, USA). The extension line was coiled, covered with bandage, and catheters then were flushed with heparinized saline (10 ml, 30 iu ml⁻¹) to prevent intracannula clotting. Males were placed in a 1.8 m × 0.9 m stall where blood was to be collected via remote catheterization the next day beginning at 08:00 h. On the day of blood collection (07:30 h), two animal handlers entered the collection stall, accessed the short extension line (76 cm line) from underneath the bandage, and connected another saline-filled extension line (150 cm) to permit remote removal of blood from outside the stall. Individual blood samples were collected while stags were fully conscious (at least 18 h after full recovery from anaesthesia). During the sampling interval (about 08:00–20:00 h), stags were able to stand, recline and turn around comfortably; alfalfa hay and water were provided *ad libitum* during this time. To obtain each blood sample, a syringe attached to a three-way stopcock was used to aspirate saline through the line until undiluted blood was collected. Blood samples (about 3 ml) were transferred into heparinized tubes, placed on ice, centrifuged (1500 g, 20 min) within 2 h, and plasma was stored at –70°C until analysed. The extension line was then flushed with either 25 ml heparinized (5 iu ml⁻¹) or nonheparinized saline on alternate blood draws to prevent clot formation.

Luteinizing hormone assay

Serum LH was analysed using a heterologous, ¹²⁵I double-antibody radioimmunoassay which used a rabbit anti-ovine LH antiserum (PKC-231A, J. L. Brown) as the first antibody, purified ovine LH (LER-1056-C2, L. E. Reichert, Jr, Albany Medical College, Albany, NY) as the tracer and ovine LH (NIH-LH-S18, National Pituitary Program, Baltimore, MD) as the standard (Brown *et al.*, 1991). Serial dilutions of pooled Eld's deer plasma yielded displacement curves parallel to that obtained for the LH standard. The mean recovery of ovine LH (range 0.03–4.00 ng per tube) added to Eld's deer plasma, after subtraction of endogenous hormone, was 99.3 ± 3.8% ($y = 1.03x + 0.005$, $r^2 = 0.98$, $P < 0.001$). Assay sensitivity, calculated as 90% of maximum binding, was 0.03 ng per tube. The interassay coefficients of variation for two separate internal controls were 10.0% and 9.9%; intra-assay variation was < 10%.

Follicle-stimulating hormone assay

Serum FSH was measured using a heterologous, ^{125}I double-antibody radioimmunoassay with a rabbit anti-ovine FSH antiserum (JADLER 178, J. A. Dias, Wadsworth Research Institute, Albany, NY), purified ovine FSH (LER-1976-A2, L. E. Reichert, Jr) tracer and ovine FSH (NIH-FSH-88, National Pituitary Program) standards (Brown *et al.*, 1987). Serial dilutions of pooled Eld's deer plasma yielded displacement curves parallel to that obtained with the standard preparation. The mean recovery of ovine FSH (range 0.78–100 ng per tube), after subtraction of endogenous hormone, was $102.3 \pm 4.4\%$ ($y = 1.06x + 0.34$, $r^2 = 0.99$, $P < 0.001$). Assay sensitivity was 0.5 ng per tube. The interassay coefficients of variation for two separate internal controls was 11.5% and 14.4%, respectively; intra-assay variation was $< 10\%$.

Testosterone assay

Serum testosterone was analysed in unextracted plasma using a ^{125}I radioimmunoassay kit (ICN, Costa Mesa, CA). Serial dilutions of plasma yielded displacement curves parallel to that obtained with the standard preparation. The mean recovery of testosterone (range 0.1–10 ng ml $^{-1}$) added to pooled Eld's deer plasma was $99.4 \pm 3.1\%$ ($y = 1.01x - 0.002$, $r^2 = 0.99$, $P < 0.001$). Assay sensitivity was 0.1 ng ml $^{-1}$. The interassay coefficient of variation was 12.9%, and intra-assay variation was $< 10\%$.

Statistical analyses

Hormone concentrations are represented as means $\pm$ SEM. All statistical comparisons were performed using Statview 512+ (BrainPower, Inc., Calabasas, CA) on a Macintosh computer. Among-season comparisons of pulse detection data, as well as among- and within-season comparisons of hormone concentrations before and after GnRH treatment were determined by repeated measures analysis of variance. Multiple pairwise contrasts were performed using Fisher's protected least significant difference test. The net increase in hormone concentrations was defined as the mean hormone concentration after GnRH treatment ($n = 12$ samples) minus the mean basal hormone concentration ($n = 3$ samples before GnRH administration). Because of within-animal fluctuations in hormone secretion patterns, the baseline before GnRH treatment was calculated as the mean of the three samples collected immediately before GnRH administration. The proportional increase in hormone concentration was defined as the mean hormone concentration after GnRH minus the mean basal hormone concentration, divided by the mean basal hormone concentration, expressed as a percentage. Correlation coefficients were calculated to determine the correspondence between LH and FSH profiles.

Cluster Analysis (Veldhuis and Johnson, 1986; version 5.0), a statistically-based pulse detection algorithm, was used to analyse pulsatile LH, FSH and testosterone secretion before GnRH administration. A 2×1 (2 nadir points $\times$ 1 peak point) cluster size was used for LH (constant coefficient of variation = 10%) and a 2×2 cluster configuration was chosen for FSH and testosterone (constant coefficients of variation, FSH = 12%;

testosterone = 10%). Pooled t statistics of 2.0 were chosen for pulse detection analyses for all hormones. The specific cluster configuration and t statistic were selected to limit the false pulse detection rate to about 5%. This program estimated all possible increases in the data series when successive nadir clusters (two data points) were compared against immediately successive potential pulse regions (one or two data points). All significant decreases within the data series were also analysed with reference to this particular cluster configuration. The mean pulse height as a percentage increase represents the percentage increase of the mean pulse amplitude above the preceding nadir value. The largest pulse height above basal represented the incremental increase of the largest value expressed as the algebraic difference between the largest value and the preceding nadir.

Results

All Eld's deer stags in the present study exhibited marked ($P < 0.05$) seasonal variations in basal concentrations of LH, FSH and testosterone. Basal secretory patterns and responsiveness to GnRH from two representative individuals (Fig. 1a, b) were typical of seasonal profiles observed in all stags. During the winter transition into the breeding season, distinct testosterone surges (mean increase above basal 1.8 ± 0.3 ng ml $^{-1}$; mean duration 149.2 ± 6.8 min; mean duration range 130–175 min) were always preceded by 30–40 min by LH pulses. During the spring rut, high amplitude (mean increase above basal 3.3 ± 1.0 ng ml $^{-1}$) testosterone surges (mean duration 120.0 ± 18.0 min; mean duration range 72–168 min) were also observed, but only 56% (9 of 16) of these surges were preceded by LH pulses. Mean basal LH secretion decreased ($P < 0.05$) from 3.3 ± 0.4 ng ml $^{-1}$ during the spring rut to 1.9 ± 0.1 ng ml $^{-1}$ during the summer, after the breeding season. Spontaneous testosterone pulses or surges were absent during the summer, but small testosterone pulses were observed coincident with LH pulses (see insets, Fig. 1) during the autumn. For all seasons, only 37% of LH pulses were secreted coincidentally with FSH pulses, and the latter were generally less prominent than were LH pulses. Overall mean correlations between FSH and LH secretion during 24, 8-h basal sampling intervals was $r = 0.38 \pm 0.05$. Correspondence between FSH and LH was greater ($P < 0.05$) in the autumn than in the summer (seasonal r values: autumn, 0.56 ± 0.09 ; winter, 0.35 ± 0.08 ; spring, 0.41 ± 0.09 ; summer, 0.20 ± 0.07).

Quantitative pulse detection analyses were performed for LH, FSH and testosterone for each 8-h data series before GnRH treatment (Table 1). LH pulse frequency was greater during the summer ($P < 0.05$) than in all other seasons, whereas no seasonal differences ($P > 0.05$) were detected in the mean pulse heights expressed as a percentage increase over basal concentrations, or in the largest observed pulse height above basal concentrations. FSH pulse frequencies during the summer and autumn were greater ($P < 0.05$) than during the winter, but not the spring ($P < 0.05$); pulse frequencies during winter and spring were similar ($P > 0.05$). No seasonal differences were found in the mean FSH pulse heights expressed as a percentage increase over basal concentrations, or in the largest observed pulse height above basal concentrations ($P > 0.05$). For testosterone, pulse frequency observed during the winter and spring was greater than that in summer. Although pulse height above

Fig. 1. Ten-hour serial blood sampling profiles for two representative stags (a and b) sampled within 2 weeks of the summer solstice, autumn equinox, winter solstice and spring equinox. (○) LH; (●) testosterone. GnRH ($1 \mu\text{g kg}^{-1}$) was administered i.v. after the 480 min sample (arrow). Hormone concentration axes are expanded for the autumn sampling interval to reveal small, but distinct fluctuations in plasma testosterone relative to LH pulses. For inset panels: LH ($0\text{--}4 \text{ ng ml}^{-1}$) and testosterone ($0\text{--}0.5 \text{ ng ml}^{-1}$) between 0 and 480 min.

basal was higher ($P < 0.05$) during the spring than in autumn, there were no differences ($P > 0.05$) between spring and winter. Furthermore, no seasonal variation in mean testosterone pulse heights, expressed as a percentage increase above basal concentrations, was observed ($P > 0.05$).

Increases in LH and FSH after GnRH treatment were significant ($P < 0.05$) for all seasons (Fig. 2a, b) while testosterone concentrations were increased after GnRH treatment ($P < 0.05$)

during all seasons except summer (Fig. 2c). The rise in LH after GnRH treatment was rapid, increasing $\geq 33\%$ within 10 min (21 of 24, 87.5%) regardless of season ($P < 0.05$). Basal LH was highest during the winter and spring, whereas the net LH increase (Table 2) after GnRH was similar ($P > 0.05$) for all seasons. In contrast, the proportional increase in LH was greatest ($P < 0.05$) during the summer and autumn when basal concentrations were lowest. The time required for FSH to increase

Table 1. Comparison of pulse detection data (mean $\pm$ SEM) for LH, FSH and testosterone during an 8 h period (before GnRH treatment) within 2 weeks of the summer solstice, autumn equinox, winter solstice and spring equinox for 6 Eld's deer stags

Hormone	Summer	Autumn	Winter	Spring
LH				
Number of pulses in 8 h	4.0 $\pm$ 0.4 ^e	2.5 $\pm$ 0.2 ^f	2.0 $\pm$ 0.9 ^f	1.8 $\pm$ 0.3 ^f
Mean pulse height as % increase ^a	122 $\pm$ 6	118 $\pm$ 3	119 $\pm$ 3	117 $\pm$ 2
Largest pulse height above basal (ng ml ⁻¹) ^b	0.8 $\pm$ 0.2	1.1 $\pm$ 0.2	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1
FSH				
Number of pulses in 8 h	2.8 $\pm$ 1.0 ^e	3.3 $\pm$ 0.5 ^e	0.5 $\pm$ 0.3 ^f	1.7 $\pm$ 0.9 ^{ef}
Mean pulse height as % increase ^c	122 $\pm$ 4	120 $\pm$ 2	111 $\pm$ 1	119 $\pm$ 4
Largest pulse height above basal (ng ml ⁻¹) ^c	6.4 $\pm$ 1.0	7.1 $\pm$ 1.2	16.0 $\pm$ 1.3	8.4 $\pm$ 2.3
Testosterone				
Number of pulses in 8 h	0.7 $\pm$ 0.7 ^e	0.8 $\pm$ 0.3 ^{ef}	2.0 $\pm$ 0.0 ^g	2.5 $\pm$ 0.4 ^g
Mean pulse height as % increase ^d	170	269 $\pm$ 136	298 $\pm$ 49	155 $\pm$ 13
Largest pulse height above basal (ng ml ⁻¹) ^d	0.3	0.3 $\pm$ 0.1 ^e	2.2 $\pm$ 0.2 ^{ef}	4.0 $\pm$ 1.4 ^f

^aThe mean pulse height as a % increase represents the % increase of the mean peak amplitude above preceding nadir value.

^bThe largest pulse height above the basal value represents the incremental increase of the largest value expressed as the algebraic difference between the largest value and the preceding nadir.

^cWinter data were excluded from seasonal comparisons because FSH pulses were detected only in 2 of 6 males.

^dSummer data were excluded from seasonal comparisons because testosterone pulses were detected only in 1 of 6 males.

Within row means with different superscripts are significantly different ($P < 0.05$).

$\geq 33\%$ after GnRH was greatest ($P < 0.05$) during the winter (78.3 $\pm$ 18.5 min) than in the summer, autumn and spring (13.3 $\pm$ 2.1; 16.7 $\pm$ 4.9; 28.3 $\pm$ 11.4 min, respectively). Basal FSH concentrations were greatest ($P < 0.05$) during the winter than in all other seasons, whereas the net FSH increase after GnRH treatment ranged from only 20 to 40 ng ml⁻¹ during all seasons. The proportional FSH increase during autumn was greater ($P < 0.05$) than that during winter and spring, but was not greater than the increase in summer. During the summer and autumn, testosterone concentrations after GnRH treatment did not increase $\geq 33\%$ until 50.0 $\pm$ 12.6 min and 70.0 $\pm$ 12.6 min, respectively. In contrast, testosterone concentrations after GnRH treatment increased faster ($P < 0.05$) during the winter (25.0 $\pm$ 5.6 min) and spring (33.3 $\pm$ 5.6 min). Testosterone concentrations before GnRH treatment and net increase in testosterone after GnRH, were highest ($P < 0.05$) during the spring. Although there were no seasonal differences ($P > 0.05$) in the proportional increase in testosterone after GnRH treatment the net increase after GnRH was lower ($P < 0.05$) during the summer and autumn than during the winter and spring. Age, weight and antler size varied substantially among males, but there were no between-male differences ($P > 0.05$) in testosterone concentrations before or after GnRH treatment, or in the net or proportional increases in testosterone after GnRH treatment (Table 2). During the winter and spring, Eld's deer stags exhibited 1–3 distinct testosterone surges (Fig. 2c) during the 8-h basal sampling interval (09:00–17:00 h) before GnRH treatment. In 10 of 12 sampling periods, stags exhibited at least one surge in both the morning and afternoon.

Discussion

Although the testicular cycle in the Eld's deer is phase-shifted approximately 6 months compared with most temperate cervid

species (Monfort *et al.*, 1993), the interactive dynamics regulating the pituitary–gonadal axis were similar to those of red deer (Lincoln and Kay, 1979; Suttie *et al.*, 1984) and fallow deer (Asher *et al.*, 1989; Asher and Peterson, 1991). Eld's deer stags exhibited marked seasonal variations in basal concentrations of LH, FSH and testosterone. Although most LH pulses were not secreted coincidentally with FSH pulses, the trends of secretion of these two hormones were generally correlated. The lack of coincidental LH/FSH pulses may have been related to the higher FSH radioimmunoassay coefficients of variation used to perform the cluster analysis which made FSH pulse detection inherently more stringent. Other factors that probably dampened FSH pulsatility and hindered pulse detection were related to the greater seasonal increase in basal FSH secretion (summer to winter: FSH, 11-fold; LH, 2-fold) and the presumably longer half-life of FSH relative to LH.

As with Eld's deer, red deer stags exhibited high amplitude, low frequency (4–6 h duration) testosterone surges (amplitude > 10.0 ng ml⁻¹) during both the transition into the breeding season and during the rut that were always clearly associated with conspicuous LH pulses (Lincoln and Kay, 1979; Suttie *et al.*, 1984). Fallow deer bucks (Asher *et al.*, 1989; Asher and Peterson, 1991) also produced testosterone surges of 4–6 h duration (amplitude 10–25 ng ml⁻¹), but these often occurred in the absence of detectable LH pulses. Asher *et al.* (1989) suggested that these prolonged testosterone secretory episodes reflected 'maximal' testosterone secretion in response to chronically increased, nonpulsatile LH secretion. This theory suggested that 4–6 h was required to replenish testicular testosterone stores before the next secretory episode occurred. However, in Eld's deer stags, pharmacological doses of GnRH administered during or immediately after spontaneous testosterone surges induced further increases in testosterone, indicating that testicular reserves were not depleted. It appears more likely that testicular

Fig. 2. Mean ($\pm$ SEM, $n = 6$ males per season) (a) LH, (b) FSH and (c) testosterone profiles plotted by season (summer, ●; autumn, ○; winter, ■; spring, □). Arrow denotes time of GnRH administration.

sensitivity to LH increased during the rut compared with the transition period before the rut, perhaps because of increased Leydig cell LH receptor concentrations. Thus, very modest LH pulses (difficult to detect using radioimmunoassay) may have been sufficient to induce substantial, sustained increases in testosterone. This assertion was supported by our Eld's deer data because (i) basal LH concentrations actually declined during the spring (rut) as testosterone concentrations were increasing, and (ii) a constant exogenous GnRH challenge across seasons resulted in a greater testosterone response during spring compared with winter.

Prolonged testosterone surges in the morning and afternoon have been reported to be synchronous among fallow bucks

(Asher and Peterson, 1991). Asher and Peterson (1991) suggested that this synchrony among males reflected entrainment of testicular secretory activity to a diurnal rhythm. The small standard errors associated with the mean testosterone surges in the study reported here during the winter and spring also suggests synchrony amongst Eld's deer stags. However, our sampling interval was insufficient to examine the possible existence of a definitive diurnal rhythm in testosterone secretion. Activity or social cues cannot be excluded as causes for this apparent synchrony (Asher and Peterson, 1991).

Both basal testosterone concentrations and pulse frequency were reduced during the hypogonadal period in the study reported here. Nonetheless, small broad-based, spontaneous testosterone pulses were first detected during the autumn that were closely associated with conspicuous LH pulses. The percentage increase in testosterone over basal concentrations at these times was substantial, suggesting that the secretory response dynamics of the testis to LH secretion was becoming re-activated. Thus, it appears that soon after the hypothalamic-pituitary axis re-develops the capacity to secrete high LH concentrations during the autumn, steroidogenic pathways also become partially activated, although peak testosterone concentrations are not observed until winter (Monfort *et al.*, 1993). This continuum of long-duration episodic testosterone pulses appears to vary seasonally in frequency and amplitude, and larger increases in testosterone apparently reflect increased testicular responsiveness to LH as well as increased testicular steroidogenic capacity.

The pituitary-gonadal axis in male deer is well known to be responsive to exogenous GnRH (red deer, Lincoln and Kay, 1979; Suttie *et al.*, 1984, 1989; Fennessy *et al.*, 1988; white-tailed deer, Plotka *et al.*, 1984; Bubenik *et al.*, 1987; rusa deer, van Mourik *et al.*, 1986). Lincoln and Kay (1979) first observed that testicular sensitivity to GnRH-induced LH (as measured by testosterone production) in red deer stags fluctuated with season and that maximum responsiveness occurred 1–2 months before the rut. Suttie *et al.* (1984) demonstrated that red deer stags could respond to exogenous GnRH during all of the year, and that responsiveness was greatest when endogenous LH secretion was at nadir. Similarly, Fennessy *et al.* (1988) reported maximal pituitary and testicular responsiveness to GnRH in red deer stags when males were developing antler, but before rut, suggesting that there is a greater releasable pool of LH at this time. Responsiveness was diminished during rut, and this has been attributed to a suppressing impact of high, endogenous testosterone (Fennessy *et al.*, 1988). A negative feedback role for testosterone has been supported by the finding that castrated males exhibit higher basal LH with increased pulse frequency and amplitude of LH secretion (red deer, Lincoln and Kay, 1979; fallow deer, Asher *et al.*, 1989). In white-tailed bucks (Bubenik *et al.*, 1987), maximal GnRH-induced pituitary LH secretion was greatest during the spring, whereas maximal testosterone secretion after GnRH occurred during the autumn rut. Similarly, rusa deer exhibited maximal GnRH-induced LH and testosterone secretion during the autumn and winter (rut), respectively (van Mourik *et al.*, 1986). In the present study, basal LH and the proportional increase in LH after GnRH treatment was greatest during the summer and autumn (pre-rut), when basal concentrations were lowest. However, net GnRH-stimulated LH secretion was similar for all seasons. Thus, although basal LH secretion increased, the releasable pool of LH apparently

Table 2. Mean ($\pm$ SEM) hormone concentrations before ($n = 3$ samples) and after ($n = 12$ samples) GnRH and the net and proportional increases in hormones from samples collected during an 8 h period within 2 weeks of the summer solstice, autumn equinox, winter solstice and spring equinox for six Eld's deer stags

Hormone	Summer	Autumn	Winter	Spring
LH (ng ml ⁻¹)				
Pre-GnRH	1.9 $\pm$ 0.1 ^c	2.0 $\pm$ 0.1 ^c	3.9 $\pm$ 0.3 ^d	3.3 $\pm$ 0.4 ^d
Post-GnRH	7.2 $\pm$ 0.3 ^c	6.8 $\pm$ 0.4 ^c	9.4 $\pm$ 0.6 ^d	8.9 $\pm$ 0.6 ^d
Net increase (ng ml ⁻¹) ^a	5.4 $\pm$ 0.3	4.9 $\pm$ 0.4	5.5 $\pm$ 0.6	5.6 $\pm$ 0.6
Proportional increase (%) ^b	293 $\pm$ 22 ^c	251 $\pm$ 32 ^c	146 $\pm$ 21 ^d	180 $\pm$ 29 ^d
FSH (ng ml ⁻¹)				
Pre-GnRH	9.4 $\pm$ 0.6 ^c	16.9 $\pm$ 3.3 ^{cd}	103.4 $\pm$ 20.9 ^e	48.2 $\pm$ 15.5 ^d
Post-GnRH	28.9 $\pm$ 1.3 ^c	57.6 $\pm$ 8.7 ^{cd}	133.4 $\pm$ 19.0 ^e	72.5 $\pm$ 16.4 ^d
Net increase (ng ml ⁻¹)	19.5 $\pm$ 1.2 ^c	40.8 $\pm$ 8.8 ^d	29.9 $\pm$ 5.7 ^{cd}	24.1 $\pm$ 2.9 ^c
Proportional increase (%)	214 $\pm$ 23 ^{cd}	313 $\pm$ 109 ^c	37 $\pm$ 11 ^f	72 $\pm$ 18 ^{de}
Testosterone (ng ml ⁻¹)				
Pre-GnRH	0.31 $\pm$ 0.16 ^c	0.16 $\pm$ 0.05 ^c	1.23 $\pm$ 0.29 ^c	2.90 $\pm$ 0.74 ^d
Post-GnRH	0.70 $\pm$ 0.26 ^c	0.24 $\pm$ 0.07 ^c	3.07 $\pm$ 0.49 ^d	6.80 $\pm$ 0.77 ^e
Net increase (ng ml ⁻¹)	0.39 $\pm$ 0.16 ^c	0.08 $\pm$ 0.03 ^c	1.83 $\pm$ 0.47 ^d	3.86 $\pm$ 0.71 ^e
Proportional increase (%)	330 $\pm$ 230	51 $\pm$ 14	213 $\pm$ 83	184 $\pm$ 48

^aNet increase represents the mean hormone concentration after-GnRH minus the mean hormone concentration before GnRH.

^bProportional increase represents the mean hormone concentration after GnRH minus the mean hormone concentration before GnRH divided by the mean hormone concentration before GnRH, expressed as a percentage.

Within row means with different superscripts are significantly different ($P < 0.05$).

remained relatively constant during all seasons, despite marked changes in basal testosterone secretion. The pituitary LH response was rapid (within 10 min) during all seasons. Furthermore, unlike the situation in red deer (Lincoln and Kay, 1979; Fennessy *et al.*, 1988), white-tailed deer (Plotka *et al.*, 1984; Bubenik *et al.*, 1987) and rusa deer (van Mourik *et al.*, 1986), no differences were observed in either the proportional or net increase in GnRH-induced LH secretion during the pre-rut and rutting season.

Seasonal variation in pituitary responsiveness to GnRH in red deer has been reported using a wide range of GnRH doses (0.5 μ g, Lincoln and Kay, 1979; about 3 μ g, Suttie *et al.*, 1984; 95 μ g, Fennessy *et al.*, 1988). Pituitary challenge studies in rusa stags (van Mourik *et al.*, 1986) revealed that LH responsiveness after GnRH-treatment was diminished during spring rut compared with the autumn (nonbreeding season); however, the relatively low dose (about 0.5 μ g) of GnRH using in that study may partially explain the attenuated pituitary responsiveness during the rut. In white-tailed bucks (Plotka *et al.*, 1984; Bubenik *et al.*, 1987), pituitary responsiveness was also maximal during the pre-rut period and minimal during rut in response to administration of a pharmacological dose of GnRH (100–200 μ g). Consequently, disparities in pituitary responsiveness among species cannot be explained solely by differences in GnRH doses.

Despite apparent species differences in pituitary sensitivity, Eld's deer exhibited maximal testicular responsiveness to GnRH-induced LH during rut which was similar to that observed in red deer stags (Suttie *et al.*, 1984) and white-tailed bucks (Plotka *et al.*, 1984). Although no seasonal trend in the proportional increase in testosterone was observed after GnRH

treatment among Eld's deer stags, the net increase in testosterone rose in parallel with the increase in basal testosterone secretion. Thus, during the pre-rut and rutting seasons, both basal testosterone concentrations and testicular testosterone reserves were increasing. In addition, the increase in testosterone secretion after GnRH treatment generally occurred more rapidly as basal concentrations increased. Because both basal LH and the net releasable pool of LH remained relatively constant, increased speed and magnitude of testicular responsiveness to LH secretion induced by GnRH most likely represented increased testicular sensitivity to LH.

In white-tailed bucks, Bubenik *et al.* (1987) suggested that 'high quality' prime antler-bearers exhibited greater testicular responsiveness to exogenous GnRH challenges (100 μ g) than did 'low quality' weak antler-producers. These authors suggested that increased testicular responsiveness could be used as a screening test to pre-select superior antler-producing potential in juvenile bucks. In the present study, despite substantial differences in age, weight and antler size among males, there were no between-male differences in either testosterone secretion before and after GnRH treatment. Our data indicated that GnRH-induced testosterone secretion may not be useful for pre-selecting superior antler-producers in all cervid species.

To our knowledge, this is only the second report describing pituitary FSH responsiveness to a GnRH challenge in any cervid. GnRH stimulated the release of FSH from the pituitary gland during all seasons. The proportional increase in FSH induced by GnRH was lowest during the winter and spring when basal FSH concentrations were highest. The net increase in FSH tended to be somewhat higher during the autumn and winter (before the rut), although during all seasons the average

range in net increases only varied from 20 to 40 ng ml⁻¹. In white-tailed bucks (Bubenik *et al.*, 1987), FSH increased slightly after GnRH treatment during the autumn rut; however, FSH increased markedly after GnRH treatment during the spring. In contrast to white-tailed bucks (Bubenik *et al.*, 1987), the capacity of the pituitary to secrete FSH in Eld's deer reached its peak during the winter. Although the FSH response to GnRH treatment was usually rapid (about 10 min) during winter, the time required to achieve a 33% rise above already high basal concentrations was greatest at this time. Thus, like LH, the amount of FSH available to be released in response to a pharmacological GnRH dose remained relatively constant during all seasons. It therefore appeared that pituitary gonadotrophs in Eld's deer seasonally adjusted their secretory 'set point', but the reserve capacity to respond above basal concentrations to an exogenous GnRH challenge was constrained by a relatively constant releasable pool regardless of season.

Finally, this study demonstrated that intensive endocrine investigations are feasible even in fully conscious, highly excitable endangered species like the Eld's deer. This is particularly important because our knowledge about seasonal reproduction in tropical-zone species is generally lacking. Such information has been largely derived from studies in domestic sheep, temperate deer species and nonmurroid rodents. Thus, zoo-maintained species serve as a valuable resource that provides researchers with unique opportunities to explore reproductive processes in nontraditional animal models.

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