The effect of rearing under a long or short photoperiod on testis growth, plasma testosterone and prolactin concentrations, and the development of sexual behaviour in rams*

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Summary. Two groups of 6 rams were maintained under constant photoperiodic conditions consisting of short days (8 h light: 16 h dark; Group S) and long days (16 h light; 8 h dark; Group L) from 4 to 20 months of age. Five other rams were reared under a photoperiod representative of that occurring naturally (Group N). Testis size, plasma testosterone and prolactin concentrations were monitored weekly and sexual behaviour tests were carried out at regular intervals. Over the treatment period Groups S and L did not differ in terms of testis growth or plasma testosterone. Both groups had a phase of testis growth and increased testosterone followed by a decline and the temporal patterns for the two groups were equivalent. Sexual behaviour was slower to develop in Group L than in Group S, indicating that photoperiod can affect the development of sexual behaviour irrespective of peripheral plasma testosterone concentrations. Plasma prolactin levels showed a cyclic variation in Group L and were significantly higher overall than in Group S rams. This, together with a trend towards negative correlations between prolactin concentrations and sexual behaviour in Group L, indicates that prolactin may be involved in the effect of photoperiod on sexual behaviour. The presence of a cycle of testicular growth and of hormone concentrations in young animals under constant photoperiod tentatively suggests that these cycles are endogenous. The constant photoperiod did, however, affect the animals because the cycles which occurred in Groups S and L were out of phase with those of Group N by about 4 months.

Introduction

There appears to be marked species variation in the effect of different photoperiods on the sexual development of young males. For example, growth and function of the testis are inhibited for a time in Djungarian hamsters and voles reared from birth or weaning under short photoperiods (Clarke & Kennedy, 1967; Grocock & Clarke, 1974; Hoffmann, 1978; Grocock, 1979), while testis development is relatively unaffected by short photoperiod in lemmings and golden hamsters (Gaston & Menaker, 1967; Reiter, Sorrentino & Hoffman, 1970; Hasler, Buhl & Banks, 1976). To the best of our knowledge these studies have been only of small mammals in which reproductive function is stimulated in the adult male by long photoperiods. The aim of the

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experiments described here was to study the effect of different photoperiods on testis growth, plasma testosterone concentrations, and the development of sexual behaviour in a 'short-day breeder', the ram. Because prolactin secretion is known to be influenced by photoperiod in the ram (Ravault, 1976) and has a high degree of involvement in testicular development in other species (Bartke, Hafez, Bex & Dalterio, 1978), concentrations of this hormone in blood plasma were also measured to examine whether photoperiodic effects on the testis might be mediated through this hormone.

Materials and Methods

Animals; general management and routine data collection

A group of 17 Suffolk × Swaledale/Border Leicester ram lambs was reared on pasture until 4 months of age. At this time, two groups of 6, selected at random, were transferred to rooms (6 × 3 m) with controlled lighting in a wooden shed. Illumination was by fluorescent strip lights (4 per room) giving about 300 lux at sheep head height. Temperature was not controlled but the rooms were ventilated by driven air which could be refrigerated during excessively hot weather. One group (S) was placed on constant short days (8 h light: 16 h dark) and the other (L) on long days (16 h light: 8 h dark). Lights went on at 08:00 h (BST) for both groups. The transfer took place 2 weeks after the summer equinox, i.e. July. Thus Group L was kept in a light regimen approximating to that outside at the time of housing, while Group S was subjected to an abrupt change in photoperiod, the light being decreased suddenly by about 8 h. The remaining 5 rams were brought from pasture 19 weeks later and placed in a similar room within the same building. These animals (Group N) were kept under a changing photoperiod corresponding to the season, the lighting being altered weekly to accord with daylength information given in the 'Daily Telegraph'. All animals were allowed free access to hay and water and were given a standard concentrate sheep ration each day between 08:30 and 11:30 h. The length and width of the testes were measured weekly (every Tuesday between 09:00 and 10:30 h) by using calipers. No correction was made for scrotal skin thickness. Weekly blood samples were collected into heparinized containers by jugular venepuncture immediately before testis measurement. Blood was centrifuged soon after collection and the plasma stored at -20°C until required for hormone assays. Serial blood samples were taken at 30-min intervals for 12 h from each animal every 6–8 weeks using indwelling jugular vein catheters. These were inserted at least 8 h before collection of the first sample. The serial bleeds were started at 24:00 h and a 20-W red bulb was used to aid collection during the dark period. Blood was processed as for the weekly samples.

Sexual behaviour tests

From Week 33 a series of behavioural tests were carried out (for intervals see Text-fig. 5). Each ram was tested in his home room by recording his behaviour towards a ewe. The ewes were ovariectomized and brought into oestrus as described by Robinson (1954). Three ewes were available and were randomly allocated throughout. Other rams in the group were separated from the animal under test by a gate which allowed visual and olfactory contact. Behaviour recorded was based on the categories described by Banks (1964) as follows: (1) courtship behaviour—(a) licks, consisting of tongue flicking associated with pushing the shoulder of the ewe and making short forward darts with the head along the ewe's flank, (b) grunts, a characteristic low vocalization made by the ram, (c) pawing, where the ram strikes the ewe with a forefoot; (2) copulation, consisting of attempted mounts, mounts with successful intromission and mounts culminating in ejaculation.

Animals were observed from outside the room, through an aperture well above sheep height, and the number of times per minute that a ram indulged in each of the specified behaviours was

recorded. A test lasted for 20 min unless the ram had shown no interest for a consecutive 10 min, such tests were abandoned.

Hormone assays

Testosterone. This assay was carried out using the technique and reagents as described by Purvis, Illius & Haynes (1974) but with the following modifications. Duplicate aliquots of plasma (usually 50 μ l) were diluted with 100 μ l assay buffer. A series of standards (doubling dilutions from 2000 to 15.6 pg/100 μ l assay buffer) was prepared and 50 μ l plasma from a castrated ram were added to each standard tube. All tubes were heated to 70°C in a water bath for 30 min. When cool, 100 μ l antiserum at a dilution of 1:7500 and 100 μ l of [1, 2, 6, 7-³H]testosterone (containing ~20 000 c.p.m.) were added to each tube and the mixture was agitated and incubated for at least 12 h. The bound and free fractions were separated with dextran-charcoal. The limit of assay sensitivity, expressed as the value of twice the s.d. from the binding obtained with zero concentration of testosterone, was 22 pg/tube.

The coefficient of variation (CV) between duplicate pairs (Snedecor, 1952) incorporating both inter- and intra-assay variation was 8.6% over the range 0.7–4.0 ng/ml ($n = 50$) and 6% over the range 4–11.0 ng/ml ($n = 50$). Inter-assay CV between reference plasmas was 12% for a standard of mean value 4.2 ng/ml, and 25% for a standard of mean value 1.2 ng/ml. The same reference samples were assayed routinely by the method of Purvis *et al.* (1974), which involved extraction of testosterone from the plasma with ether before assay, and mean $\pm$ s.e.m. values of 4.3 ± 0.2 ($n = 20$) and 1.4 ± 0.3 ($n = 40$) ng/ml were obtained. As a check that assays for testosterone with or without extraction were equivalent, serial blood samples from rams in different physiological states were assayed by both methods. The profiles obtained were similar and a typical example is shown in Text-fig. 1.

Text-fig. 1. Peripheral plasma testosterone concentrations measured in ether extracts of plasma (■) or in plasma without an extraction step (□). The ram was given 100 μ g Gn-RH in 2 ml saline intravenously and later castrated (C).

Prolactin. Antisera against NIH-P-S10 were raised in rabbits by using the procedure described by Lynch & Shirley (1975). Blood taken from one rabbit was characterized and used as antiserum (R3B5) in the assay. The assay procedure was as described for bovine prolactin (Webb, Lamming, Haynes & Foxcroft, 1980) with the following modifications. A 5 μ g aliquot of sheep prolactin (NIH-P-S10) was iodinated. Standards (double dilutions from 20 to 0.16 ng/tube) were prepared in 500 μ l assay diluent. Antiserum at an initial dilution of 1:200,000 in assay diluent was added in 200 μ l aliquots to each tube. This was followed immediately by the addition of 100 μ l ¹²⁵I-labelled prolactin (~20 000 c.p.m.).

The antiserum bound 40–50% ¹²⁵I-labelled sheep prolactin at a dilution of 1:200 000.

Sensitivity, defined as for testosterone, was 250 pg/tube. For an assessment of accuracy known amounts (0.16–10 ng) of NIH-P-S10 were assayed after addition to a ram plasma pool. After adjustment for 115 ng prolactin/ml measured in this pool, the mean $\pm$ s.e.m. recovery figure was $104.0 \pm 2.1\%$. Cross-reactions, calculated as the amount of other hormones (w/w with prolactin) giving 50% inhibition in the assay, were 2% for NIH-GH-S7 and <0.2% for NIH-LH-S18 and NIH-FSH-S11. Inhibition curves for 2 ram plasma samples at dilutions from 1:1 to 1:32 were parallel to the prolactin standard curve. The intra-assay CV for randomly selected duplicate pairs was 7.2% ($n = 50$). The inter-assay CV was 8.4%.

Statistical analysis

This was by analysis of variance or Student's *t* test unless otherwise stated.

Results

Testicular changes and testosterone

Mean testis volume changes for the three groups with time are shown in Text-fig. 2. Between Weeks 15 and 20 there were some instances when the mean testis volume was significantly greater in Group L than in Group S. Mean testis volume then followed a similar pattern in Groups S and L, increasing markedly up to Week 50 and then declining, with no overall significant difference between the groups. Group N rams showed little change in mean testis volume until Week 50, after which it increased. There were 2 occasions before Week 25 (Text-fig. 3), when the mean weekly plasma testosterone concentrations were significantly higher ($P < 0.05$) in Group L than in Group S rams. The pattern of testosterone then fluctuated in both groups, but did not differ significantly between the groups, following a general pattern of change equivalent to that of the testes. Between Weeks 21 and 50, the mean plasma testosterone concentrations for Group N were significantly lower ($P < 0.01$) than those for Groups S and L.

Text-fig. 2. Mean testis volume (expressed as the volume of a cylinder, incorporating both length and width measurements) for rams in Groups S (●), L (□) and N (▲). The s.e.m. values ranged from 9 to 31 cm³ and were generally proportional to the mean. The s.e.m. value is shown for Groups S and L when the means were significantly different (*, $P < 0.01$).

Table 1. Mean $\pm$ s.e.m. concentrations, peak heights and number of peaks for testosterone in plasma collected every 30 min for 12 h for each group of rams

Week of sampling	Group S			Group L			Group N		
	Conc. (ng/ml)	Peak height (ng/ml)	No. of peaks	Conc. (ng/ml)	Peak height (ng/ml)	No. of peaks	Conc. (ng/ml)	Peak height (ng/ml)	No. of peaks
2	1.5 $\pm$ 0.1	3.2 $\pm$ 0.2	1.2 $\pm$ 0.3	1.1 $\pm$ 0.1	2.7 $\pm$ 0.2	1.7 $\pm$ 0.5			
9	3.4 $\pm$ 0.5	7.4 $\pm$ 0.8	2.0 $\pm$ 0.3	2.5 $\pm$ 0.4	6.8 $\pm$ 0.7	2.5 $\pm$ 0.3			
15	0.8 $\pm$ 0.1 ^a	6.4 $\pm$ 1.2	1.2 $\pm$ 0.2	1.4 $\pm$ 0.2 ^a	7.0 $\pm$ 1.1	1.4 $\pm$ 0.2			
20	1.7 $\pm$ 0.3	5.4 $\pm$ 0.9	1.2 $\pm$ 0.2	1.8 $\pm$ 0.2	4.6 $\pm$ 0.8	1.2 $\pm$ 0.2			
27 (29)	1.8 $\pm$ 0.4	5.1 $\pm$ 0.5	1.5 $\pm$ 0.3	1.7 $\pm$ 0.2	4.5 $\pm$ 0.4	1.7 $\pm$ 0.2	1.7 $\pm$ 0.5	5.7 $\pm$ 0.8	1.3 $\pm$ 0.3
39 (41)	1.5 $\pm$ 0.2	3.7 $\pm$ 0.4	1.8 $\pm$ 0.3	2.2 $\pm$ 0.3	4.8 $\pm$ 0.5	2.7 $\pm$ 0.3	1.2 $\pm$ 0.1	3.6 $\pm$ 0.6	1.2 $\pm$ 0.4
47 (49)	2.9 $\pm$ 0.6 ^a	4.7 $\pm$ 1.1	1.8 $\pm$ 0.5	3.2 $\pm$ 0.4 ^b	7.0 $\pm$ 0.4	1.6 $\pm$ 0.2	1.8 $\pm$ 0.3	4.0 $\pm$ 0.6	1.8 $\pm$ 0.4
56	1.5 $\pm$ 0.3 ^a	2.7 $\pm$ 0.6 ^x	1.0 $\pm$ 0.3	3.2 $\pm$ 0.4 ^{ab}	6.6 $\pm$ 1.6 ^y	1.4 $\pm$ 0.4	1.7 $\pm$ 0.4 ^{ab}	4.4 $\pm$ 0.7	1.3 $\pm$ 0.3
63	1.8 $\pm$ 0.4 ^a	4.5 $\pm$ 0.4 ^x	1.2 $\pm$ 0.4	3.3 $\pm$ 0.2 ^b	3.8 $\pm$ 0.8 ^y	1.2 $\pm$ 0.4	1.4 $\pm$ 0.1 ^b	3.8 $\pm$ 1.0	1.0 $\pm$ 0.3
				1.5 $\pm$ 0.2 ^b		1.2 $\pm$ 0.4	5.4 $\pm$ 0.5 ^{ab}	11.0 $\pm$ 0.6 ^{xy}	1.7 $\pm$ 0.3

Weeks in parentheses refer to Group N for which, because of technical limitations, profiles were obtained at a different time from those for Groups S and L. For a particular row, values with the same superscript are significantly different ($P < 0.05$).

From Weeks 50 to 65 plasma testosterone concentrations in Group N rose in parallel with the increase in testicular volume. The summary of the testosterone data obtained from the serial blood samples is shown in Table 1: basically the data accord with those from the weekly testosterone measurements.

Text-fig. 3. Mean plasma testosterone concentrations for rams in Groups S (●), L (□) and N (▲). The s.e.m. values ranged from 0.1 to 2.2 ng/ml and were generally proportional to the mean. The s.e.m. value is shown for Groups S and L when the means were significantly different (*, $P < 0.05$).

Prolactin

Mean plasma prolactin concentrations for the rams are shown in Text-fig. 4. Overall, plasma prolactin was significantly higher ($P < 0.01$) in Group L than in Group S and the pattern of secretion over time was also significantly different ($P < 0.01$). The periods when prolactin in Group L rams was significantly higher ($P < 0.05$) than that in Group S rams and the relatively constant prolactin concentrations in the latter suggests that prolactin values in Group L rams showed a cyclic pattern. A cycle of prolactin concentration was also observed in Group N, but the cycles were 4 months out of phase with those in Group L.

Behaviour

The numbers of courtship patterns (licks, grunts and pawing combined) and numbers of mounts performed by each ram per test are shown in Text-fig. 5. Group L rams were slower overall to develop courtship and mounting behaviour (χ^2 test, $P < 0.001$) than those in Group S. However, there was some overlap in the behavioural development between the groups and towards the end of the testing period a number of animals were behaviourally equivalent. The greatest number of behaviour patterns per test was observed during the phase of rapid testicular growth. Courtship and mounts observed for Group N rams were low throughout and mounts were not seen until the period of rapid testicular development and increased plasma testosterone. Behaviour data for individual animals in relation to mean plasma testosterone and prolactin concentrations for each animal are shown in Table 2. The correlation coefficients (r) for mean plasma prolactin experienced, against numbers of behaviours observed for rams in Groups S and L respectively were: courtship, 0.1 and -0.61 ; mounts, -0.05 and -0.76 ; ejaculations, 0.19

and -0.88 . The correlation coefficient had to be >0.81 before it reached a level of significance of $P < 0.05$.

Text-fig. 4. Mean plasma prolactin concentrations for rams in Groups S (●), L (□) and N (▲). The s.e.m. values ranged from 1.0 to 27.0 ng/ml and were generally proportional to the mean. The s.e.m. value is shown for Groups S and L when the means were significantly different ($P < 0.05$).

Text-fig. 5. Numbers of courtship behaviours (licks, grunts and pawing combined) and mounts shown by each ram (Nos 1–5 or 6 from left to right in each week) towards a ewe during one 20-min behavioural test at the week indicated. The total for 2 tests carried out in the same day is given at *. Rams in Group N were not tested until Week 41.

Table 2. Total behaviour patterns observed and mean $\pm$ s.e.m. weekly plasma testosterone and prolactin concentrations for each ram over the period of study (Weeks 5 (19 for Group N)–60)

Ram	Courtship	Mounts	Ejaculations	Hormone conc. (ng/ml)	
				Testosterone	Prolactin
Group S					
1	59	1	0	2.2 ± 0.3	18.5 ± 2
2	154	108	6	3.0 ± 0.9	16 ± 3
3	32	0	0	2.8 ± 0.6	12 ± 2
4	131	52	8	1.7 ± 0.4	17 ± 2
5	85	24	6	2.6 ± 0.5	24 ± 4
6	121	32	10	2.3 ± 0.5	16 ± 3
Group L					
1	69	18	5	3.0 ± 0.3	48 ± 7
2	15	0	0	2.4 ± 0.3	56 ± 7
3	29	0	0	3.5 ± 0.4	61 ± 8
4	55	52	5	2.9 ± 0.4	32 ± 4
5	30	14	5	1.4 ± 0.5	31 ± 6
6	1	0	0	2.5 ± 0.4	70 ± 16
Group N					
1	0	0	0	1.6 ± 0.2	61 ± 16
2	4	0	0	1.9 ± 0.3	48 ± 16
3	14	0	0	2.0 ± 0.7	82 ± 20
4	31	6	1	1.5 ± 0.4	38 ± 15
5	3	0	0	1.1 ± 0.1	62 ± 17

Discussion

Over the period studied, developing rams exposed to either constant long or short days did not show major differences in patterns of testicular growth or plasma testosterone concentration. This 'short-day breeding' species therefore behaves like the golden hamster and lemming and differs from the Djungarian hamster and vole (see 'Introduction'). However, constant photoperiod, whether short or long, produced developmental differences compared with rams kept in simulated natural daylength. In accord with the observations of Illius, Haynes, Purvis & Lamming (1976) and Alberio & Colas (1976), testicular growth and plasma testosterone concentrations in Group N slowly increased from birth, to a plateau from the winter of the first year until an accelerated phase of testicular growth in the 2nd year at a time when daylength was decreasing. The rapid increase in testicular growth and plasma testosterone concentrations in Groups S and L during February to July therefore occurred at a time when testis growth was depressed in Group N, presumably by increasing photoperiod. It appears from this study that after 25 weeks on a constant photoperiod (when the relative immaturity of the reproductive system may have limited an effect of long versus short periods of light) and in the absence of a new photoperiodic cue, a young ram has an accelerated phase of testicular growth regardless of whether the photoperiod is 8 or 16 h light. Lincoln & Davidson (1977) have demonstrated that gonadotrophin secretion and consequent testicular development in the ram are depressed by long days, but will increase again in animals maintained in constant long days for a prolonged period. From this, and related evidence, Lincoln & Davidson (1977) postulate that the role of photoperiod in sheep may be to entrain the time of the sexual cycle, rather than cause it. The results reported here support this hypothesis. The fact that these developing rams showed their first testicular cycle of growth and regression under conditions of constant photoperiod is further tentative evidence for the cycle being endogenous.

The gradual increase in behaviour in Groups S and L may have resulted merely from experience gained during repeated testing as this is a well established phenomenon (Campbell, Finkelstein & Turek, 1978). On the other hand, it may have been facilitated by the increase in testosterone which occurred over the testing period. Although comparisons with Group N are confounded by the fact that behaviour tests started later in this group, sexual behaviour was markedly depressed compared to that in Groups S and L and testosterone levels were significantly lower over the testing period. Possibly a threshold level of testosterone, barely reached in Group N, is necessary for normal overt sexual behaviour to occur. Factors other than plasma testosterone levels, however, must have influenced the sexual responses shown. Firstly, some animals in Groups S and L showed little sexual behaviour, yet had plasma testosterone concentrations greater than the average (see Table 2). Photoperiod was obviously not responsible for the within-group differences and what caused the variability is not known. However, the experimental design was predisposed towards low sexual drive, since, apart from the behaviour tests, the rams were reared in all-male groups, a procedure which can lead to repressed sexual activity in rams (Banks, 1964; Pretorius, 1967; Anderson & Zenchak, 1976; Zenchak, 1977). Secondly, there was a latency in behavioural development in Group L rams relative to those in Group S. Although differences in plasma testosterone were apparent between the groups on isolated occasions in the first 25 weeks, the higher values did occur in Group L and it would therefore be surprising if these were responsible for the subsequent delay in the development of sexual behaviour in these animals. If these are discounted as influential factors, the lack of overall difference in testosterone patterns implies that factors other than plasma testosterone concentrations *per se* are instrumental in causing this difference and such factors operate under photoperiodic influence. It has been suggested that the sensitivity of behaviour centres in the brain to hormones may be more important than plasma hormone levels in regulation of behaviour and that this sensitivity may be altered by photoperiod (Hutchison, 1978). Circumstantial evidence for this exists from various experiments. In canaries, the ability of exogenous oestrogen to induce nest building after gonadectomy is significantly enhanced by exposing the birds to long photoperiods (Steel & Hinde, 1972, 1976). Similarly, ovariectomized ewes show a seasonal variation of behaviour in response to oestrogen (Raeside & McDonald, 1959; Fletcher & Lindsay, 1971). There is also evidence to suggest that photoperiod interacts with androgens in the control of sexual behaviour in some male mammals. For example, castrated hamsters maintained in short days had a reduced sexual response to systemic implants of testosterone propionate compared to animals in long days and given an equivalent implant (Campbell *et al.*, 1978; Morin & Zucker, 1978) and castrated red deer show a seasonal decline in sexual responsiveness to implanted androgen (Lincoln, Guinness & Short, 1972). The observations recorded in the current experiments are a demonstration in the intact animal of photoperiod affecting sexual behaviour independently of plasma testosterone concentrations. The differences in prolactin concentrations in rams in Groups S and L and the negative correlations found between plasma prolactin levels and behaviour suggest that prolactin may be involved in mediating the behavioural response to photoperiod and other data accord with this. Hyperprolactinaemia has been associated with suppressed levels of sexual behaviour in male rats and mice (Doherty, Michael & Svare, 1978), and elevation of prolactin levels reduced libido in rabbits, overriding pretreatment with testosterone propionate (Hartmann, Endröczy & Lissák, 1966). Hyperprolactinaemia has also been associated with decreased libido in man (Thorner & Besser, 1977). That a causal relationship exists in the current study is, however, merely speculative and mechanisms by which prolactin may affect behaviour are unknown.

Prolactin is a necessary adjunct for maximum testicular steroidogenesis in rats and hamsters (Bartke *et al.*, 1978) and on this basis it has been suggested that the high seasonal level of prolactin which normally precedes testicular growth and increased testosterone output in rams may be a prerequisite to prime the testis for maximum seasonal activity (Buttle, 1974). This is not substantiated by the present studies which indicate no more than a permissive role for

prolactin since testicular growth and plasma testosterone concentrations were similar in two groups of animals previously experiencing markedly different prolactin levels. A cycle in plasma prolactin concentration similar to that seen in Group L has been reported for mature ewes kept in a constant photoperiod (Ortavant, Pelletier, Revault & Thimonier, 1978). Peak prolactin levels always occurred, however, when ambient temperature was high and these authors concluded that this was the external cue because prolactin is known to be elevated by high temperature (Tucker & Wetteman, 1976). This would not explain the results of the present study. Although temperature was not controlled, peak plasma concentrations in Group L rams occurred in mid-winter when the rooms were at their coolest and some 4 months out of phase with peak concentrations in Group N; again, tentative evidence that a rhythm in these animals is endogenous. The rams are now being maintained under the same conditions for a prolonged period to determine whether the rhythms presently observed persist.

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References

- Alberio, R. & Colas, G. (1976) Influence of photoperiodism on the sexual development of the young ile-de-France ram. *Proc. 8th Int. Congr. Anim. Reprod. & A.I., Krakow* 3, 326–329.
- Anderson, G.C. & Zenchak, J.J. (1976) Homosexual behaviour in rams (*Ovis aries*) and rearing experience. *Proc. 8th Int. Congr. Anim. Reprod. & A.I., Krakow* 5, 1196.
- Banks, E.M. (1964) Some aspects of sexual behaviour in domestic sheep. *Behaviour* 23, 249–279.
- Bartke, A., Hafez, A.A., Bex, F.J. & Dalterio, S. (1978) Hormonal interactions in regulation of androgen secretion. *Biol. Reprod.* 18, 44–54.
- Buttle, H. (1974) Seasonal variations of prolactin in plasma of male goats. *J. Reprod. Fert.* 37, 95–99.
- Campbell, C.S., Finkelstein, J.S. & Turek, F.W. (1978) The interaction of photoperiod and testosterone on the development of copulatory behaviour in castrated male hamsters. *Physiol. Behav.* 21, 409–415.
- Clarke, J.R. & Kennedy, J.P. (1967) Effect of light and temperature upon gonad activity in the vole (*Microtus agrestis*). *Gen. comp. Endocr.* 8, 474–488.
- Doherty, P.C., Michael, S.D. & Svare, B.B. (1978) Hyperprolactinaemia suppresses mating behaviour in male rats and mice. *Biol. Reprod.* 18, Suppl. 1, Abstr. 134.
- Fletcher, I.C. & Lindsay, D.R. (1971) Effect of oestrogen on oestrous behaviour and its variation with season in the ewe. *J. Endocr.* 50, 685–696.
- Gaston, S. & Menaker, M. (1967) Photoperiodic control of hamster testis. *Science, N.Y.* 158, 925–928.
- Greenwood, F.C., Hunter, W.M. & Glover, J.S. (1963) The preparation of ¹³¹I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 89, 114–123.
- Grocock, C.A. (1979) Testis development in the vole, *Microtus agrestis*, subjected to long or short photoperiods from birth. *J. Reprod. Fert.* 55, 423–427.
- Grocock, C.A. & Clarke, J.R. (1974) Photoperiodic control of testis activity in the vole, *Microtus agrestis*. *J. Reprod. Fert.* 39, 337–347.
- Hartmann, G., Endröczy, E., & Lissák, K. (1966) The effect of hypothalamic implantation of 17 β -oestradiol and systemic administration of prolactin on sexual behaviour in male rabbits. *Acta Physiol. Acad. Sci. Hung.* 30, 53–59.
- Hasler, J.F., Buhl, A.E. & Banks, E.M. (1976) The influence of photoperiod on growth and sexual function in male and female collared lemmings (*Dicrostonyx groenlandicus*). *J. Reprod. Fert.* 46, 323–329.
- Hoffmann, K. (1978) Effects of short photoperiods on puberty, growth and moult in the Djungarian hamster (*Phodopus sungorus*). *J. Reprod. Fert.* 54, 29–35.
- Hutchison, J.B. (1978) Hypothalamic regulation of male sexual responsiveness to androgen. In *Biological Determinants of Sexual Behaviour*, pp. 277–317. Ed. J. B. Hutchison. John Wiley & Sons, Chichester.
- Illius, A.W., Haynes, N.B., Purvis, K. & Lamming, G.E. (1976) Plasma concentrations of testosterone in the developing ram in different social environments. *J. Reprod. Fert.* 48, 17–24.
- Lincoln, G.A. & Davidson, W. (1977) The relationship between sexual and aggressive behaviour and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. *J. Reprod. Fert.* 49, 267–276.
- Lincoln, G.A., Guinness, F. & Short, R.V. (1972) The way in which testosterone controls the social and sexual behaviour of the red deer stag. *Horm. Behav.* 3, 375–396.
- Lynch, S.S. & Shirley, A. (1975) Production of specific antisera to follicle-stimulating hormone and other hormones. *J. Endocr.* 65, 127–132.
- Morin, L.P. & Zucker, I. (1978) Photoperiodic regulation of copulatory behaviour in the male hamster. *J. Endocr.* 77, 249–258.

- Ortavant, R., Pelletier, J., Ravault, J.P. & Thimonier, J. (1978) Annual cyclic variations in prolactin in sheep. In *Environmental Endocrinology*, pp. 75–78. Eds I. Assenmacher & D. S. Farner. Springer-Verlag, Berlin.
- Pretorius, P.S. (1967) Libido and mating dexterity in rams reared and left in isolation from ewes. *Proc. S. Afr. Soc. Anim. Prod.* **6**, 208–212.
- Purvis, K., Illius, A.W. & Haynes, N.B. (1974) Plasma testosterone concentrations in the ram. *J. Endocr.* **61**, 241–253.
- Raeside, J.J. & McDonald, M.F. (1959) Seasonal changes in the oestrous response by the ovariectomized ewe to progesterone and oestrogen. *Nature, Lond.* **184**, 458–459.
- Ravault, J.P. (1976) Prolactin in the ram: seasonal variations in the concentration of blood plasma from birth until three years old. *Acta endocr., Copenh.* **83**, 720–725.
- Reiter, R.J., Sorrentino, S. & Hoffman, R.A. (1970) Early photoperiodic conditions and pineal anti-gonadal function in male hamsters. *Int. J. Fert.* **15**, 163–170.
- Robinson, T.J. (1954) The necessity for progesterone with estrogen for the induction of recurrent estrus in the ovariectomized ewe. *Endocrinology* **55**, 403–408.
- Snedecor, G.W. (1952) Query 92. *Biometrics* **8**, 85–86.
- Steel, E. & Hinde, R.A. (1972) The influence of photoperiod on oestrogenic induction of nest building in canaries. *J. Endocr.* **55**, 265–278.
- Steel, E. & Hinde, R.A. (1976) Effect of a skeleton photoperiod on the daylength-dependent response to oestrogen in canaries (*Serinus canarius*). *J. Endocr.* **70**, 247–254.
- Thorner, M.O. & Besser, G.M. (1977) Hyperprolactinaemia and gonadal function: results of bromocriptine treatment. In *Prolactin and Human Reproduction*, pp. 285–302. Eds P. G. Crosignani & C. Robyn. Academic Press, London.
- Tucker, H.A. & Wetteman, R.P. (1976) Effects of ambient temperature and relative humidity on serum prolactin and growth hormone in heifers. *Proc. Soc. exp. Biol. Med.* **151**, 623–628.
- Webb, R., Lammie, G.E., Haynes, N.B. & Foxcroft, G.R. (1980) Plasma progesterone and gonadotrophin concentrations and ovarian activity in post-partum dairy cows. *J. Reprod. Fert.* **59**, 133–143.
- Zenchak, J.J. (1977) *Early socialisation effects on adult ram sexual behaviour*. Ph.D. thesis, West Virginia University.

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