Effect of age and time of day on the timing of the surge in luteinizing hormone, behavioural oestrus and mating in red deer hinds (*Cervus elaphus*)

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Summary. The oestrous cycles of fourteen red deer hinds (six yearling; eight more than 2 years old) were synchronized during the early breeding season by removal of a progesterone-containing intravaginal device and blood samples were taken at intervals of 3 h commencing 13 or 25 h later and continued for 54 h. The controlled internal drug release devices (CIDRs) were removed at 08:00 h (group 1; three yearlings and four adults) or 12 h later at 20:00 h (group 2; three yearlings and four adults). There was no significant effect of time of removal of CIDR on the interval to the onset of oestrus (group 1, 34.5 ± 4.05 h; group 2, 42.14 ± 7.8 h) on the time of peak concentration (group 1, 41.81 ± 5.69 h; group 2, 41.71 ± 7.81 h) or on duration of the luteinizing hormone (LH) surge (group 1, 15.00 ± 0.95 h; group 2, 14.57 ± 0.78 h). The six yearling animals exhibited oestrus and LH surge significantly later than the adults (55 ± 4.2 versus 32 ± 6.3 h for the LH surge for yearling and adult females, respectively). In a further experiment, 20 hinds were synchronized during the breeding season by removal of CIDR at two times of day 12 h apart and placed with a stag. Mating took place at a mean time of 42.1 ± 2.4 h and 37.0 ± 1.3 h later in the two groups. There was no significant effect of time of removal of CIDR upon time to onset of oestrus. These data indicate that the timing of ovulation and onset of sexual behaviour in this species is unlikely to be controlled by a circadian gate. The significant delay in onset of oestrus in young animals suggests that either social or developmental factors may play an important role in timing ovulation in red deer following the decline of progesterone.

Keywords: red deer; oestrus; luteinizing hormone surge; circadian rhythm

Introduction

Artificial insemination (AI) protocols have been well established for a few selected domesticated species. The development of deer as an alternative farmed species over the past 15 years has led to an interest in the use of AI as a potential means of rapid genetic improvement (Asher *et al.*, 1988a, 1990) or creation of economically useful new hybrids (Asher *et al.*, 1988b). Detailed information on the timing of the luteinizing hormone (LH) surge and behavioural oestrus is available only for two species of deer, fallow deer (Asher *et al.*, 1988a) and Père David's deer (Loudon *et al.*, 1990). For red deer, the most common farmed deer species, there is no information.

In a number of mammals, principally rodents, the LH surge is controlled by a circadian gate mediated by the suprachiasmatic nuclei (Coen & MacKinnon, 1980). Such gating confines the surge and subsequent ovulation to a specific time of day (Stetson & Gibson, 1977; Swann & Turek, 1982, 1985). The possibility that the LH surge may be gated by a circadian mechanism has not to

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our knowledge been systematically investigated in ruminants. Such a possibility would seriously compromise attempts to time ovulation by synchronization protocols intended to coincide the timing of the LH surge and behavioural oestrus between individuals, applied at different times of the day. In this study, we compared the timing of the LH surge and onset of behavioural oestrus in postpubertal yearlings (16 months old) with that in mature adult animals (>2 years old) which were synchronized at two times of day 12 h apart by withdrawal of an intravaginal progesterone containing device (controlled internal drug release device: CIDR). In addition, we compared the natural mating interval in another two groups of hinds put to the stag after removal of CIDR at the same two time intervals.

Materials and Methods

Experiment 1

Animals. Fourteen red deer hinds were maintained at pasture at the Institute of Zoology's facility at Whipsnade, Bedfordshire (52°N). Blood samples were collected for the measurement of progesterone from 31 August to confirm a normal onset to the breeding season. During the study, animals were housed indoors to facilitate handling under natural photoperiods. Six yearlings and eight mature hinds were treated with an intravaginal CIDR on 25 October 1990 (CIDR Type-G, 0.3 g progesterone; Agricultural Division, CHH Plastic Products Group Ltd, Hamilton, NZ). One week later, all animals were treated with 20 µg of a prostaglandin analogue, cloprostenol (Estrumate: Coopers Animal Health, Crewe, Cheshire, UK). On 5 November, CIDRs were withdrawn at 08:00 h (group 1: three yearlings, four mature hinds) and at 20:00 h (group 2: three yearlings, four mature hinds).

Blood sampling and behaviour. From 09:00 h on 6 November blood samples were collected at intervals of 3 h for a period of 54 h from all animals. At the time of each blood sample, animals were assessed for behavioural oestrus as previously described (Curlewis *et al.*, 1988). Thus, blood sampling covered the periods 25–79 h and 13–67 h following CIDR withdrawal for groups 1 and 2, respectively.

Experiment 2

Animals. Twenty mature hinds (more than 2 years old) were fitted with CIDRs on 15 October 1990. Nine days later, hinds were treated with 0.75 ml Estrumate and CIDRs were withdrawn on 29 October. CIDRs were withdrawn at 08:00 h for group 1 ($n = 10$) and at 20:00 h for group 2 ($n = 10$). At the time of withdrawal of CIDRs, animals were fitted with large numbered collars and large numbers were painted onto each flank and the rump to aid identification in the dark. The animals were then placed with a stag in one of two large indoor pens of 3 m × 28 m (ten hinds per stag). From this time, the two stags and 20 hinds were observed continuously for 48 h from an elevated hide and all matings recorded. The building was naturally illuminated but four red neon strip lights were fitted to the roof to aid visibility at night (<1 lx at pen level).

Hormone measurement. Progesterone was measured in a direct radioimmunoassay as described by McLeod *et al.* (1991). Within this study, the limit of sensitivity of the assay was 0.8 ng ml⁻¹ and intra-assay coefficients of variation were 7.6% and 5.14% at 0.8 and 3.7 ng ml⁻¹, respectively. Interassay coefficients of variation were 6.9% and 7.3% at 0.8 and 1.7 ng ml⁻¹, respectively. LH was determined in an assay as described by Loudon *et al.* (1990). Within this study, the limit of sensitivity of the assay was 0.2 ng NIH-LH-S24 equivalents ml⁻¹ plasma, and the intra- and interassay coefficients of variation were 8.9 and 9.4%, respectively. The beginning and end of the LH surges of individual hinds were defined as the first and last sampling times at which LH concentrations exceeded mean basal values by twice the standard deviation. The onset of behavioural oestrus was recorded as the first time at which hinds responded to dorsal pressure with lordosis. A two-way ANOVA was used to test for the effects of time and age.

Results

Experiment 1

All animals were undergoing repeated oestrous cycles at the beginning of the study as assessed by measures of progesterone concentrations in excess of 0.8 ng ml⁻¹ for four or more consecutive samples (data not shown). Six out of seven hinds in group 1 and all hinds in group 2 exhibited an LH surge and behavioural oestrus following CIDR withdrawal. One yearling in group 1 did not exhibit either, although she had commenced ovarian cyclicity before the study. There was no significant difference between the groups with respect to the timing of the LH surge (group 1,

41.81 ± 5.69 h; group 2, 41.71 ± 5.55 h; $F_{1,9} = 0.04$, $P > 0.5$) and onset of oestrous behaviour (group 1, 34.5 ± 4.05 h; group 2, 42.14 ± 5.55 h; $F_{1,9} = 32.65$, $P > 0.05$) relative to CIDR withdrawal. Accordingly, the data from groups 1 and 2 were pooled and analysed with respect to the time of CIDR withdrawal. There was a highly significant difference between yearlings and mature hinds with respect to both the time of onset of the LH surge ($F_{1,9} = 37.62$, $P < 0.01$) and behavioural oestrus ($F_{1,9} = 32.65$, $P < 0.01$) relative to CIDR withdrawal (Fig. 1). In mature animals, the peak of the LH surge occurred at 32.3 ± 2.63 h; in yearlings, the surge occurred later at 55.0 ± 1.9 h. Within each group, the LH surge was closely associated with the onset of behavioural oestrus (Fig. 2) and this occurred significantly earlier in yearlings ($P < 0.001$). Thus, in all recorded parameters the yearling animals undergoing their first breeding season were delayed by approximately 24 h.

Fig. 1. Models of the luteinizing hormone (LH) surge in mature (—) and yearling (---) hinds following controlled internal drug release device (CIDR) withdrawal representing mean peak and basal LH concentrations and mean times for the onset of the peak and end of the LH surge ($\pm$ SEM). The horizontal bars indicate the mean onset and end of behavioural oestrus for the mature and yearling groups.

Since there was no effect of time of day of withdrawal of CIDR on the timing of the LH surge and behavioural oestrus, data for both groups have been pooled and normalized with respect to the time of the LH peak (Fig. 3). Pre-surge baseline and peak surge LH concentrations were similar in both age groups (mature: 2.58 ± 1.33 ng ml $^{-1}$ and 51.87 ± 5.78 ng ml $^{-1}$; yearlings: 3.40 ± 1.27 ng ml $^{-1}$ and 42.50 ± 5.22 ng ml $^{-1}$). The mean duration of the LH surge (as assessed by the criteria of Loudon *et al.*, 1990) was 15.5 ± 1.2 h with a mean peak of 38 ± 2.1 ng ml $^{-1}$. There was no significant difference for these parameters between yearling and adult animals (15.1 h and 15.8 h for yearling and adult animals, respectively).

Experiment 2

There was no significant difference between groups 1 and 2 with respect to the time of mating following CIDR withdrawal (group 1, 42.1 ± 2.4 h; group 2, 37.1 ± 1.3 h). Eight out of ten and nine out of ten hinds mated in groups 1 and 2, respectively: eight hinds were mated once, six mated twice and three hinds on three occasions. In hinds that mated more than once, the average interval between mating was 24 ± 2 min (Fig. 4). All matings were fertile and viable calves were born 233.6 ± 1.7 days later, the normal gestation period for this species.

Fig. 2. Association of the onset of behavioural oestrus and the LH surge peak for mature (●) and yearling (○) hinds.

Fig. 3. Mean changes ($\pm$ SEM) in luteinizing hormone (LH) concentration for all of the hinds normalized around the peak of the LH surge.

Discussion

The results of Expts 1 and 2 indicate that it is extremely unlikely that there is any photoperiodic constraint on the timing of the LH surge, behavioural oestrus and mating in this seasonally breeding ruminant. It is therefore unlikely that there is a role for the circadian system in controlling ovulation in red deer hinds. The study does provide a further indication of the very close temporal correlation between the timing of the LH surge and behavioural oestrus as previously reported for

Fig. 4. Time of mating for hinds in (a) group 1 and (b) group 2 with respect to time of withdrawal of controlled internal drug release device (CIDR) and time of day ($\square$) and night ($\boxtimes$). Vertical lines represent actual mating times. Histograms indicate the number of hinds mated in intervals of 3 h.

other species of deer (fallow: Asher *et al.*, 1988a; Père David's deer: Loudon *et al.*, 1990). It is clear that oestrous behaviour provides a convenient and sensitive indication for the timing of the LH surge (and probably ovulation) within individual animals.

The differences between yearlings and adult animals were unexpected and may offer an explanation for the general failure of AI in red deer yearlings (C. M. Argo, W. Holt, P. Goddard, J. Milne and A.S.I. Loudon, unpublished data). It may also explain the wide range of oestrus dates observed in a study of Père David's deer reported by Curlewis *et al.* (1988). In this study all animals were cycling and post-pubertal at the beginning of the experiment. There are several possible explanations for the delay in the onset of the LH surge in yearling animals. The dynamics of follicular development may be different in young animals inducing a delay in oestrogen feedback and hence induction of the surge. Resolution of this issue would require further investigations of ovarian physiology. A second possibility is that there may be a social or developmental factor delaying the neuronal response to oestrogen feedback in young animals, perhaps when they are co-housed with adult animals.

The duration of the LH surge in red deer is similar to that of the related Père David's deer, although maximum LH concentrations are approximately three times higher as measured in the same radioimmunoassay (Loudon *et al.*, 1990). Asher *et al.* (1988a) reported that, in fallow deer, the duration of the LH surge extended over approximately 15 h. Thus, in these three species of deer, the LH surge exhibits a similar dynamic change following progesterone decline. In this respect, deer would appear to exhibit a longer LH surge than those reported for cattle (Rahe *et al.*, 1980; Peters, 1984; Walters & Schallenberger, 1984) or goats (BonDurant, 1981) but similar to that for sheep (McLeod *et al.*, 1982, 1983; McLeod & Haresign, 1984).

The animals in this study (Expt 2) exhibited impressive fecundity since all 17 of the mated hinds successfully conceived and carried calves to term (in one case, stags mated up to 17 times in 27 h).

Such high fecundity is not commonly observed in domesticated species (Lamming *et al.*, 1989). It is possible that the very close association of timing of sexual behaviour and the LH surge contributes to such a high fecundity in a species where there are strong evolutionary pressures for an optimal time of birth in a seasonal environment (Guinness *et al.*, 1978).

We thank A. Hartley, C. Layram, S. Redrobe and A. Cutler for help with blood sampling and observations of animals, and D. Thomas and Y. Fasawe for assistance with radioimmunoassays. The work was supported in part by a joint programme grant from the MRC and AFRC and by a grant from the Ministry of Agriculture, Food and Fisheries.

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Revised manuscript received 27 April 1992