Journal of Reproduction and Fertility (1994) **100**, 607–611

Influence of prenatal photoperiod on postnatal reproductive development in male red deer (*Cervus elaphus*)

C. L. Adam, C. E. Kyle and P. Young

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK

Male red deer calves, whose mothers had been kept for the last 14 weeks of gestation in long days (18 h light:6 h dark) (group L, $n = 7$) or short days (6 h light:18 h dark) (group S, $n = 5$), were kept in constant intermediate daylength (12 h light:12 h dark) from birth to 75 weeks of age. Both groups showed the same live-weight gain. Mean plasma LH concentrations were higher in group L than in group S from birth to 20 weeks of age (averaging 1.55 versus 0.48 ng ml^{-1}, $P < 0.001$), from 21 to 45 weeks (1.65 versus 1.32 ng ml^{-1}, $P < 0.05$) and from 46 to 50 weeks (1.84 versus 1.27 ng ml^{-1}, $P < 0.001$); thereafter, there was no significant difference between the groups (1.81 ng ml^{-1}). Mean concentration of plasma testosterone was relatively low from birth to 30 weeks (averaging 0.38 and 0.27 ng ml^{-1} ($P < 0.05$) in groups L and S, respectively), but thereafter increased to a maximum which was greater (2.78 versus 1.46 ng ml^{-1}, $P < 0.01$), and occurred earlier (47 versus 68 weeks of age, $P < 0.001$) and at lower body weight (82 versus 96 kg, $P < 0.01$) in group L compared with group S. Growth of antlers started in both groups at 25 weeks, but they hardened earlier in group L than in group S (42 versus 47 weeks of age, $P < 0.05$). These results provide evidence that in male red deer postnatal photoperiodic change is not required to trigger puberty, that prenatal photoperiodic history influences postnatal reproductive development and that the timing of reproductive maturation in deer raised on 12 h light:12 h dark is advanced by long days experienced prenatally.

Introduction

Short-day breeders normally achieve puberty in decreasing daylength provided a threshold body size has been attained. In the summer-born red deer this does not occur until the second autumn of life at about 16 months of age (Lincoln, 1971a; Guinness *et al.*, 1971). However, with radically altered birth dates, the threshold live weight may be achieved earlier and puberty expressed in the first autumn (8–9 months, Adam *et al.*, 1992a). Improved nutritional status without altered birth dates may also accelerate attainment of pubertal live weight but at a time when natural photoperiod is inhibitory in the spring/summer; puberty may then be advanced by 6 months by administration of melatonin to mimic short daylengths, provided that this is preceded by exposure to artificial long daylengths (Adam *et al.*, 1989). Postnatal photoperiodic history is therefore important for the red deer calf, as in the lamb (Yellon and Foster, 1985). However, prenatal photoperiod has also been shown to influence reproductive maturation in other mammals (e.g. meadow voles: Lee *et al.*, 1987; Djungarian hamsters: Stetson *et al.*, 1986). An earlier report from our group (Adam *et al.*, 1992b) provided evidence that red deer fetuses respond to photoperiodic information thereby acquiring a photoperiodic history *in utero* that influences postnatal prolactin secretion for up to 14 weeks of age. This paper reports the subsequent reproductive development, in terms of antler development and circulating concentrations of LH and testosterone, of these same male calves kept in constant intermediate daylength from birth (12 h light:12 h dark) following long (18 h light:6 h dark) or short (6 h light:18 h dark) daylength prenatally. A preliminary report has been published (Adam *et al.*, 1992c).

Revised manuscript received 6 August 1993.

Materials and Methods

Animals and treatments

Pregnant red deer hinds which subsequently gave birth in June to male (stag) offspring were individually penned in light-sealed rooms with controlled photoperiod from about 19 weeks of gestation until term (about 33 weeks). Fluorescent lights, regulated by electric time clocks, provided a daytime light intensity of about 200 lux. Seven hinds (group L mothers) were exposed to long daylength (18 h light:6 h dark) and five hinds (group S mothers) were exposed to short daylength (6 h light:18 h dark). From the day of birth, all twelve calves (singletons) were kept throughout in constant intermediate daylength (12 h light:12 h dark). They were suckled until 14 weeks of age then given, to appetite, a good quality balanced diet (comprising concentrates and chopped hay).

Measurements

Live weight was recorded weekly from birth to 75 weeks of age and visual assessments were made of antler development.

Blood samples were collected once a week by jugular venepuncture into heparinized vacutainer tubes, 1–2 h after lights-on, and plasma was stored at −20°C.

Radioimmunoassays

LH. Plasma LH concentrations were measured using a radioimmunoassay based on the method of Bernard *et al.* (1983). The antiserum (UCB Bioproducts, Shield Diagnostics Ltd, The Technology Park, Dundee) was raised in rabbits to bovine LH and the tracer was prepared by labelling bovine LH (UCB Bioproducts) with ^{125}I using the chloramine-T method of Greenwood *et al.* (1963) and subsequent purification on a 25 cm × 0.9 cm Sephadex G50 column (Pharmacia Biosystems Ltd, Milton Keynes). The same bovine LH preparation was used to prepare the standard curve and was calibrated against the First International Standard for bovine LH (EHC-bLH-1) (Loeber *et al.*, 1987). All solutions were made up in pH 7.5 phosphate buffer (0.05 mol phosphate l^{-1}, 0.1% w/v BSA and 0.1% NaN_3).

Samples (100 µl) were incubated for 16–20 h with 100 µl buffer, 100 µl antiserum solution (1:20 000 dilution) and 100 µl of tracer solution (20 000 d.p.m.). A precipitating second antibody solution made up of 5 µl donkey anti-rabbit serum and 0.5 µl normal rabbit serum (both from Scottish Antibody Production Unit, Law Hospital, Carluke, Lanarkshire) in 1000 µl buffer was added to each tube with mixing. Incubation was continued for a further 2–4 h. Incubations were carried out at 20°C. The tubes were centrifuged at 3000 *g* at 4°C for 20 min and the radioactivity of the bound fraction remaining in the tube after decanting the supernatant was measured on a Packard Cobra gamma counter. LH concentrations were calculated using Packard RiaSmart software.

Crossreactivity of the antiserum, as reported by the suppliers on their data sheet, was 0.5% with bovine FSH and 0.4% with bovine TSH. Serial dilutions of deer plasma demonstrated no deviation from parallelism with the standard curve. The mean sensitivity of the assay (determined as the hormone concentration at 2 SD from the binding of the zero standard) was 0.02 ng per tube (0.2 ng ml^{-1} sample). The inter- and intra-assay coefficients of variation were 11.6% ($n = 5$) and 1.5% ($n = 5$), respectively (at 3.6 ng ml^{-1}). The mean recovery of bLH standard added to sample was 110%.

Testosterone. Concentrations of plasma testosterone were measured after extraction using a radioimmunoassay based on the method of Corker and Davidson (1978). The antiserum was raised in rabbit against testosterone-3-*O*-carboxymethoxyloxime–BSA, as described by Rowe *et al.* (1974) and the tracer was [1,2,6,7-^{3}H]testosterone (Amersham International, Amersham). Testosterone (Sigma Chemical Co. Ltd, Poole, Dorset) was used to prepare the standard curve. All solutions were made up in phosphate–gelatin buffer (0.1 mol phosphate l^{-1}, 0.1% w/v gelatin and 0.1% NaN_3) pH 7.0.

Samples (100 µl) were shaken for 20 min in a sealed tube with 100 µl of phosphate–gelatin buffer, 200 µl of carbonate buffer (0.85 mol l^{-1}, pH 10.5) and 2.5 ml diethyl ether (Normapur: Prolabo, Manchester). The supernatant ether layer was decanted into a polypropylene assay tube after briefly centrifuging the extraction tube and freezing the aqueous layer by immersion in a bath at −40°C. The ether was evaporated from the assay tube under a stream of air on a dry block at 40°C and 100 µl of assay buffer was then added to the extract.

Antiserum (100 µl of 1:20 000 dilution) was added to the sample extract or standard, followed by 100 µl of tracer (8 nCi), and tubes were incubated for 16–20 h at 4°C. Cold charcoal suspension (700 µl of a mixture of 0.625 g Norit GSX and 0.0625 g Dextran T70 in 1 l of 0.01 mol phosphate–saline buffer l^{-1}) was added rapidly to each tube and incubation was continued for a further 15 min at 4°C. The tubes were centrifuged at 3000 *g* at 4°C for 10 min and the bound fraction in the supernatant was decanted into scintillation vials. Scintillant (2.5 ml of Packard Ultima Gold; Canberra-Packard Ltd, Pangbourne, Berkshire) was added and the radioactivity of vials measured on a Packard Minaxi liquid scintillation counter.

Extraction efficiency was determined for each batch of samples by preparing two tubes with 100 µl of tracer solution instead of the phosphate–gelatin buffer and comparing the amount of radioactivity recovered after extraction with that originally added. The values obtained ranged from 87 to 95%. Crossreactivity of the antiserum was measured as 74% with 5α-dihydrotestosterone, 1.6% with androstenedione, 0.1% with androsterone and less than 0.01% with other steroids. Serial dilutions of deer plasma demonstrated no deviation from parallelism with the standard curve. The mean sensitivity of the assay (determined as the hormone concentration at 2 SD from the binding of the zero standard) was 0.004 ng per tube (0.04 ng ml^{-1} sample) and the inter- and intra-assay coefficients of variation were 9.8% (1.28 ng ml^{-1}, $n = 9$) and 8.5% (0.55 ng ml^{-1}, $n = 9$), respectively. The mean recovery of testosterone added to samples before extraction was 92.0%.

Statistical analysis

Results are presented as group means with SEM or SED. Live weights and ages at antler hardening were compared by Student's *t* test. LH and testosterone data were subjected to analysis of variance using GENSTAT 5 whereby, to minimize the effects of large variabilities, group means were compared after averaging individual values over 5 weeks. Maximum testosterone values (identified from the individual 5-week averages) and the age at which they occurred were also compared as indices of reproductive maturation.

Results

Both groups of stags showed similar mean live weight and live-weight gain throughout the study (Fig. 1).

Mean concentrations of plasma LH (Fig. 2) were significantly higher in group L than in group S from birth to 20 weeks of age ($P < 0.001$), from 21 to 45 weeks of age ($P < 0.05$) and from 46 to 50 weeks of age ($P < 0.001$); thereafter, there was no significant difference between the groups.

Weekly mean concentrations of plasma testosterone were relatively low from birth but increased markedly at about 41 and 64 weeks, respectively, in groups L and S (Fig. 3). Analysis of values averaged over 5 weeks revealed that mean concentrations were significantly higher ($P < 0.05$) in group L than in group S from 1 to 30 weeks of age and from 46 to 55 weeks

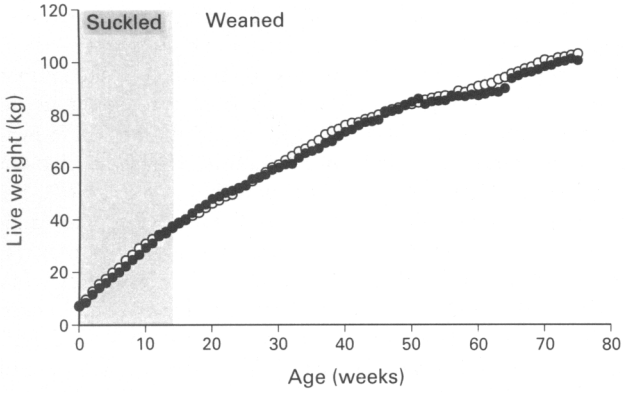

Fig. 1. Mean live weight of stags kept in constant intermediate daylength (12 h light:12 h dark) whose mothers had been kept for the last 14 weeks of gestation in long days (○, $n = 7$) or short days (●, $n = 5$) (SED = 4.0).

of age, and that the maximum testosterone concentration was greater (2.78 versus 1.46 ng ml^{-1}, SED 0.419, $P < 0.01$) and occurred earlier (47 (45–49) versus 68 (66–70) weeks of age, SED 3.3, $P < 0.001$) and at a lower live weight (82 versus 96 kg, SED 4.0, $P < 0.01$) in group L compared with group S.

Antler growth was initiated at 25 ± 0.9 weeks in both groups (Fig. 3) but antlers hardened earlier for group L (42 ± 1.4 weeks of age) than for group S (47 ± 1.2 weeks of age; $P < 0.05$).

Discussion

Both groups of young stags showed similar live-weight gain in the constant intermediate daylength irrespective of their prenatal photoperiodic experience; any differences in the timing of reproductive development were therefore not due to differences in body weight at any given age.

In our earlier paper (Adam *et al.*, 1992b), we reported differences in prolactin secretion between these groups of stags, and these differences persisted until at least 14 weeks of age. The data reported here provide evidence for a longer term influence of prenatal photoperiod on the secretory patterns of pituitary and gonadal hormones during the period up to reproductive maturation.

Puberty in the male cannot be measured with the same degree of accuracy as that in the female since many definitions are used and it is generally regarded as a phase of development rather than a sudden event (Dýrmundsson, 1973). The culmination of pubertal development in stags is shown by increased testis size, increased testosterone secretion and spermatogenesis (Lincoln, 1971a). These changes are similar to those shown annually in the adult breeding season (Lincoln, 1971b) and they are preceded by high circulating concentrations of LH and accompanied by maximal circulating testosterone (Lincoln and Kay, 1979). For the purposes of the present study, 'puberty', or the completion of reproductive maturation, was regarded as the time at which the mature endocrine function of the testes became evident from a marked increase in plasma testosterone to maximum concentrations. This event apparently occurred some 21 weeks earlier for the stags exposed prenatally to long rather than to short days, despite identical photoperiodic conditions and live-weight gains postnatally. Interestingly the latter stags showed a similar delay in activating pituitary LH secretion and developing appreciable plasma concentrations of this hormone. Although it is generally accepted that testosterone is released largely in response to LH stimulation, the pattern of circulating concentrations of LH may not alone have been responsible for the changes in testosterone secretion, as the testes also develop heightened responsiveness to LH during the breeding season (Lincoln and Kay, 1979). Thus the testes of stags exposed prenatally to long as opposed to short days may have developed this increased sensitivity to LH at an earlier age. Furthermore, high LH secretion may have accounted for the higher baseline plasma testosterone values from birth to 30 weeks of age and a combination of high LH and heightened testicular sensitivity may have accounted for the greater maximum testosterone concentration shown by stags exposed prenatally to long as opposed to short days.

The annual cycle of antler growth provides a unique outward sign of reproductive quiescence (growing or velvet antler) or activation (hard antler) in the male deer (Lincoln, 1971a, b) and is thought to be primarily controlled by testosterone (Suttie *et al.*, 1984). In the study reported here growth of the first set of antlers began when the appropriate body weight was reached, as expected (Suttie and Kay, 1983), and thus at the same age irrespective of prenatal photoperiod. The fully grown antlers then hardened earlier for stags exposed to long rather than short days prenatally, in agreement with their earlier achievement of maximum testosterone secretion, although the difference in timing between the groups was considerably less for the former event. Thus, although the antlers of the stags exposed to long days prenatally hardened around the time that testosterone secretion was increasing to its maximum, paradoxically those of stags exposed to short days prenatally hardened several weeks in advance of the maximum testosterone values detected by the relatively infrequent sampling protocol. However, earlier changes of significance in the episodic testosterone secretory profile cannot be ruled out, as baseline values became more variable from around 30 weeks of age. Nonetheless the data suggest that sustained maximal testosterone concentrations are not the sole trigger for antler hardening, but that very slight increases in the episodic secretory pattern, perhaps allied to differences in developing sensitivity to testosterone, may play a role.

These results demonstrated that in constant postnatal conditions of 12 h light:12 h dark, and with identical body growth curves, exposure to long days for the last 14 prenatal weeks induced precocious reproductive development in stags (at 10 months of age), whereas exposure to short days for the same period did not (with puberty occurring at about the normal age of 16 months). If puberty in the male is an inevitable phase of growth and development, perhaps the former treatment triggered *in utero* an earlier start to a progressive reproductive maturation process, or indeed the latter treatment delayed it. In this respect, the photoperiodic change in mid-pregnancy (from natural short winter daylengths) may have been more critical than the change at birth; certainly the fetus has receptors for melatonin and may thus receive photoperiodic information from maternal melatonin before this time (Helliwell *et al.*, 1991). It is likely that the influence of prenatal photoperiod may be

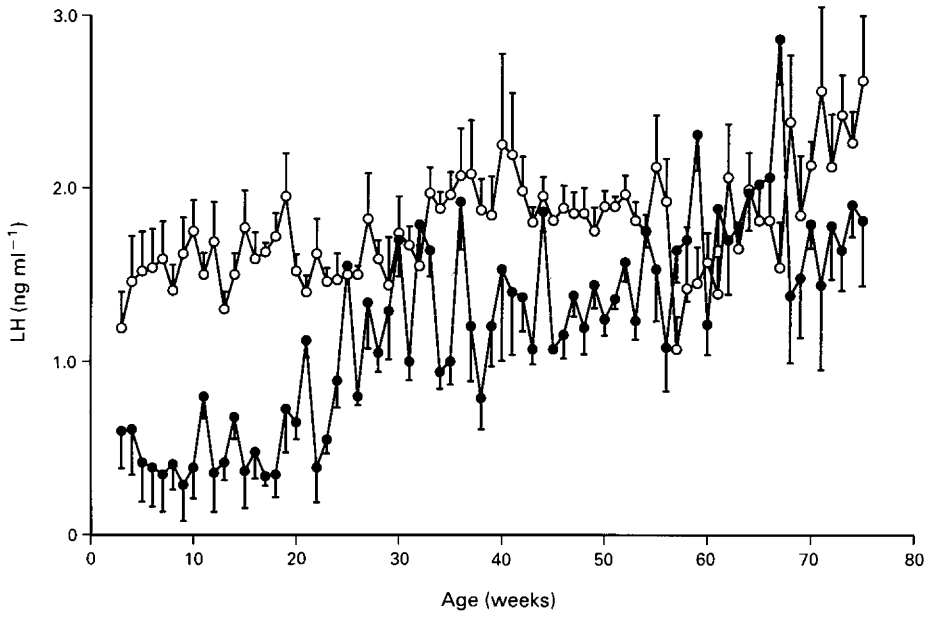

Fig. 2. Plasma concentrations of LH (mean ± SEM) in stags kept in constant intermediate daylength (12 h light:12 h dark) whose mothers had been kept for the last 14 weeks of gestation in long days (○, $n = 7$) or short days (●, $n = 5$).

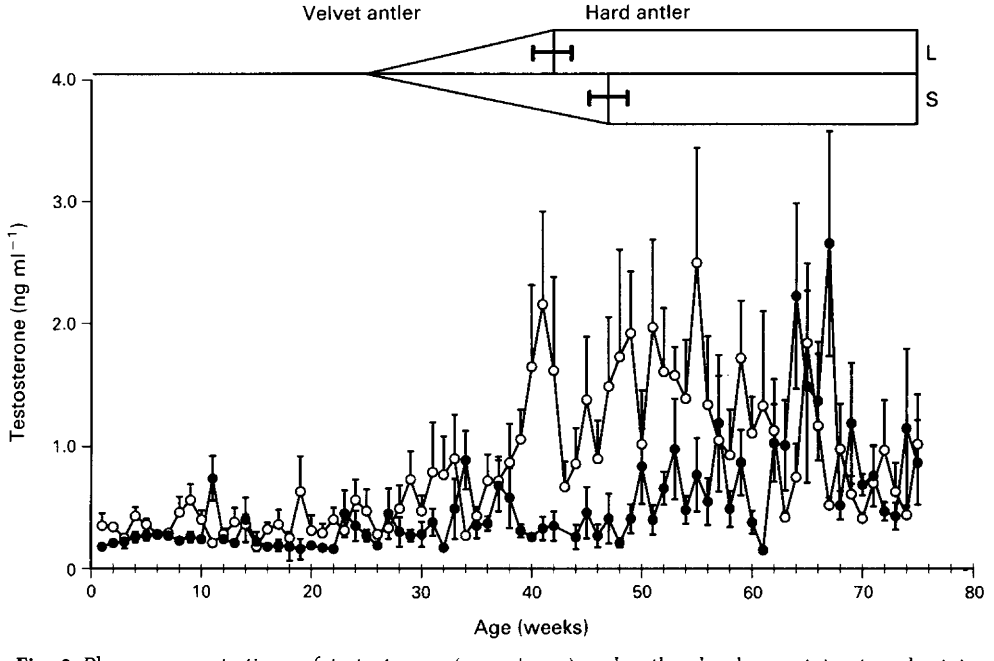

Fig. 3. Plasma concentrations of testosterone (mean ± SEM) and antler development in stags kept in constant intermediate daylength (12 h light:12 h dark) whose mothers had been kept for the last 14 weeks of gestation in long days (L, ○, $n = 7$) or short days (S, ●, $n = 5$).

modified and the rate of reproductive maturation altered by overriding postnatal photoperiodic changes not experienced in the present trial since, for example, puberty is delayed in autumn-born male goats in natural photoperiod by long days experience *in utero* (Deveson *et al.*, 1992). Normally stags born in the summer will have experienced increasing long days prenatally and yet their pubertal breeding activity is delayed to 16 months by the natural seasonal pattern of postnatal photoperiod, whereas such stags kept from birth in constant 12 h light:12 h dark achieve precocious puberty at 10 months (C.L. Adam, C.E. Kyle and P. Young, unpublished) much as those given constant long days prenatally. Furthermore, the present results provide evidence that, in the absence of photoperiodic change, the culmination of reproductive development in male

deer demonstrated by antler hardening and high testosterone secretion is not simply expressed upon attainment of a threshold age or live weight since the stags exposed to short days prenatally were both older and heavier when this occurred than those exposed to long days prenatally. This finding suggests a more subtle interplay between photoperiod, photoperiodic history and as yet unidentified signals of metabolic body size in their influence on the maturing hypothalamo–pituitary–gonadal axis.

The authors thank D. J. Hirst of Scottish Agricultural Statistics Service for help with statistical analysis, A. Thomson and staff of the Duthie Experimental Farm for the excellent daily care of the animals, and T. Atkinson for advice on radioimmunoassay methods. The Rowett Research Institute is financed by the Scottish Office Agriculture and Fisheries Department.

References

Adam CL, Moir CE and Shiach P (1989) Effect of melatonin on puberty in female red deer *Journal of Reproduction and Fertility Abstract Series* **3** Abstract 110

Adam CL, Kyle CE and Young P (1992a) Growth and reproductive development of red deer calves (*Cervus elaphus*) born out-of-season *Animal Production* **55** 265–270

Adam CL, Kyle CE and Young P (1992b) Influence of prenatal photoperiod on postnatal prolactin secretion in red deer (*Cervus elaphus*) *Journal of Reproduction and Fertility* **95** 959–964

Adam CL, Kyle CE and Young P (1992c) Prenatal photoperiod influences timing of puberty in red deer *Journal of Reproduction and Fertility Abstract Series* **9** Abstract 5

Bernard C, Valet JP, Béland R and Lambert RD (1983) Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH *Journal of Reproduction and Fertility* **68** 425–430

Corker CS and Davidson DW (1978) A radioimmunoassay for testosterone in various biological fluids without the use of chromatography *Journal of Steroid Biochemistry* **9** 373–374

Deveson S, Forsyth IA and Arendt J (1992) Retardation of pubertal development by prenatal long days in goat kids born in autumn *Journal of Reproduction and Fertility* **95** 629–637

Dýrmundsson OR (1973) Puberty and early reproductive performance in sheep II. Ram lambs *Animal Breeding Abstracts* **41** 419–427

Greenwood FC, Hunter WM and Glover JS (1963) The preparation of ^{131}I labelled human growth hormone of high specific radioactivity *Biochemical Journal* **89** 114–123

Guinness F, Lincoln GA and Short RV (1971) The reproductive cycle of the female red deer, *Cervus elaphus* L *Journal of Reproduction and Fertility* **27** 427–438

Helliwell RJA, Adam CL, Hannah LT, Kyle CE and Williams LM (1991) The distribution of central 2-[^{125}I]-iodomelatonin binding sites in the adult and fetal red deer (*Cervus elaphus*) *Journal of Reproduction and Fertility Abstract Series* **8** Abstract 117

Lee TM, Smale L, Zucker I and Dark J (1987) Influence of daylength experienced by dams on post-natal development of young meadow voles (*Microtus pennsylvanicus*) *Journal of Reproduction and Fertility* **81** 337–342

Lincoln GA (1971a) Puberty in a seasonally breeding male, the red deer stag (*Cervus elaphus* L) *Journal of Reproduction and Fertility* **25** 41–54

Lincoln GA (1971b) The seasonal reproductive changes in the adult red deer stag (*Cervus elaphus* L) *Journal of Zoology* **163** 105–123

Lincoln GA and Kay RNB (1979) Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags (*Cervus elaphus*) *Journal of Reproduction and Fertility* **55** 75–80

Loeber JG, van de Wiel DFM and Gaines Das RE (1987) An international collaborative study on the First International Standard of bovine luteinizing hormone for immunoassay *Journal of Reproduction and Fertility* **79** 145–152

Rowe PH, Lincoln GA, Racey PA, Lehane J, Stephenson MJ, Shenton JC and Glover TD (1974) Temporal variations of testosterone levels in peripheral blood plasma of men *Journal of Endocrinology* **61** 63–73

Stetson MH, Elliott JA and Goldman BD (1986) Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters (*Phodopus sungorus sungorus*) *Biology of Reproduction* **34** 664–669

Suttie JM and Kay RNB (1983) Influence of nutrition and photoperiod on the growth of antlers of young red deer. In *Antler Development in Cervidae* pp 61–71 Ed. RD Brown. Caesar Kleberg Wildlife Research Institute, Kingsville, Texas

Suttie JM, Lincoln GA and Kay RNB (1984) Endocrine control of antler growth in red deer stags *Journal of Reproduction and Fertility* **71** 7–15

Yellon SM and Foster DL (1985) Alternate photoperiods time puberty in the female lamb *Endocrinology* **116** 2090–2097