Induction of oestrus in the ovariectomised red deer with exogenous progesterone and oestradiol benzoate

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ABSTRACT

Exogenous progesterone and oestradiol benzoate (OB) were used in long-term ovariectomised red deer to investigate a simple technique for induction of oestrus. Up to 12 days of intravaginal progesterone (CIDR-S; replaced at 4 day intervals) was followed 24 h later by a single intramuscular injection of OB. Continual observations (for behavioural oestrus) and blood sampling every 6 h (for plasma LH concentrations) were conducted over the subsequent 48-h period.

In Experiment 1 (December), 12 days of progesterone preceded 0, 1.0, 2.5 or 5.0 mg OB. Onset of oestrus (28-44 h post-OB; mean 33 h) was similar in all treated hinds. Plasma LH concentrations fell ($P<0.01$) after OB administration, followed 30 to 36 h later by a dramatic increase (to 6.4 ng/ml) indicative of a pre-ovulatory-like LH surge. Hinds receiving no OB exhibited 4% of all mounting interactions but showed no increases in LH concentration.

Experiment 2 (January) consisted of no treatments or 12 days of progesterone followed by 0, 0.1 or 1 mg OB resulted in suppression of plasma LH after OB. However, all other plasma LH and behaviour responses elicited were anomalous.

In Experiment 3 (February), 0, 4, 8, or 12 days of progesterone pretreatment were terminated 24 hours prior to administration of 1 mg OB. Plasma progesterone concentrations were elevated above 1 ng/ml during all progesterone treatments. Onset of oestrus was observed later ($P<0.5$) after 4 days of progesterone treatment (33.4 h; sem 1.2 h). Tonic plasma LH secretion was elevated ($P<0.01$) from Experiment 1 through to Experiment 3 but the plasma LH suppression in response to OB was similar in all 3 experiments. These results indicate that oestrus can be induced in the red deer hind by at least four days of intravaginal progesterone treatment followed by i.m. oestradiol benzoate administration 24 h after progesterone removal.

Keywords Red deer; ovariectomy; oestrus; progesterone; oestradiol benzoate; LH

INTRODUCTION

Red deer (*Cervus elaphus*) are seasonally polyoestrous, with the onset of the breeding season occurring in autumn. Oestrus lasts 12-24 hours and, in the absence of conception, recurs at 18-21 day intervals. Calving occurs in early summer after a 233-day gestation period (Guinness *et al.*, 1971; Kelly and Moore, 1977; Adam *et al.*, 1985).

In order to manipulate these reproductive patterns, exogenous progestagens are frequently used to induce early seasonal breeding (to take advantage of seasonal trends in farm pasture production) and to synchronise oestrus and ovulation (to facilitate breeding programmes such as artificial insemination). Progesterone-releasing devices, designed primarily for use in sheep, are an effective way of administering exogenous progestagens in red deer (Adam *et al.*, 1985; Jopson *et al.*, 1990). However, peripheral plasma progesterone concentrations resulting from some of these devices were not maintained at luteal phase levels in the hind (Jopson *et al.*, 1990) bringing into question the efficacy of these devices for oestrous control.

Inadequate exposure to progesterone prior to oestrus and ovulation has an adverse effect on a number of factors affecting fertility in the ewe and could also affect the time of onset of oestrus (Robinson, 1982). Progesterone also appears to play a role in potentiating oestrous behaviour when administered as a pre-treatment to oestradiol, but when given at the same time as oestradiol, progesterone inhibits oestrous behaviour (Robinson, 1959; Scaramuzzi *et al.*, 1971).

The objective of the present study was to develop a simple technique for inducing oestrus in ovariectomised red deer, by following ovariectomised sheep models (Robinson *et al.*, 1956; Robinson, 1959;

Scaramuzzi *et al.*, 1971), in order to quantify the requirements for progesterone and oestrogen.

Three experiments were conducted; the first two to define an appropriate dose of oestrogen (Experiments 1 and 2), and the third to establish the duration of progesterone pretreatment (Experiment 3), required to induce behavioural oestrus.

MATERIALS AND METHODS

Animals

An 8 year old red deer stag was run with 19 long term ovariectomised (see Jopson *et al.*, 1990) adult red deer hinds (mean live weight 105.5; sem 2.5 kg) in December 1988 for Experiment 1, and 18 hinds in January 1989 for Experiment 2 and again in February 1989 for Experiment 3.

Experiments 1 and 2: Effect of OB dose level on induction of oestrous behaviour.

In Experiment 1, all hinds were pre-treated for 12 days with intravaginal progesterone CIDR devices (CIDR [Controlled Internal Drug Release]-type S, 9% w/w progesterone; AHI Plastic Moulding Co; Hamilton, New Zealand). CIDR devices were renewed every 4 days to maintain elevated peripheral plasma progesterone concentrations. An i.m. injection of 0 (n=5), 1 (n=5), 2.5 (n=5) or 5 (n=4) mg OB (Intervet [Aust] Pty, NSW, Australia) was administered 24 h after final CIDR device withdrawal. The group was then observed for 48 h under field conditions during daylight, and artificial lighting indoors at night. The onset of oestrus was defined as the time when a hind first stood immobile when mounted by another hind or the stag.

Experiment 2 varied from Experiment 1 in that one group received neither progesterone pretreatment nor OB (n=5) while the other groups received 12 days (CIDR device renewed every 4 days) of progesterone followed 24 h later by 0 (n=4), 0.1 (n=5) or 1.0 (n=4) mg OB.

Blood samples (10 mls) were collected by jugular venepuncture while the hinds were restrained mechanically at CIDR device insertion (day 0), renewal (days 4 and 8) and removal (day 12), and every 6 h for 48 h after OB injection.

Plasma was recovered after centrifugation and stored frozen until analysed for progesterone and LH concentrations.

Experiment 3: Effect of duration of progesterone pretreatment on induction of oestrous behaviour.

Hinds were pre-treated with progesterone for either 0 (n=4), 4 (n=5), 8 (n=5) or 12 (n=4) days with CIDR device replacement at 4 day intervals. An i.m. injection of 1.0 mg OB was administered to all groups 24 h after final CIDR device removal. Blood samples were collected as for Experiment 1, but the observations were reduced to cover a 30-h period commencing 24 h after OB administration.

Analysis

Plasma progesterone concentrations were determined by solid phase ^{125}I radioimmunoassay as described by Jopson *et al.*, (1990) and plasma LH by a heterologous double antibody radioimmunoassay (Fisher *et al.*, 1989).

Analysis of variance and Student's t-test were used to examine the log transformed LH data and the interval from OB to the onset of oestrus was subjected to analysis of variance. All means are presented ± standard error of the mean (sem).

RESULTS

Experiment 1

All hinds treated with OB exhibited the onset of behavioural oestrus at a mean time of 33 ± 1.5 h (range 28 - 45) with no dose/response relationships evident. The first signs of oestrous behaviour included tail wagging, followed by circling, rubbing and attempting to mount the stag. The stag bunted, bit and kicked hinds which were seeking his attention, but he would not instigate courtship interest in them. Eventually the hinds concentrated on each other, with only occasional attention paid to the stag. At times, the stag became so intimidated by the behaviour of the hinds that he hid behind a horse that was also in the paddock. Two of the 5 hinds receiving no OB (ie, controls) exhibited oestrus at 35.5 and 38 hours after injection. However, these hinds showed no prior interest in the stag, and their mounting

behaviour accounted for only 4% of all mounts observed.

Plasma LH concentrations (Figure 1) averaged 0.32 ± 0.08 ng/ml during the period from progesterone withdrawal to OB administration. Concentrations then declined (P<0.01) in all OB treated animals to 0.08 ± 0.03 ng/ml for 12 to 18 h. This was followed by a dramatic 10 to 13- fold increase to a mean of 3.6 ± 0.5 ng/ml. Plasma LH then declined to pre-OB concentrations (0.24 ± 0.05 ng/ml) by 42 h after OB treatment. LH concentrations in the control group fluctuated around 0.60 ± 0.05 ng/ml throughout the sampling period without any apparent pattern.

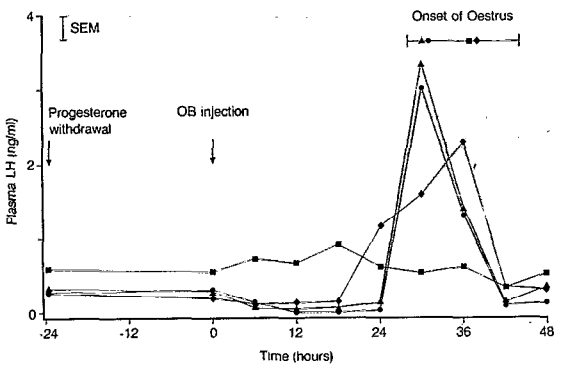

FIG 1 Onset of behavioural oestrus (treatment mean and group range) and plasma LH concentrations in response to 12 days of progesterone pre-treatment followed by 0 (■), 1 (▲), 2.5 (◆) or 5 (●) mg OB (0 hours).

Experiment 2

Oestrous behaviour was observed in only 2 of the 5 0.1 mg OB-treated hinds, one of which stood once and the other 14 times to be mounted, and in none of the 1 mg OB-treated hinds. Initial plasma LH concentrations of 0.86 ± 0.08 ng/ml prior to OB administration were significantly (P<0.01) higher than those recorded in the same period in Experiment 1. Although the initial plasma LH concentrations (Fig. 2) were suppressed immediately after injection, only 2 hinds given 0.1 mg OB (one of which showed oestrus and one that did not) produced any resemblance to a pre-ovulatory like LH surge, 30 hours after administration. In the 1.0 mg OB group, after initial suppression, plasma LH concentrations rose slowly to concentrations seen in the 0 mg OB groups without a pre-ovulatory like LH surge. No oestrus behaviour or trends in plasma LH were evident in either of the 0 mg OB groups.

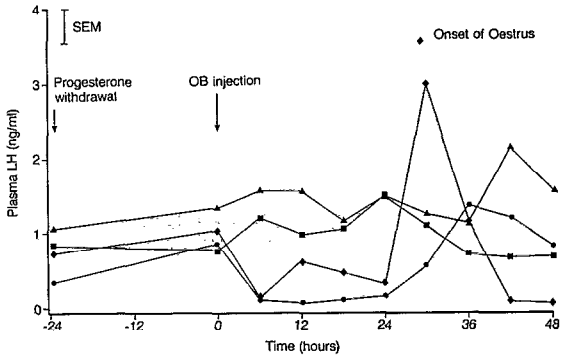

FIG 2 Onset of behaviour oestrus (treatment mean and group range) and plasma LH oncentrations in response to no treatments (■), or 12 days of progesterone pretreatment followed by 0 (▲), 0.1 (◆) or 1.0 (●) mg OB (time 0).

Experiment 3

One of the hinds receiving 12-day progesterone pretreatment (Y716) had a mean plasma progesterone concentration of 12.0 ± 1.14 ng/ml during the pretreatment period and 2.8 to 5.4 ng/ml throughout the rest of the study. This animal was removed from all experimental analyses to avoid confounding of the quantitative results.

A mean plasma progesterone concentration of 0.23 ± 0.03 ng/ml occurred immediately before CIDR device insertion. Peripheral progesterone concentrations were elevated above 1.0 ng/ml by 4 days after initial CIDR device insertion and continued until final CIDR device removal. Progesterone concentrations declined to 0.15 ± 0.05 ng/ml 24 h after final removal of the device. Control hind (no CIDR device) plasma progesterone concentrations fluctuated around 0.22 ± 0.09 ng/ml. A progesterone treatment of 4 days resulted in a longer interval to the onset of behavioural oestrus than 8 or 12 days (33.4 ± 1.2 h versus 29.0 ± 0.7 h; P<0.05). The stag showed more interest in this experiment by exhibiting flehmen and non-copulatory mounting in response to repeated hind attention. None of the hinds

in the control group (no progesterone) displayed any signs of oestrus.

Initial plasma LH concentrations of 1.56 ± 0.15 ng/ml prior to OB administration were significantly ($P<0.01$) higher than those recorded in the same period in Experiment 2. Following OB, plasma LH concentrations fell ($P<0.01$) to 0.37 ± 0.03 ng/ml for 18 h followed by an increase to peak at a mean concentration of 6.05 ± 0.75 ng/ml, 30 to 36 h after OB. Plasma LH then declined to similar concentrations (0.56 ± 0.09 ng/ml) recorded immediately prior to the preovulatory-like surge by 48 h after OB administration. No LH surges were detected in the control group.

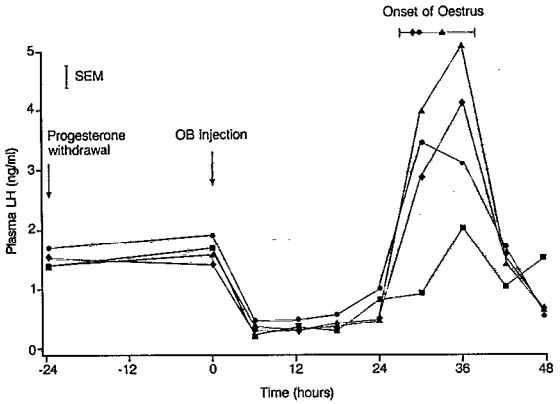

FIG 3 Onset of behavioural oestrus (treatment mean and group range) and plasma LH concentrations in response to 0 (■), 4 (▲), 8 (◆) or 12 (●) days of progesterone pre-treatment followed by 1 mg OB (0 hours).

DISCUSSION

Although not mated by the stag, the oestrous behaviour of hinds was similar to that reported during the breeding season (Guinness *et al.*, 1971; Clutton-Brock, *et al.*, 1982; Veltman, 1985). This indicated that oestrus can be induced in the ovariectomised red deer hind, approximately 30 to 36 hours after a treatment regime of 4 to 12 days of intravaginal progesterone, followed by 1 to 5 mg oestradiol benzoate administered 24 hours after progesterone withdrawal. From a practical viewpoint, 4 days of progesterone pretreatment followed 24 hours later by 1 mg OB may be a convenient way to induce oestrus. However, it is evident from the results in Experiment 2, where progesterone-OB treatments failed to elicit uniform hormonal or behavioural responses, that the timing of treatment relative to the breeding season may affect the minimum dosage of progesterone and OB to induce oestrus. Preliminary data from a similar experiment performed by the authors in January 1990 using a 12 day progesterone period followed, 24 hours later, by 0, 0.1, 0.5 or 1.0 mg OB also failed to elicit uniform behavioural oestrus. As in the same experiment performed a year earlier (Experiment 2) only 2 hinds in the 0.1 mg OB treatment group showed any sign of oestrous behaviour. This response in red deer highlights the need for further studies to investigate possible seasonal components. Stags may also need to be reproductively advanced (eg by melatonin or photoperiodic treatments) to induce premature rutting behaviour (Fisher and Fennessy, 1990) and seasonal testicular development (Lincoln *et al.*, 1984) if pre-season semen collection and libido testing is to be contemplated.

The regime used in the present study is similar to that used in sheep (Robinson, 1954; Robinson *et al.*, 1956; Scaramuzzi *et al.*, 1971), cattle (Carrick and Shelton, 1969) and fallow deer (H.N. Jabbour, pers. comm.). However, as 1 mg OB is higher (on a liveweight basis) than that required to induce oestrous behaviour in the ovariectomised ewe (10-17 μg OB; Robinson *et al.*, 1956) and cow (500 μg OB; Cook *et al.*, 1986) there is a need to further define the lowest dose, according to season, of OB in red deer before direct comparison with other species can be made. Progesterone pretreatment was found to be as critical in red deer as in sheep (Robinson, 1959) as 1 mg OB produced no oestrus response without exposure to progesterone.

Renewal of CIDR devices every 4 days kept progesterone concentrations consistently elevated above 1 ng/ml over all pretreatment periods. The 4-day period of progesterone pretreatment produced similar behavioural and hormonal effects as longer pretreatment periods, indicating 4 days of progesterone to be adequate to induce oestrus. However, plasma progesterone concentrations observed during the mid-oestrous cycle in entire red deer of 2.2-3.1 ng/ml (Jopson *et al.*, 1990) were not maintained in any of the pretreatment periods. This, therefore, may have had an effect on the timing of

the onset of oestrus.

The unusually high and anomalous plasma progesterone concentrations exhibited by one of the hinds in Experiment 3, necessitating its removal from analysis, was probably caused by endogenous secretion of progesterone from the adrenal glands (Meikle 1988; Jopson *et al.*, 1990). A previous experiment involving the same hind (Jopson *et al.*, 1990) also recorded high progesterone concentrations (2.7-5.0 ng/ml) similar to those obtained in the present experiment.

Tonic plasma LH concentrations increased significantly from December to January to February, both in the presence of exogenous progesterone and in the period from CIDR device withdrawal to OB administration (OB administration caused LH concentrations to fall to similar concentrations in all Experiments). This apparent increase in tonic plasma LH secretion in ovariectomised hinds indicates that a change in sensitivity to oestradiol is not the sole determinant of LH secretion. The castrate fallow buck (Asher *et al.*, 1989) also exhibits a similar seasonal pattern in LH secretion, unlike the continuously high concentrations noted in the ovariectomised ewe (Worthy and Haresign, 1983; Karsch *et al.*, 1984). This phenomenon is being investigated in the ovariectomised red deer hind more fully at the present time.

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