Seasonal pattern of LH and testosterone secretion in adult male fallow deer, *Dama dama*

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Summary. At monthly intervals during the year blood samples were collected every 20 min for 12 h from 4 entire and 2 prepubertally castrated adult fallow deer bucks. In the entire bucks there were seasonal changes in mean concentrations and pulse frequencies of plasma LH. Mean concentrations in late summer and autumn were 3–6 times higher than during other seasons. LH pulse frequency was low (0–1 pulses/12 h) during most of the year and increased only during the 2-month period (January and February) that marked the transition from the non-breeding season to the autumn rut. During this period there was a close temporal relationship between pulses of LH and testosterone. However, during the rutting period (March and April) episodic secretion of testosterone, manifest as surges in plasma concentrations of 4–6 h duration, was not associated with any detectable pulses in LH although mean plasma concentrations of LH remained elevated. During the rut, the surges of plasma testosterone occurred at similar times of the day. Plasma profiles in May indicated very low concentrations of LH and testosterone secretion in the immediate post-rut period. Castrated bucks exhibited highly seasonal patterns of LH secretion, with mean plasma LH concentrations and LH pulse frequency being lowest in November (early summer) and highest in February and March (late summer–early autumn). Mean concentrations and pulse frequency of LH in castrated bucks were higher than for entire bucks at all times of the year.

Keywords: fallow deer; reproduction; pituitary; testis; LH; testosterone

Introduction

Adult male fallow deer (*Dama dama*) exhibit marked annual changes in liveweight and testicular function, with clearly defined periods of reproductive activity and of sexual quiescence (Chaplin & White, 1972; Chapman & Chapman, 1975; Asher *et al.*, 1987). Several workers have demonstrated testicular-mediated changes in secondary sexual characteristics such as antlers (Chapman & Chapman, 1975), accessory glands (Chapman & Chapman, 1979), the prepuce (Kennaugh *et al.*, 1977) and neck muscles (Field *et al.*, 1985; Asher *et al.*, 1987). While it is generally accepted that the seasonal cycle of testicular activity is controlled, in part, by changes in the pituitary secretion of luteinizing hormone (LH), the pattern of release of this hormone, and the resultant effects on testicular secretion of testosterone, have not been investigated for this species.

The purpose of the present study was to define seasonal changes in the secretion pattern of LH and testosterone in entire and castrated adult fallow bucks.

Materials and Methods

Animals and management. Four polled entire bucks and 2 prepubertally castrated bucks, all born in December 1981 and 55 months old at the start of the trial (July 1986), were kept in high-fenced paddocks at the Ruakura

Agricultural Centre (37°46'S, 175°20'E) and grazed on ryegrass-white clover pastures. They were maintained as a bachelor group with other male fallow deer except during April and May 1987 when the entire bucks were run individually with groups of does for mating.

Measurement of liveweight, neck girth and testis diameter. The bucks were mustered into a covered handling shed at monthly intervals from July 1986 to November 1987 and liveweight, neck girth and testis diameter were recorded (Asher *et al.*, 1987).

Blood sampling. At monthly intervals from August 1986 to July 1987 inclusive, on the day after the recording of liveweight, neck girth and testis measurements, the bucks were again mustered into the handling shed. Each animal was repeatedly restrained in a cradle (Asher, 1986) at 20-min intervals from 08:00 to 20:00 h (New Zealand Standard Time) and blood (5 ml) was withdrawn into heparinized Vacutainers by jugular venepuncture. The bucks were well habituated to such handling and were kept indoors, under natural lighting, for the duration of each 12 h blood sampling session. Water and meadow hay were provided *ad libitum*.

Blood samples were centrifuged immediately after collection and the plasma was stored at -10°C until assayed.

Hormone assays. LH concentrations were determined in duplicate using a heterologous radioimmunoassay procedure described for sheep plasma by Scaramuzzi *et al.* (1970) and validated for fallow deer plasma (Asher *et al.*, 1986). The inter-assay coefficient of variation, calculated from determinations of deer control samples (mean concentration = 4.16 ng/ml) in each assay ($n = 6$) was 16.1%. The intra-assay coefficient of variation for multiple determinations of the same control sample was 5.7%. All samples from an individual buck ($n = 444$) were included within a single assay. The least amount distinguishable from zero was 0.30 ng/ml.

Plasma testosterone concentrations were determined in duplicate using an extraction radioimmunoassay similar to that described by Peterson *et al.* (1978) but omitting the chromatographic step used to separate androgens. The antiserum used in the assay ('Trillion'; Department of Clinical Biochemistry, Christchurch Hospital, New Zealand) was raised in a rabbit against testosterone-3- O -carboxymethyloxime-BSA conjugate and used at a final dilution of 1:20 000. Cross-reactivities at the 50% displacement level were as follows: 5 α -androstan-17 β -ol-3-one 27.5%, 11 β -hydroxytestosterone 13.8%, epitestosterone 5.3%, oestrone 5.3%, androstenedione 2.2%, androstendiol 1.3%, androsterone 0.6%, oestradiol-17 β 0.3%, cholesterol 0.3%, 5-androsten-3 β -ol-17-one, cortisol and all progestagens tested <0.01%. The inter-assay coefficients of variation, calculated from determinations of low (mean concentration = 0.89 ng/ml) and high (4.44 ng/ml) control samples (entire buck plasma) in each assay ($n = 38$) were 12.7% and 13.5% respectively. The intra-assay coefficients of variation for multiple determinations of the same control samples were 11.8% and 10.7% respectively. The mean ($\pm$ s.d.) recovery of testosterone added to charcoal-stripped plasma from a castrated buck (10 determinations) was $88.5 \pm 2.3\%$ at 1.0 ng/ml and $82.4 \pm 5.8\%$ at 4.0 ng/ml. Plasma values were not corrected for recovery. The least amount distinguishable from zero was 0.05 ng/ml.

Statistical analyses. LH and testosterone profiles were analysed separately for each buck at each sampling session using the PULSAR pulse identification routine (Merriam & Wachter, 1982). Pulse analyses were carried out for 37 points. Inputs for PULSAR included intra-assay standard deviation and the smoothing time was set at 360 min for LH and 180 min for testosterone. A cut-off value of 2.7 standard deviation units was used for peak splitting.

Results

Seasonal changes in liveweight, neck girth and testis diameter

There was a steady increase (142 g/day) in mean liveweight of the entire bucks from August to February (early spring to late summer) to a pre-rut maximum of 86.3 kg (Fig. 1). During the autumn rut (i.e. March to May) the entire bucks showed a precipitous decline (425 g/day) in mean liveweight to a post-rut minimum of 58.0 kg. Mean liveweights increased at 99 g/day over the subsequent winter period between May and August.

Mean neck girth of entire bucks was lowest (35.5 cm) during November and December (early summer) despite rapid liveweight gains at this time (Fig. 1). Neck girths increased from January to maximum dimensions (48.0 cm) in early April (i.e. immediately before the rut). There was a decline over the rutting period, coincidental with a marked decline in liveweight, but a slight increase through June and July. Thereafter until October the values remained constant. Mean testis diameter followed similar trends with mean neck girth being minimal (23 mm) in November and maximal (52 mm) in March (Fig. 1).

Individual liveweight and neck girth profiles of the 2 castrated bucks show small seasonal changes and such changes probably reflect variation in pasture quality. Liveweights were lower than the mean values of the entire bucks except towards the end of the rut in early May. Neck girths of castrated bucks never exceeded the mean values for the entire bucks (Fig. 1).

Fig. 1. Seasonal profiles of mean ($\pm$ s.e.m.) liveweight, neck girth and testis diameter of the 4 entire bucks ($\bullet$) superimposed on the individual liveweight and neck girth profiles of the 2 castrated bucks, G34 ($\circ$ — $\circ$) and G37 ($\circ$ — $\cdot$ — $\circ$). Arrows indicate the dates of intensive blood sampling.

Seasonal changes in LH and testosterone secretion in entire bucks

Mean LH concentrations of entire bucks were lowest from May to December and highest between February and April (Fig. 2a). The seasonal patterns of mean plasma testosterone concentrations were similar to those of LH but the difference between seasonal minimal and maximal values was considerably greater. Peak mean plasma testosterone concentrations (5–9 ng/ml) occurred during the rut (late April) in all but one buck (i.e. G10; Fig. 2a).

There were few pulses of LH in the entire bucks for most of the year (Table 1). However, there was a marked increase in the frequency of pulses in January and February (mid-late summer), the period preceding the autumn rut (Table 1). This is particularly apparent for Buck G22 (Fig. 3). Apart from this short episode of increased LH pulse frequency, the main seasonal difference in LH secretion in entire bucks was the changing basal concentrations, which were lowest (0.2–0.5 ng/ml) in November and December and highest (3.0–4.0 ng/ml) in March and April.

The relationship between LH and testosterone secretion varied seasonally. During the months from August to December (late winter to mid-summer) plasma testosterone concentrations were generally low (<0.5 ng/ml) except for single low amplitude (2–3 ng/ml) pulses of 4–5 h duration

Fig. 2. Seasonal profiles of mean ($\pm$ s.e.m.) plasma LH (●) and testosterone (○) concentrations for each of 4 entire bucks (a) and 2 castrated bucks (b). $n = 37$ samples per buck/day. Note the different scales for the ordinate axes of (a) and (b).

occurring for 3 bucks (G3 (Fig. 3), G10 and G32) in September (early spring) and occasional elevations (<1.5 ng/ml) at the start of the sampling sessions of some bucks (e.g. August and September, Buck G22, Fig. 3). These events appeared to be unrelated to any detectable LH pulses. However, all 4 entire bucks exhibited a single low-amplitude (<2 ng/ml) plasma testosterone pulse of about 4 h duration in January that was preceded by one or more LH pulses. In February (late summer), 2 entire bucks (G22 (Fig. 3) and G32) exhibited recurrent high-amplitude (3–8 ng/ml) testosterone pulses of short duration (1.5–2 h) that were each preceded by a single LH pulse. In these cases, the pulse peaks for LH and testosterone were displaced by 20–40 min.

For all 4 entire bucks, the plasma testosterone profiles in March and April (rut) were characterized by long-duration (5–6 h), high-amplitude (10–25 ng/ml) surges that were not preceded by any detectable LH pulses as confirmed by the PULSAR algorithm. Mean LH concentrations were uniformly high (3–4 ng/ml) during these months.

Table 1. Number of defined LH pulses occurring during each 12-h sampling period for each of the 4 entire and 2 castrated fallow bucks

Date	Entire bucks				Castrated bucks	
	G3	G10	G22	G32	G34	G37
21 August	0	0	0	1	0	5
23 September	0	0	0	0	4	1
23 October	0	0	0	0	3	3
20 November	0	0	0	0	2	1
23 December	0	0	0	0	8	4
22 January	1	2	2	2	9	7
22 February	2	1	5	3	8	10
24 March	0	0	0	1	8	6
22 April	0	0	0	0	3	6
29 May	0	0	0	0	4	2
24 June	0	0	0	0	4	1
22 July	0	0	0	1	2	4

Fig. 3. Twelve-hour profiles of plasma LH (●) and testosterone (○) concentrations of 2 entire bucks for each month. The rutting period is represented by the April profiles.

Fig. 4. Twelve-hour profiles of plasma testosterone concentrations of the 4 entire bucks for each month. The rutting period is represented by the April profiles.

In May (after the rut) plasma testosterone and LH concentrations were low (<0.5 ng/ml) throughout the 12 h period, and were comparable to profiles occurring earlier during late spring and summer. However, by June and July (early–mid-winter) there was increased testosterone secretion, with some bucks exhibiting occasional pulses of 1–2 h duration, particularly at the start and end of the sampling period (e.g. June sample for Buck G22; Fig. 3). Again, there was little evidence of a direct relationship between LH pulses and testosterone secretion.

The changes in plasma testosterone concentrations during the year (Fig. 4) were uniform among bucks with a common pattern evident during some months. In particular, in March and April there were two clear high-amplitude surges of plasma testosterone concentrations, one having peaked by the start of blood sampling (08:00 h) and the other in the afternoon. This pattern was also evident in February for Bucks G3 and G10, and, to a lesser extent, in June for bucks G10 and G22 (Fig. 4).

Seasonal changes in LH secretion in castrated bucks

Mean plasma testosterone concentrations of the two castrated bucks ranged between 0.01 ng/ml (below assay sensitivity) and 0.10 ng/ml (Fig. 2b), being lower than the mean values for entire bucks during all months except November. Mean plasma LH concentrations of castrated bucks were always much higher than those of entire bucks and exhibited marked seasonal trends. The mean values declined from September to reach their lowest levels in November. There was a significant increase in December and the highest concentrations occurred in February and March. Values were again declining in April (Fig. 2b).

The plasma LH profiles for the 2 castrated bucks (Fig. 5) reveal that the seasonal changes in mean LH concentration reflect changing pulse frequency and amplitude. Both these parameters

Fig. 5. Twelve-hour profiles of plasma LH concentrations of the 2 castrated bucks for each month. Asterisks identify pulses. Note that the ordinate scale differs from that in Fig. 3.

were lowest in November and the significant increase in mean values in December reflected an increase in pulse frequency of LH (Table 1).

Discussion

Adult entire fallow bucks demonstrate marked seasonal variation in LH and testosterone secretion. The dynamic interaction between these two hormones cause the profound changes in testis size, neck girth and liveweight associated with the highly seasonal pattern of breeding in this species. This relationship has been well documented for other seasonally breeding ruminants such as sheep (Katongole *et al.*, 1974; Sanford *et al.*, 1974; Lincoln, 1976; Schanbacher & Ford, 1976; Wilson & Lapwood, 1978) but has received little scrutiny in deer, mainly due to difficulties associated with handling these animals.

The seasonal variation in LH secretion was characterized by changes in the mean plasma concentrations and also by changes in pulse frequency, duration and amplitude. Similar results have been reported for rams (Lincoln & Short, 1980), and adult male red (Lincoln & Kay, 1979) and white-tailed (Mirachi *et al.*, 1978) deer. The LH pulse frequency in the bucks was low for most of the year and increased only during the 2-month period (January and February) that marked the transition from the non-breeding season to the autumn rut. This transition was also characterized by a close temporal relationship between LH and testosterone secretion, with high-amplitude LH pulses preceding high-amplitude testosterone pulses. This relationship is observed throughout the entire year in rams (Lincoln, 1976; Lincoln *et al.*, 1977). This indicates that the testis of fallow deer is extremely sensitive and responsive to episodic pituitary stimulation during the period of

resurgence of sexual activity, but clearly responds in a different manner at other stages of the annual sexual cycle. A direct causal relationship between LH and testosterone secretion was not apparent in fallow bucks during the rut in March and April. Despite massive fluctuations in plasma testosterone concentrations over 4–6 h, plasma LH concentrations remained elevated but with no evidence of pulsatile secretion. Similar results have been reported for red deer stags (Lincoln & Kay, 1979). This suggests that a resetting in the dynamics of the negative feedback of testosterone upon LH secretion occurs at this time. The elevated LH concentrations could be maximally stimulating the secretion of testosterone but the testes are unable to maintain this production requiring 4–6 h to replenish testosterone stores. The fact that the episodic plasma testosterone surges occurred at similar times of the day during the rut may reflect a diurnal rhythm. Such a rhythm could be entrained by exogenous factors such as day:night patterns, daily activity patterns or social facilitation effects. To define the extent to which testosterone secretion in fallow bucks is truly rhythmic during the rut will require longer sampling durations than were used in the present study.

The LH profiles of the castrated bucks indicated that in male fallow deer there is a direct effect of season upon gonadotrophin release independent of any testicular feedback. These bucks had been castrated at 5 months of age, well before the onset of puberty, and at least 4 years before the present trial. It is doubtful therefore that any residual imprinting of photoperiod (Poulton, 1986) would have remained. This seasonal variation in LH concentrations in castrated fallow bucks contrasts with observations on male red deer (Lincoln & Kay, 1979) but is similar to that reported in a variety of other seasonal breeders (Turek & Campbell, 1979), including some breeds of rams (Pelletier & Ortavant, 1975). Both the LH pulse frequency and mean concentrations in the castrated bucks were always higher than in the entire bucks, indicating the negative feedback of testicular hormones on LH secretion in fallow bucks. The presence of the testis does modulate the annual LH profile although it is not clear from our data whether testosterone by itself is involved in the apparent change in sensitivity of the negative gonadal feedback throughout the year; something well documented in male hamsters (Turek, 1979) and sheep (Lincoln, 1984).

Although it is assumed that the stimulus to gonadotrophin secretion in autumnal breeding animals is decreasing daylength and, conversely, inhibition occurs during increasing daylength, the profile of LH concentrations in the castrated bucks seems at variance with this. The major increase in LH, both in pulse frequency and amplitude, occurred by 23 December, only 2 days after the summer solstice. Moreover, the decline in pulse frequency and amplitude occurred in April despite the continuing occurrence of decreasing daylength until the winter solstice in June. Thus, although it is tempting to ascribe the post-rut (May) decline in LH, and subsequently testosterone, in the entire bucks to their deteriorating body condition, as postulated for rams (Alkass *et al.*, 1982), the same decline in LH secretion occurred in the castrated bucks which, for obvious reasons, did not exhibit rut-related liveweight losses. Various authors have proposed, on the basis of fixed light intervals, that seasonal breeders become refractory to long or short days (e.g. Lincoln & Short, 1980), thus explaining observed changes in gonadotrophin secretions in times of increasing and decreasing daylengths. Perhaps, however, these animals are responding to the rate of change of daylength, which could explain the decline in LH about the autumnal equinox.

Three of the 4 entire bucks had a brief resurgence of LH and testosterone secretion in spring (September) and 1 of the castrated bucks also had an increased pulse frequency of LH at this time. West & Nordan (1976) discussed the possibility of a resurgence of gonadotrophin and testosterone release occurring in the spring in Columbian black-tailed deer, relating it to similar light–dark ratios occurring in autumn as in spring. Similar rates of change of daylength also occur at these times and a brief resurgence of testosterone secretion has been observed, not only in fallow deer, but also in entire male roe deer (Gimenez *et al.*, 1975) and wapiti (Haigh *et al.*, 1984).

We thank M. Langridge, O. C. Chitty and H. H. Humble, and other staff at the Ruakura Deer Unit, for technical assistance; Robyn Shanley and Gillian Lintz for hormonal analyses; and D. Duganzich for statistical advice.

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Received 1 August 1988