

# The measurement of daylength in the Ile-de-France ram

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**Summary.** Two groups of 12 adult Ile-de-France rams were exposed to artificial 6-month light cycles in which daily illumination was provided in one or two photofractions. In Group I, daylength increased linearly from 8 to 16 h in 3 months and decreased similarly from 16 to 8 h. The daily increment or decrement (5–33 min/day) was constant. In Group II, 8 h of light were given in two parts: the main one, 7 h, began at the time of dawn in Group I and an additional 1 h light pulse was coincident with the last hour of the former group. The onset of the pulse changed therefore each day and the interval between dawn of the first block and dusk of the second block of light increased from 8 to 16 h in 3 months and declined from 16 to 8 h the next 3 months. Testicular weight was estimated by an orchidometer every 2 weeks for 2 ( $N = 12/\text{group}$ ) or 3 consecutive light cycles ( $N = 6/\text{group}$ ). The testicular weight variations were identical in both groups. In the 6 rams of each group studied during 3 light cycles, variations of testicular weight were submitted to an harmonic regression analysis according to time and the computed values for the mean, amplitude, period and phase were, respectively, 260 g, 66 g, 185 days and 120 days in Group I and 262 g, 65 g, 181 days and 111 days in Group II. Analysis performed for each ram gave very similar values in all individuals. It is concluded (1) that daylength is not measured by the total duration of light exposure but between two limits represented here by dawn and the pulse of light and (2) that the measurement is not limited to a particular photosensitive phase but is effective throughout the entire light cycle.

## Introduction

Rams submitted to artificial light regimens mimicking annual photoperiodic changes in 6, 4 or 3 months present testis variations with the same periodicity as that of the light cycle (Pelletier *et al.*, 1985). Animals are therefore able to measure daylength and to adjust the activity of the hypothalamo–hypophysial system accordingly. In addition, previous experiments indicate that a stimulation of LH secretion and testicular development was obtained in Ile-de-France and Préalpes-du-Sud rams receiving 8 h of light in two photofractions, one main fraction of 7 h and one extra hour of light (pulse) given 16–17 h after the onset (dawn) of the first photofraction (Pelletier *et al.*, 1981). LH and testes were not stimulated if the pulse was positioned elsewhere relative to dawn. These results suggest the existence of a rhythm of photosensitivity in the ram comparable to those described for gonadotrophin and/or testicular weight in some other mammals (Elliott *et al.*, 1972; Grocock & Clarke, 1974; Turek & Campbell, 1979). The position of the stimulatory light pulse relative to dawn corresponds to the conditions of the summer solstice under our latitude (47°N), i.e. at time at which LH pulsatility is greatest (Pelletier *et al.*, 1982) and testicular weight is close to its maximum (Pelletier & Ortavant, 1970). These results suggest that full lighting between dawn and dusk may be unnecessary for the measurement of daylength. However, the demonstration was limited in the ram, as in the other species of mammals, to very few positions of the light pulse. In addition, when the stimulatory light pulse was given repeatedly at the same interval after dawn, the stimulation tended to vanish in few weeks. Finally, in all experiments done until now in mammals, the results indicate only that a pulse of light set up at a convenient delay after dawn can stimulate (or inhibit)

gonadotrophin and gonadal activities. It is therefore uncertain whether animals exposed to a skeleton photoperiod really measure daylength by the interval between the dawn of the main light phase and the dusk of the pulse, whatever the interval between them.

The present study was therefore undertaken to compare the measurement of daylength in rams exposed to light regimens in which illumination was given in one or two photofractions. The position of the second photoperiod, the pulse, was adjusted each day to the last hour of the light photoperiod of controls which received illumination in one block. It was expected that these dynamic conditions would allow a more accurate description of the effects of light fractionation than the static paradigms used previously in other species of mammals. Preliminary results have been published in abstract form (Thimonier *et al.*, 1985).

## Materials and Methods

**Animals.** Young adult Ile-de-France rams (2–3 years old) were housed in a light-proofed building in four rooms of 10 m<sup>2</sup> each (6 animals/room) where the light was provided by four fluorescent tubes giving 350–400 lx at eye level. The animals, previously reared outdoors under natural photoperiodic conditions, were allowed to adjust to the new environment for 1 month in December 1983 during which they were exposed to natural daylength changes. On 1 January 1984, rams of Group I (two rooms,  $N = 12$ ) were placed in a light regimen in which the period was 6 months and the amplitude of daylength varied progressively in 3 months from 8 to 16 h and conversely from 16 to 8 h in the 3 subsequent months (Fig. 1a). The daylength was adjusted each day and the daily increment (or decrement) was constant (5.33 min/day) modifying both dawn and dusk. The animals of Group II, in two other rooms, were exposed to a light regimen with a similar 6-month period but in which the total lighting was equal to 8 h given in two fractions. The onset of the first light photoperiod of 7 h duration was adjusted to the time of dawn of Group I. Similarly, the end of the second light photoperiod, the pulse of 1 h, was adjusted to dusk (Fig. 1c). All animals were exposed to two consecutive light cycles, but, because of the availability of light-proofed rooms, only 6 out of 12 animals of each group were exposed to an additional third light cycle. As one animal of Group I and another of Group II died in mid-December 1984 and early January 1985 respectively, 6 animals of each group were kept in the other room during the third light cycle. The onset (dawn) and the end (dusk) of lighting were controlled by an electronic clock.

Each morning the rams were fed a diet of 300 g lucerne pellets, 400 g wheat and 30 g mineral supplement with water and wheat straw *ad libitum*. Animals were weighed each month.

Testicular weight was estimated every 2 weeks using an orchidometer, a set of standard testis-shaped beads ranging from 100 to 400 ml. The correlation with the actual testis weight was +0.96 (Oldham *et al.*, 1978).

The testicular weight was chosen as a representative measure integrating gonadotrophin changes after daylength variations. Since daylength measurements might not be identical in all rams, special attention was devoted to testicular weight changes of individual rams.

**Statistical analysis.** Differences in body and testicular weights between groups were subjected to one-factor variance analysis. Two-factor analysis of variance (room and group) was also performed. Furthermore, for the 6 animals of each group followed during 3 light cycles, variations in testicular weight according to time ( $t$ ) were submitted to an harmonic regression analysis following the model  $\hat{y}(t) = \mu + a \sin(2\pi t/\tau + \phi)$  where  $\mu$ ,  $a$ ,  $\tau$ ,  $\phi$  were mean, amplitude, period and phase respectively, estimated according to the Gauss–Marquardt algorithm (Marquardt, 1963). Amplitude was the difference between mean and maximal or minimal value. The first 3 months of the experiment were considered as the time of adjustment of animals to their new light regimen and were not used in the computation.

## Results

### Animal weight

The weight of animals was homogeneous at the start of the experiment (Group I =  $75 \pm 3$  kg and Group II =  $78 \pm 2$  kg) and did not differ thereafter between groups until the end of the experiment.

### Testicular weight

In Group I each increase in testicular weight was associated with a decrease of daylength and, conversely, testicular regression corresponded mainly to an increase in daylength (Fig. 1b). During the first two light cycles (12 rams/group) there was no difference in the testicular weights between rooms or groups. Similarly, there was no difference between groups during the third light cycle

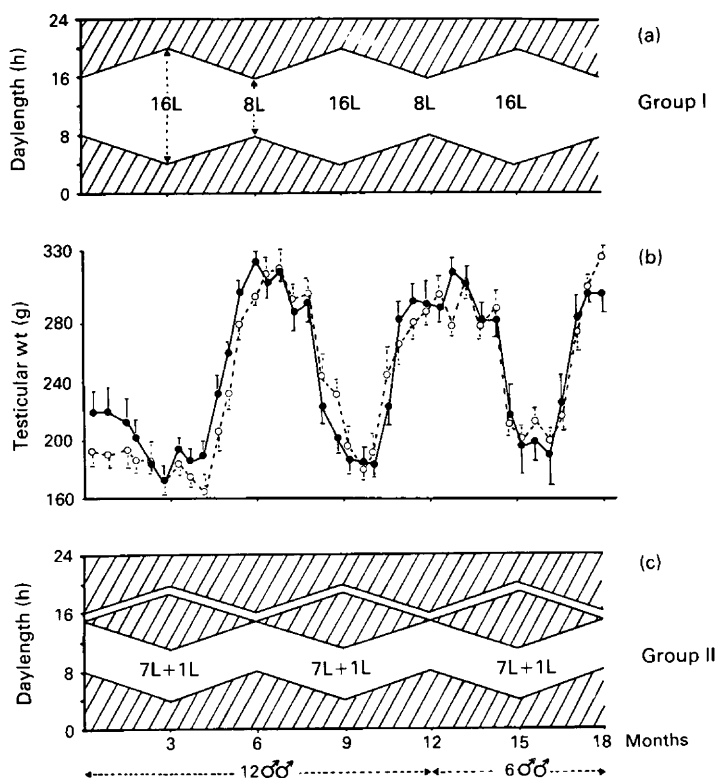


Fig. 1. Testicular weight variations (b) in control (Group I, ●—●) and experimental (Group II, ○---○) rams according to photoperiodic daylength changes (a and c). In Group II, dawn of the main light phase was adjusted to dawn of the control group while the 1 h light pulse was coincident with the last hour of daylight (shaded area = darkness).

(6 rams/group). The two subgroups of 6 rams within each group had homogeneous testicular weights and so the decrease from 12 to 6 animals between the second and the third light cycles did not change the patterns of the curves (Fig. 1b).

Testicular weights were low and identical in both groups at 3, 9 and 15 months after the onset of the experiment, i.e. when the light schedules were 16L:8D in Group I and 7L:8D:1L:8D in Group II. The overall mean testicular weights were very close,  $240 \pm 8$  and  $235 \pm 8$  g, for Groups I and II respectively. During the first 3 months, the animals appeared to be still under the influence of the previous light regimen but thereafter the testicular weight varied according to a 6-month periodicity.

After adjustment, and using the means of the 6 individuals of each group kept under experimental conditions for 3 light cycles, the computed means of testicular weight and amplitude variations were very similar between groups (Table 1). More importantly, the fitted periods of the testicular weight rhythms were also similar between groups: that of Group II fitted exactly with the imposed period of the light regimen, 181 days. The phase shift between the light and the testicular weight changes was also not significantly different between groups.

The fitting of individual data of the 6 rams of each group exposed to 3 light cycles (Table 2) shows that if the mean testicular weight and the amplitude of the rhythm varied to some extent within groups, the coefficients of variation were similar in both groups for these two measures, 8.7 and 4.3% for testicular weight and 14.3 and 16.4% for amplitude in Groups I and II respectively. Furthermore, the computed periods were very close between all individuals. Finally, the phase and its range of variation did not differ significantly between groups.

**Table 1.** Determination coefficient ( $R^2$ ) between the observed and the computed curves, mean, amplitude, period and phase of computed curves from the mean testicular weight of 6 rams of Group I (light photoperiod in one block) and 6 rams of Group II (light photoperiod in two blocks)

Group	$R^2$	Mean (g)	Amplitude (g)	Period (days)	Phase (days)
I	0.89	260 (254–266)	65.9 (57.4–74.3)	185 (181–190)	120 (112–128)
II	0.82	262 (254–270)	64.5 (53.5–75.8)	181 (174–187)	111 (101–121)

Values in parentheses are confidence limits ( $P = 0.05$ ).

**Table 2.** Determination coefficient ( $R^2$ ) between the observed and the computed curves, mean, amplitude, period and phase of computed curves from the testicular weight of 6 individuals of Group I (light photoperiod in one block) and Group II (light photoperiod in two blocks)

Group	Ram	$R^2$	Mean (g)	Amplitude (g)	Period (days)	Phase (days)
I	1	0.84	238	74.2	188	110.38
	2	0.63	255	53.1	185	97.70
	3	0.68	290	62.2	179	91.94
	4	0.78	245	73.9	187	111.24
	5	0.69	294	59.0	186	109.51
	6	0.82	242	79.5	193	115.86
	Mean		261	67.0	186	106.10
II	1	0.70	268	58.6	178	92.8
	2	0.82	267	73.7	187	105.20
	3	0.73	255	57.0	176	98.86
	4	0.82	240	84.9	181	87.90
	5	0.48	266	54.1	182	95.39
	6	0.80	275	68.5	183	100.87
	Mean		262	66.1	181	96.83

## Discussion

The experimental system was chosen because it offered an acute and well stereotyped waxing and waning of testicular weight different from that observed in the natural environment, a condition favourable to the study of daylength measurement by animals.

In the present experiment, the daily increment and decrement in daylength were constant in the control group. However, the pattern of testicular weight was similar to that reported for previous studies in which a sinusoidal pattern of daylength was imposed (Pelletier & Ortavant, 1975; Lindsay *et al.*, 1984). In the 6-month light regimens, the testicular weight changes depended strictly on LH pulse frequency (Lindsay *et al.*, 1984; Thimonier *et al.*, 1985). In all the regimens in which the period of the rhythm was shortened, a strong photoperiodic drive of LH release was observed during the decreasing daylength stage and so the testicular weight was maximum in short days and was almost out of phase with daylength. This latter finding was observed again in the present study. These results differ markedly with those observed in rams kept under natural daylength since, in this

case, testicular growth occurred at the time of summer solstice and testicular weight was maximum in summer (Pelletier & Ortavant, 1970).

Rams which received light in two photofractions presented testicular weight variations that were similar to those of controls exposed to changes of light given in one block. The similar patterns of testicular weight during the experiment in both groups indicate that daylength was measured by animals of Group II not only during a particular part of the light cycle, such as a photosensitive phase, but also throughout the entire light cycle. The animals therefore measured daylength between two limits without regard to whether the interval between them was fully illuminated or not. The first set point could be dawn since LH pulse frequency throughout the day appears to be correlated to it (Ortavant *et al.*, 1982). Dusk or a part of the moving light pulse is the obvious candidate for the second set point. Furthermore, the measurement of daylength is independent of a moving dawn or dusk *per se* since a regimen with a static dawn (or dusk) and a twice higher increment or decrement of daylength affecting only dusk (or dawn) is measured by rams as the actual total daylength (unpublished data). This reinforces the concept of two set points to measure daylength.

It is of a particular interest to consider whether all animals used the same limits to measure daylength and so special attention was given to individual variations in testicular weight. For example, one individual could consider the onset of the pulse as dawn and the end of the main light photoperiod as dusk. For such an animal daylength changes would be out of phase compared with controls. For the same reason it is unlikely that rams, neglecting the pulse of light, measured daylength between the dawn and the dusk of the main block only. From analysis of individual changes in testicular weight, it can be ascertained that none of the rams of Group II had measured the photoperiod differently from controls. It is also possible that rams changed the set points for measuring daylength during a given light cycle. Although this possibility cannot be ruled out, such changes must have been negligible. Indeed, it is expected that in animals exposed to light regimens with a short period, any temporary change in the perception of increasing and decreasing daylengths would have resulted in a modified periodicity of the testicular weight rhythm and a loss of coincidence with the rhythm of controls.

The present results therefore indicate that Ile-de-France rams measure daylength day after day, between two limits, dawn and the light pulse, whatever the interval between them.

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