Diurnal variation in release of LH and testosterone in the ram

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Summary. The time of appearance of plasma LH and testosterone peaks through the day was determined in 75 Préalpes du Sud and 41 Ile-de-France rams in December and in 44 Préalpes du Sud and 11 Ile-de-France rams in June. The distribution of peaks throughout the day was non-random for the two hormones in the two breeds and for both times of the year ($P < 0.01$ at least on each occasion; $P < 0.001$ on pooled data from the two breeds). The most striking features were the occurrence of (1) a minimum of LH and testosterone peaks immediately after 'dawn' (lights on) in both months; (2) a maximum of peaks 3 h after 'dawn' in June and 4 h after 'dawn' in December.

For several hours after the increase in frequency of peaks the probability of measuring peaks of LH and testosterone remains high. This and the correlation between the LH values in December and June when adjusted for the time of 'dawn' suggest that dawn could act as a synchronizer of gonadotroph activity.

Introduction

It is well established that LH and testosterone pulsatile patterns in the ram vary with the season, the numbers of LH and testosterone peaks per unit of time increasing at the breeding season (Sanford, Winter, Palmer & Howland, 1974; Schanbacher & Ford, 1976; Wilson & Lapwood, 1978).

Terqui, Garnier, de Reviers, Huet & Pelletier (1980) and Pelletier, Garnier, de Reviers, Terqui & Ortavant (1982) observed that the numbers of LH and testosterone peaks in periods of 12 or 24 h were higher during the breeding season than during the non-breeding season and furthermore differed between the two breeds studied. By contrast we can find no reference to evidence of a true diurnal rhythm in the pattern of release of LH or testosterone (Katongole, Naftolin & Short, 1974; Purvis, Illius & Haynes, 1974; Falvo *et al.*, 1975; Wilson & Lapwood, 1978). However, Lincoln, Peet & Cunningham (1977) indicated that gonadotrophin concentrations in plasma were low during the morning and high during darkness and suggested that this could be related to body activity, while in another experiment, the number of peaks was high during the afternoon (Lincoln & Peet, 1977). On the other hand, Walton, Evins, Fitzgerald & Cunningham (1980) found a consistent release of LH after dawn and during the afternoon in anoestrous ewes, but few animals were used.

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The aim of the present study was to determine whether release of LH and testosterone is independent of the time of day and, if not, light could be involved in synchronizing the release of LH. Variations in LH and testosterone concentrations were then followed during periods of 24 h on a large number of animals of two breeds (Préalpes du Sud and Ile-de-France rams) at the time of the shortest (December) and longest (June) days.

Materials and Methods

Animals

Mature intact rams, 2–3 years old, submitted to normal seasonal changes in daylength in a light-proof building were used in the present study. Details of the housing were as given by Pelletier *et al.* (1982). The animals were allowed 1 month to adjust to the new environment before the first period of sampling and then stayed in the rooms until the completion of the experiment. The time when lights were switched on was considered as ‘dawn’ and the time they were switched off as ‘dusk’. Food was provided daily at 08:30–09:00 h.

The Préalpes du Sud or Ile-de-France rams were bled from the jugular vein at hourly intervals for 24 h (from 09:00 to 09:00 h the next day) on two occasions, at the end of December or early January (‘December group’) and at the end of June or early July (‘June group’). There were 75 Préalpes du Sud animals in December and 44 in June, and 41 Ile-de-France rams in December and 11 in June. Some of the animals were those studied by Pelletier *et al.* (1982). During the night, blood was collected in darkness. Plasma samples were kept frozen at -14°C until LH and testosterone radioimmunoassays were carried out.

Assays

Plasma LH and testosterone were measured in duplicate as described by Pelletier, Kann, Dolais & Rosselin (1968) and Garnier, Cotta & Terqui (1978), respectively. Minor changes in methodology and characteristics of assays are given by Pelletier *et al.* (1982). A peak was defined as an increase in hormone levels followed by a decrease, the magnitude of the increase being greater than 4 times the coefficient of variation of the baseline in the assays (i.e. 4.0–10.0% for LH and 7.8–8.4% for testosterone). As discussed by Pelletier *et al.* (1982) sampling every hour was considered suitable for detecting 93.5% and 98.6% of all possible LH and testosterone peaks respectively. LH and testosterone peaks were considered as coincident if the testosterone peak was simultaneous with or occurred just after an LH peak. High values of testosterone at the first bleeding or high values of LH at the last which would be insufficient evidence of coincident peaks were discarded when calculating the numbers of such peaks but were included in computing the numbers of peaks per hour throughout the day.

Statistical analysis

The non-parametric statistical Q test of Cochran (1952) was used to test for randomness of the peak distribution throughout the day. The statistical difference between minimum and maximum distribution of peaks was assessed by the same test. The homogeneity of distributions of LH and testosterone peaks throughout the day between breeds was analysed by the Kolmogorov–Smirnov test and the correlation between the numbers of peaks found in December and in June throughout the day was estimated by Spearman’s rank correlation test (Siegel, 1956). In this test a rank was attributed to each hour according to the number of peaks which were observed at that hour in all animals. In the absence of a shift, the rank obtained at 09:00 h in December was correlated with the rank at 09:00 h in June and so on for all the 24 h. When a shift of 1 h was introduced the ranks of peak number at, for example, 09:00 and 10:00 h

in June were correlated with those of 10:00 and 11:00 h in December. The influence of all possible shifts, from 0 to 23 h, was similarly examined (Kendall, 1970).

Results

Correlation between peaks of LH and testosterone

Of 603 testosterone peaks, 586 occurred simultaneously or just after an LH peak, i.e. coincident peaks represented 97.2% of the total number of peaks and the 17 isolated testosterone peaks represented only 2.8%. Similarly of 607 LH peaks only 21 were not associated with a testosterone peak. These isolated peaks were disseminated randomly in Préalpes du Sud and Ile-de-France rams, in the December as well as in the June group. Since they could not be rejected objectively they are included in the analysis, but their influence is negligible.

Distribution of peaks of LH and testosterone throughout the day

The analysis indicates that the LH and testosterone peaks were not distributed at random for both breeds and both seasons ($P < 0.001$ except for testosterone in the December group of Ile-de-France rams when $P < 0.01$). Because the distributions of peaks of LH and testosterone throughout the day were found to be identical for the two breeds in the December and June groups, the values for each breed were pooled. The diurnal patterns produced from these pooled data were significantly non-random ($P < 0.0001$).

In December the pattern of distribution of LH peaks (Text-fig. 1a) appeared multiphasic and could be schematically described as presenting three periods when peaks were at maximum, i.e. 13:00, 18:00 and 05:00 h. The greatest number of peaks was found at 13:00 h, 4 h after 'dawn'. Similarly, two periods with a minimum of peaks were found, one immediately after 'dawn', from 09:00 to 12:00 h and the other during the night between 21:00 and 04:00 h. In June (Text-fig. 1a) there were two definite maxima, one at 08:00 h, about 3 h after 'dawn' and another at 12:00 h. During the remainder of the day the LH peaks were erratically distributed but generally low. The minimum values were found at 17:00 and 07:00 h when the number of peaks differed significantly ($P < 0.01$) from the maxima.

A relatively comparable description of the distribution of testosterone peaks throughout the day was possible (Text-fig. 1b) except that the most prominent maximum was delayed by 1 h compared with the LH maximum. In the December group, most testosterone peaks were observed at 14:00 h and the most LH peaks at 13:00 h, while in June the maxima were at 09:00 h for testosterone and at 08:00 h for LH. The least number of peaks was found at 11:00 and 23:00 h in December and at 10:00 and 18:00 h in June, these numbers being all significantly different from the maximum values ($P = 0.01$ or less).

Distribution of LH and testosterone peaks during the light and dark phases

LH peaks were distributed between the light and dark phases of the day in both breeds according to the length of each phase. The number of LH peaks during darkness represented 67.6 and 29.7% for the December and June groups respectively when this phase was approximately 66 and 33% of the total daylength (at 47°N latitude). Similarly, the distribution of numbers of testosterone peaks was 67.9 and 30.4%.

Comparison of the distribution of the LH and testosterone peaks between December and June

Using cross rank correlation analysis the number of LH peaks observed at each hour in December was negatively correlated with the corresponding values in June ($\rho = -0.45$, $P <$

Text-fig. 1. Distribution of numbers of plasma peaks of (a) LH and (b) testosterone throughout the day in Ile-de-France and Préalpes du Sud rams (pooled data) in December and June (light schedule indicated by the horizontal bar). The broken line (---) represents the number of peaks per hour if they were evenly distributed. The number of LH and testosterone peaks per ram per 24 h is higher in June than in December, but the total numbers of peaks illustrated are similar in both seasons because the number of animals involved in June (N = 55) was less than that involved in December (N = 116).

0-02). When a shift of 4 h was introduced to change 'dawn' from 09:00 h in December to 05:00 h in June, the coefficient ρ was positive, +0.33, and significant ($P < 0.05$). For a shift of 5 h, 04:00 h in June compared with 09:00 h in December, $\rho = +0.47$ ($P < 0.02$). No other shift gave significant positive correlations between December and June. When 'dusk' was taken as the reference point, i.e. a shift of 19, 20 or 21 h there was no significant correlation between December and June. Testosterone gave similar results except that the only shift giving a positive and significant correlation was 7 h ($\rho = 0.664$, $P < 0.01$).

Discussion

In this experiment we have shown that LH and testosterone are not released randomly. Furthermore, the large number of rams used (≈ 180) enables us to show that the diurnal release is multiphasic with the highest density of peaks between 3 and 9 h after 'dawn' and, at least in December, another phase of high activity during the night. There is also a phase of low activity just after 'dawn'. Most workers who have studied the release of LH and testosterone have not demonstrated diurnal patterns for these hormones (Katongole *et al.*, 1974; Purvis *et al.*, 1974; Sanford *et al.*, 1974; Falvo *et al.*, 1975; Wilson & Lapwood, 1978). However, in Soay rams, Lincoln *et al.* (1977) showed a circadian rhythm in release of LH and testosterone with highest activity during the darkness. But Lincoln & Peet (1977) showed a minimum of peaks immediately after dawn and a maximum 4 h later, i.e. during the light. Similarly, Walton *et al.* (1980) suggested that there is maximum activity in release of LH in anoestrous ewes soon after dawn. It should be emphasized that our results (and *a fortiori* those of others) do not show that there is a rhythm but demonstrate that there is a higher probability of finding peaks of LH and testosterone at one part of the day rather than at another. This effect cannot be shown clearly with individual animals or with small numbers. We cannot be sure, either, that at latitudes different from ours or with different breeds of sheep diurnal release patterns of gonadotrophins would be similar.

At the present time there is no study in which blood has been sampled on a sufficient scale and for several days to ascertain whether there is a true diurnal rhythm of release.

Variations in locomotor activity have been proposed as a factor which could explain the

Text-fig. 2. Distributions of LH peaks throughout the day in December and in June when pooled in 3-h blocks and shifting the scale to make 'dawn' a common reference time.

release of LH and testosterone at certain times. For example, Boyar *et al.* (1972, 1973) related maximal release of gonadotrophin in prepubertal boys with sleep and Lincoln *et al.* (1977) associated minimum density of LH peaks with maximal activity and *vice versa*. However, in the present experiment, a minimum of peaks was seen when animals were at rest and immediately after 'dawn'. In fact, a first maximum of LH peaks per hour was found 3 h after 'dawn' in June and 4 h after 'dawn' in December, suggesting a possible triggering role of 'dawn'. Comparison of LH peaks in June and in December when data are arbitrarily pooled in 3-h blocks to minimize the hour-to-hour fluctuations (Text-fig. 2) also strongly suggests a synchronizing role of 'dawn'. This could act either directly as a synchronizer of the release of LH which could be at maximum 3–4 h later or initially as a short-term inhibitor of discharge of LH which would be released more intensively after the end of the inhibition phase. Whatever the role of 'dawn', stimulatory or inhibitory, animals at the beginning of the sexual season (June) appear more responsive than those at the beginning of sexual rest (December–January). The shift of 1 h observed between the maximum of LH peaks in December and in June, in addition to the normal 4 h difference between the 'dawns' in December and in June, explains why the numbers of peaks in December and June were significantly correlated with a final shift of 5 h. In fact, in no other shift (including that where 'dusk' might be a synchronizer) did December values correlate with June values.

The present data demonstrate statistically that the release of LH and testosterone in the sheep varies in a non-random way as a function of daytime. Presumably there is more than one factor involved in the mechanism of control, but 'dawn' acting as a synchronizer, seems to be particularly important.

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