

Seasonal variation in LH and testosterone release in rams of two breeds

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Summary. Blood was collected hourly for 24 h in December, February, April, June and September from Préalpes du Sud and Ile-de-France rams. Coincidence of the LH and testosterone peaks was found for 96.4% of a total of 670 LH peaks and 647 testosterone peaks. The number of LH and testosterone peaks increased by 66% in Ile-de-France rams and 200% in Préalpes du Sud rams between December and June ($P < 0.01$). Values in June and September were similar in Préalpes du Sud rams. There were no differences between breeds in December, but in June, Préalpes du Sud had significantly more peaks than did Ile-de-France rams ($P < 0.025$). The numbers of LH and testosterone peaks increased significantly ($P < 0.05$) in Préalpes du Sud rams between December and February or April. These results indicate that, although numbers of peaks of LH and testosterone increase when the animals pass from the non-breeding to the breeding season, the genotype influences the pattern of release through the year.

Introduction

In the ram, LH and testosterone are released into the blood in a pulsatile pattern (Attal, 1970; Bolt, 1971; Katongole, Naftolin & Short, 1974; Sanford, Winter, Palmer & Howland, 1974; Falvo, *et al.*, 1975), and each peak of LH is followed by a peak of testosterone (Lincoln, 1976; Sanford, Palmer & Howland, 1977; Wilson & Lapwood, 1978). There is general agreement that the number of LH and testosterone peaks increases when animals pass from the non-breeding season to the breeding season (Sanford *et al.*, 1974, 1977; Schanbacher & Ford, 1976; Wilson & Lapwood, 1978). However, the influence of genotype on the magnitude of this increase in pulsatility has until now been neglected although it may be of importance because the mean plasma testosterone concentration varies in rams of different breeds (Schanbacher & Lunstra, 1976; Garnier, Cotta & Terqui, 1978). In the present study, therefore, the influence of season on the frequency of LH and testosterone peaks has been examined in rams of the Ile-de-France and Préalpes du Sud breeds.

Materials and Methods

Animals

Mature (2–3 years old) intact rams were used. They were housed in groups of 5–6 in 10 m² rooms in a lightproof building and subjected to normal seasonal changes in daylength regulated

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by electrical clocks. These clocks were adjusted every 2–4 days according to the magnitude of the daily change of daylength.

At the latitude of Nouzilly, 47°N, daylight varies from about 8 h in December to about 16 h in June. In each room, four 40 W, white, fluorescent tubes provided about 300 lux at the level of the rams' eyes. Animals were allowed 1 month to adjust to the new environment before the first period of blood sampling and then stayed continuously in the rooms during the rest of the experiment.

Food was provided each morning at 08:30–09:00 h and consisted of 400 g dehydrated lucerne, 250 g dehydrated maize, 300 g oats and 25 g mineral supplement along with straw and water *ad libitum*.

The male sexual season extended from the end of June to the end of December in Ile-de-France rams and from the beginning of June to the end of December in Préalpes du Sud rams (Thibault *et al.*, 1966; G. Colas, personal communication).

The rams were bled in successive years with alternation and replication for each breed. The periods of blood sampling and the total numbers of animals were as follows:

end of December—41 Ile-de-France and 75 Préalpes du Sud
mid-February—11 Ile-de-France and 9 Préalpes du Sud
beginning of April—11 Ile-de-France and 9 Préalpes du Sud
beginning of June—11 Ile-de-France and 9 Préalpes du Sud

In addition, blood samples were collected at the end of September from 5 Préalpes rams on one occasion.

In each sampling period, blood was collected hourly from the jugular vein for 24 h (25 samples from 09:00 h to 09:00 h the next day). During the night, blood was collected in darkness. Plasma samples were kept frozen at -14°C until radioimmunoassays for LH and testosterone were carried out.

LH assay

LH was measured in duplicate according to the method of Pelletier, Kann, Dolias & Rosselin (1968) which was slightly modified in that: (1) ^{125}I (Radiochemical Company, Amersham, U.K.) was used for labelling instead of ^{131}I ; (2) after labelling, iodinated LH was separated from free iodine on a P-10 G25 Sephadex column, and the most radioactive fraction was repurified further on a K30 G100 Sephadex column, equilibrated with 0.025 M-veronal and 0.25% human serum albumin buffer; (3) the second antibody was anti-guinea-pig γ globulin serum obtained by immunization of a mare; (4) all the samples collected during a given year were assayed simultaneously in an automatic LKB 2071 sample processor; (5) results expressed in terms of LH-M3 (= $1.8 \times \text{NIH-LH-S1}$) were obtained on-line from an LKB 1270 Rack-gamma counter; (6) specificity was verified, especially for cross-reaction with TSH (Freychet, Pelletier & Rosselin, 1969); (7) within-assay coefficients of variation of the baseline concentration varied according to experiments between 4 and 10%; and (8) the interassay coefficients of variation were 10 and 8.3% for baseline and peak levels respectively. Sensitivity under these conditions was 0.1 ng/ml.

Testosterone assay

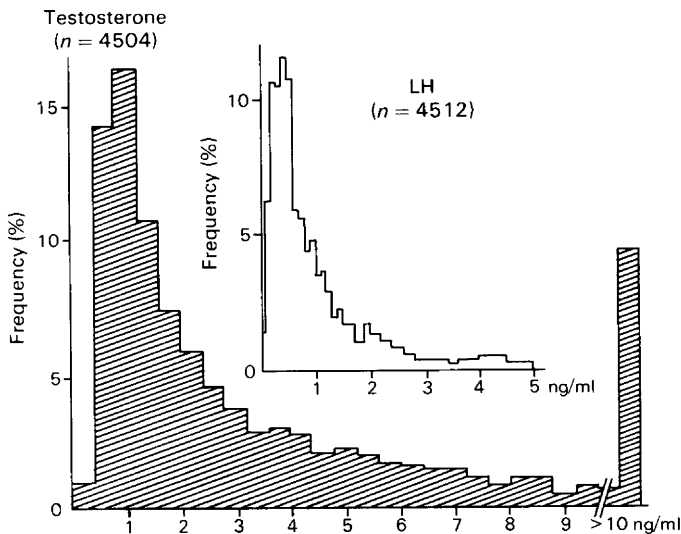
Testosterone was measured directly in 0.05 ml plasma by the radioimmunoassay described by Garnier *et al.* (1978). The within-assay coefficients of variation were 8.4, 7.8 and 7.9% for 1.0, 4.8 and 8.2 ng/ml respectively, and the inter-assay coefficients of variation were 14, 10.5 and 13%. Sensitivity was 0.2 ng/ml.

Determination of LH and testosterone peaks

Continuous bleeding experiments carried out on rams during the breeding season (Davies, Main & Setchell, 1977) or during both the non-breeding season and the breeding season (Terqui, Garnier, Pelletier, Ravault & Ortavant, 1978; Terqui, Garnier, de Reviere, Huet & Pelletier, 1980) have shown that most LH and testosterone peaks last more than 60 min. Indeed, only 19% of LH peaks and 4% of testosterone peaks have a duration of 40–60 min. The probability of detecting these shorter peaks with a sampling interval of 1 h is therefore at least 4/6 and the hourly sampling interval should give detection of at least 93.5% of all LH peaks and 98.6% of all testosterone peaks. A peak was defined as an increase in hormone concentration to a value greater than 4 times the coefficient of variation of the baseline in the assay, followed by a decrease. Because the percentage increase of the hormonal level above the baseline at the time of a peak was high (100–1000%), the differences in the within-assay coefficients of variations were not considered to have biased the comparison of the number of peaks between breeds. This is reflected by the very comparable percentage of coincident LH and testosterone peaks found in the two breeds (see 'Results'). LH and testosterone peaks were considered as coincident peaks if the testosterone peak was simultaneous with or occurred just after an LH peak.

Results

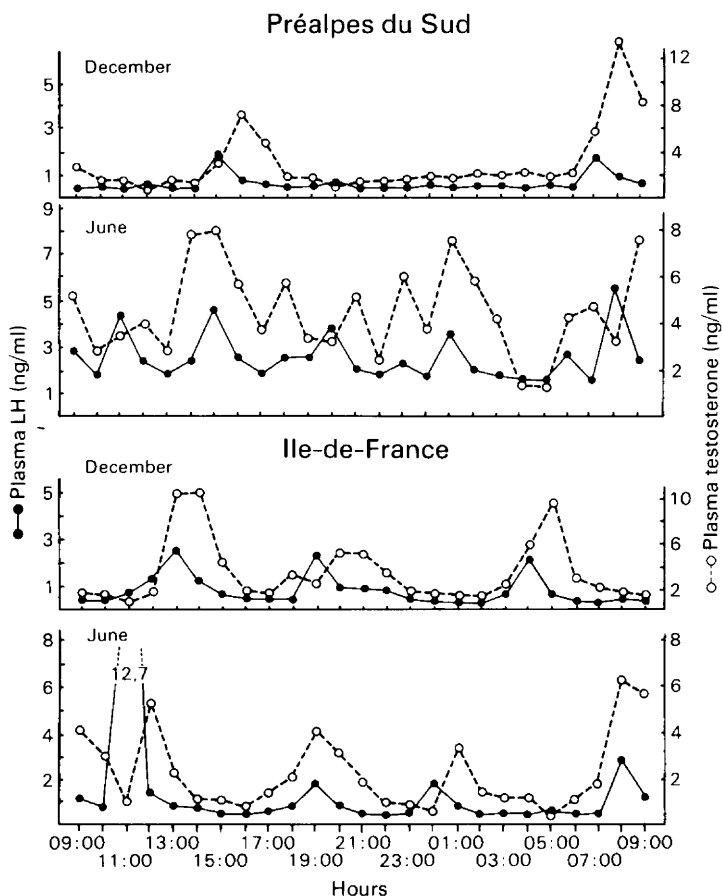
When plasma LH and testosterone levels were summarized as frequency distributions, these distributions were unimodal and strongly asymmetric (Text-fig. 1). In addition, plasma LH and testosterone for a given ram and a given hour of sampling cannot be considered as independent variables. Therefore (i) the unimodal distribution of levels could not be used for the determination of the LH and testosterone peaks as suggested by Christian, Everson & Davis (1978) who found a bimodal distribution of GH levels in cattle and (ii) classical parametric analysis, and the expression mean \pm s.e.m., was inadequate and only seasonal variation in the numbers of LH and testosterone peaks per 24 h (expressed as median values rather than mean values) are given. Effects of time of year and breed on numbers of peaks per 24 h were tested statistically by the non-parametric Mann–Whitney U test (1947).



Text-fig. 1. Distributions of plasma LH and testosterone levels expressed as relative frequencies (%).

Correlation between LH and testosterone peaks

As a general rule, a peak of LH was found simultaneously with, or followed by, a testosterone peak (Text-fig. 2). Out of a total of 670 LH peaks and 647 testosterone peaks, there were 96.4% coincident LH and testosterone peaks (404 LH and 394 testosterone, 97.5%, in Préalpes du Sud, and 94% in Ile-de-France rams). This figure included a few results, mainly occurring in June, in which two well-defined LH peaks were followed by a single steady, high level of testosterone. There were 48 isolated peaks, i.e. 3.6% of the total; 23 were peaks of LH without any testosterone peak and 25 were testosterone peaks without any LH peak.

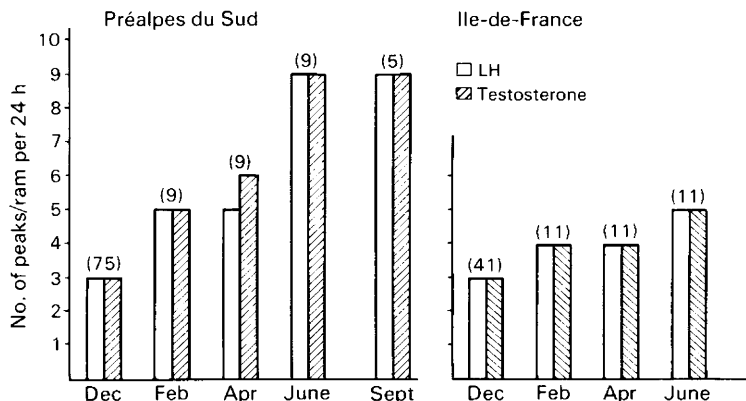


Text-fig. 2. Plasma LH and testosterone variation throughout the day in Préalpes du Sud and Ile-de-France rams in December and in June

Seasonal variation in LH and testosterone peaks: influence of genotype

Text-figure 3 shows that the frequency of LH and testosterone peaks increased between December and June for rams of both breeds. In Préalpes du Sud rams, the number of LH and testosterone peaks increased significantly in February and April ($P < 0.05$) compared with the December group. The difference between December and June was still greater—a 200% increase for both hormones ($P < 0.01$). However, the number of peaks in June was similar to that observed in September. By contrast, the increase in the number of peaks of both LH and testosterone was moderate in Ile-de-France rams and the difference was significant ($P < 0.01$) only for the 66% increase that occurred between December and June. Finally, while the numbers

of peaks were similar in the two breeds in December, they were significantly different in June ($P < 0.025$).



Text-fig. 3. Seasonal variation in the frequencies of peaks of LH and testosterone in Préalpes du Sud and Ile-de-France rams (nos in parentheses). Values are medians (see text).

Discussion

From a statistical point of view, plasma LH and testosterone values did not appear to be sampled from normally distributed populations, as shown by asymmetrical distributions, variance heterogeneity, and lack of independence of plasma hormone concentrations within any given ram. From a physiological point of view, the occurrence of large and rapid changes in plasma LH and testosterone values from 0.5–1.0 up to 10–20 ng/ml (Terqui *et al.*, 1980) also precludes the use of mean \pm s.e.m. values. On the other hand, the number of LH pulses appears to be closely related to the development of the testes (Lincoln, 1979). For this reason, we chose to express the data as the number of LH or testosterone peaks observed in blood samples collected over 24 h and to use a non-parametric test for the analysis.

From the present results, it appears likely that each LH peak is accompanied by a testosterone peak. Continuous collection of blood for 2 days has confirmed the close connection between the LH and the testosterone peaks (Terqui *et al.*, 1980). Findings of an apparent absence of relationship between LH and testosterone levels in rams may only be the result of too infrequent sampling (Falvo *et al.*, 1975). Due to the duration of LH peaks and the use of hourly sampling, some peaks could have been missed, but the high percentage of coincident LH-testosterone peaks observed excluded this as a major source of error.

The present results confirm that the numbers of LH and testosterone peaks increase from the non-breeding to the breeding season (Sanford *et al.*, 1974, 1977; Schanbacher & Ford, 1976; Wilson & Lapwood, 1978), but indicate further that the peak numbers increase before the start of the breeding season in both breeds, and rise continuously in Préalpes du Sud rams during the season of increasing daylength. An increase of plasma concentrations of FSH and testosterone before the summer solstice has been previously mentioned for Soay rams by Lincoln & Short (1980). Unlike Sanford *et al.* (1977), we did not observe any increase in the numbers of LH peaks or testosterone peaks at the end of the breeding season (December).

There was a clear genotypic influence on the pattern of release, with the percentage increase between December and June being three times as great in the Préalpes du Sud rams as in Ile-de-France rams. This result indicates that, in studies of photoperiodic effects, genotype must be taken into account.

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