Effect of progesterone and oestradiol benzoate on oestrous behaviour and secretion of luteinizing hormone in ovariectomized fallow deer (Dama dama)

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Summary. Eighteen ovariectomized fallow deer does and two adult bucks were used to investigate the effect of exogenous progesterone and oestradiol benzoate on oestrous behaviour and secretion of luteinizing hormone (LH). In Expts 1 and 2, conducted during the breeding season (April–September), does were treated with intravaginal Controlled Internal Drug Release (CIDR) devices (0·3 g progesterone per device) for 12 days and differing doses of oestradiol benzoate administered 24 h after removal of the CIDR device. The dose had a significant effect on the proportion of does that exhibited oestrus within the breeding season (P < 0·001), the incidence of oestrus being 100% with 1·0, 0·1 and 0·05 mg, 42% for 0·01 mg and 0% for 0·002 mg oestradiol benzoate. There was a significant log–linear effect of dose on the log duration of oestrus, which was 6–20, 2–14, 2–12 and 2 h after treatment with 1, 0·1, 0·05 and 0·01 mg of oestradiol benzoate, respectively. Dose had a significant effect on the peak plasma LH concentration (P < 0·01), mean (± s.e.m.) surge peaks of 27·7 ± 2·3, 25·9 ± 1·8 and 18·6 ± 3·4 ng/ml being observed following treatment with 1, 0·1 and 0·01 mg oestradiol benzoate respectively. In Expt 3, also conducted during the breeding season, progesterone treatment (0 vs. 6–12 days) before the administration of 0·05 mg oestradiol benzoate had a significant effect on the incidence of oestrus (0/6 vs. 10/12, P < 0·05), but not on LH secretion. The duration of progesterone treatment (6 vs. 12 days) had no effect on oestrus. In Expt 4, conducted in the nonbreeding season (October–March), control does were largely unresponsive to treatment with 0·1 mg oestradiol benzoate. This was manifest in a lower proportion of does exhibiting oestrous behaviour and LH surges. Melatonin treatment, with implants administered on four occasions at intervals of 28–30 days starting from 24 October, significantly increased the proportion of does that exhibited oestrus in February, during the later phase of the nonbreeding season (7/8 vs. 1/8, P < 0·05). Melatonin-treated does also exhibited significantly higher basal plasma LH concentrations after removal of CIDR devices in February (5·8 ± 0·5 vs. 2·1 ± 0·4 ng/ml, P < 0·01). While only one control doe had an LH surge, with a peak of 13·8 ng/ml, all melatonin-treated does exhibited LH surges, with a mean peak concentration of 58·0 ± 8·4 ng/ml.

Keywords: fallow deer; progesterone; oestradiol benzoate; oestrous; luteinizing hormone

Introduction

Fallow deer (Dama dama) are seasonal breeders, the onset of mating activity occurring in autumn (Chapman & Chapman, 1975). In the absence of conception, fallow deer does may exhibit up to

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six oestrous cycles, oestrus recurring every 21–22 days, each breeding season (Asher, 1985). First oestrus, observed in New Zealand within a period of 12–14 days starting in late April is preceded by 'silent ovulations' (Asher, 1985). In sheep, this phenomenon has been attributed to the lack of progesterone sensitization at the onset of the breeding season (Robinson, 1954). Progesterone is believed to condition receptor cells within the central nervous system to show an optimum response to oestrogen secreted during the subsequent oestrous cycle (Scaramuzzi et al., 1972).

In recent years, there has been a considerable interest in the manipulation of reproductive activity of farmed fallow deer, aimed mainly at advancement of the breeding season for more efficient use of pasture resources (Asher et al., 1988b) or synchronization of oestrous for application within artificial insemination programmes (Asher et al., 1988a). The following study on ovariotomized fallow deer was designed to investigate the relationship between administered progesterone and oestrogen on oestrous behaviour and changes in secretion of luteinizing hormone (LH) and to develop a standard regimen for the induction of oestrus in does that will be used to carry an internal artificial vagina for semen collection from bucks (Jabbour & Asher, in press).

Materials and Methods

Animals and management

A total of 18 ovariotomized fallow deer does and two mature bucks were used in a series of four experiments between August 1989 and March 1990. The does were ovariotomized as mature, parous animals (>3 years old) in February (n = 12) or June (n = 6) 1989. The animals were always held in two separate groups, each with a single mature buck, from the time of commencement of treatment. Each group had equal numbers of animals representative of each treatment regimen, which were balanced for liveweight. The deer were contained in high-fenced paddocks (2500 m²) and grazed on ryegrass-clover pastures. Meadow hay was provided ad libitum and occasional feeding of whole-kernel maize was used to habituate the deer to their handlers.

Hormone administration

Single Controlled Internal Drug Release (CIDR) devices (type G, 0.3 g progesterone per device; Agricultural Division, CHH Plastic Products Group Ltd, Hamilton, NZ) were inserted intravaginally as indicated for each experimental protocol. Oestradiol-17ß-3-benzoate (Sigma Chemical Co., St Louis, MO, USA) was dissolved in groundnut oil <24 h before use, concentrations being adjusted so that 1.0 ml contained the desired dose. In all experiments, oestradiol benzoate was given as a single intramuscular injection in the rump, 24 h after withdrawal of the CIDR device.

Detection of oestrus

The bucks were fitted with ram mating harnesses (Fergus; Merck. Sharpe and Dohme NZ Ltd, Auckland, NZ) containing red crayons. The crayons were replaced every 8–12 h. Does were checked for mating marks every 4 h from the time of withdrawal of the CIDR device until administration of oestradiol benzoate and then every 2 h for 48 h. Mating marks were promptly removed with 70% ethanol. The time from administration of oestradiol benzoate to onset of oestrus, and the duration of oestrus were taken to be the time at which the first mating marks were recorded, and the interval between the first and last mating marks, respectively.

Blood sampling

Every 4 h from the time of withdrawal of the CIDR device until administration of oestradiol benzoate and then every 2 h for 48 h, the does were mustered into a covered handling shed and then individually restrained in a cradle for blood sampling. Samples (≈5 ml) were withdrawn from the right external jugular vein via indwelling catheters (Intracath 3122, Deseret Company, UT, USA) inserted, without anaesthesia, 3–4 h before the start of blood sampling. Catheter patency was maintained by back-flushing with 2–3 ml heparinized saline solution after each sampling. Blood samples were centrifuged for 30 min at 1000 g immediately after collection and the plasma was stored at −10°C until required for assay.
LH assay

Plasma LH concentrations were determined in duplicate using a heterologous radioimmunoassay procedure described for ovine plasma by Scaramuzzi et al. (1970) and validated previously for fallow deer plasma (Asher et al., 1986). All samples from an individual doe were included within a single assay. The intra-assay coefficients of variation were 9.7% for the low control (mean concentration = 0.42 ng/ml, n = 3), 3.4% for the medium control (9.64 ng/ml, n = 3) and 5.8% for the high control (11.2 ng/ml, n = 3). The interassay coefficients of variation were 11.4, 4.3 and 4.7% (n = 10), respectively for the three control samples. The assay sensitivity, as defined as the first point on the standard curve that was significantly different from 0, was 0.03 ng NIH-LH-S11 (0.30 ng/ml).

Conduct of the experiments

Experiment 1. A total of 18 does each received a single intravaginal CIDR device for 12 days from 10 August. They were allocated to three treatment groups and injected with 1 (n = 6), 0.1 (n = 6) or 0.01 (n = 6) mg of oestradiol benzoate. Blood samples were collected from all does.

Experiment 2. A total of 18 does each received a single CIDR device for 12 days from 30 August. They were allocated to three treatment groups and injected with 0.05 (n = 6), 0.01 (n = 6) or 0.002 (n = 6) mg of oestradiol benzoate. Blood was not sampled from these does.

Experiment 3. Eighteen does were allocated to three treatment groups. They were each treated with a single CIDR device for 12 days from 15 September (n = 6), 6 days from 21 September (n = 6) or 0 days (n = 6). All does were injected with 0.05 mg oestradiol benzoate. Blood samples were collected from nine does, three does from each treatment.

Experiment 4. Eight does received two subcutaneous melatonin implants (<4 mm³ containing 18 mg of synthetic melatonin; Regulin, Schering Agrochemicals Ltd, NSW, Australia) on 24 October, 24 November, 21 December and 19 January. A total of 16 does (eight melatonin-treated and eight non-implanted controls) were each treated with a single CIDR device for 6 days from 20 February, and 0.1 mg oestradiol benzoate was administered on 27 February. The does were mated with two bucks treated with melatonin implants as in the above schedule. Blood samples were collected from all does.

Statistical analyses

The incidence of oestrus was subjected to $\chi^2$ analysis using log-linear modelling. Data from the combined Expts 1 and 2 on time to onset of oestrus and duration of oestrus were analysed using REML with the does used as a component of variation (Patterson & Thompson, 1975). The rest of the data were subjected to analysis of variance, with log transformation performed for data on the duration of oestrus using the Genstat package (Numerical Algorithms Group Ltd, Oxford, UK). The data are presented as nontransformed means (± s.e.m.). Mean plasma LH profiles were obtained by normalizing the data about the time of withdrawal of the CIDR device and the LH surge peak.

Results

Experiments 1 and 2

One doe treated with 1 mg oestradiol benzoate in Expt 1 lost the CIDR device and hence was excluded from the analyses. There were no significant differences between Expts 1 and 2 in the variables measured, hence the data were pooled. There was a significant linear relationship between the log dose of oestradiol benzoate and the incidence of oestrus ($P < 0.001$). The dose of 0.05 mg was the lowest that resulted in a 100% response; a dose of 0.002 mg failed to elicit any response (Table 1). The dose of oestradiol benzoate had no effect on the time to onset of oestrus within the breeding season (Table 1). The overall mean time to onset of oestrus was 21.6 ± 0.9 h, the does exhibiting oestrus over a range of 16–28 h after administration of oestradiol benzoate. There was a significant linear effect of the log dose of oestradiol benzoate on the log duration of oestrus during the breeding season (Table 1). On average, the duration of oestrus increased 2.5 ± 0.5-fold per tenfold increase in the dose administered ($P < 0.001$). The does exhibited oestrus for periods of 6–20, 2–14, 2–12 and 2 h following treatment with 1, 0.1, 0.05 and 0.01 mg oestradiol benzoate, respectively.

The dose of oestradiol benzoate had no effect on the pattern of plasma LH concentrations (Expt 1) from withdrawal of CIDR device until 34 h later, hence the data for this period were pooled across doses (Fig. 1a). The mean plasma LH concentration before administration of...
Exhibiting during times Experiment of data oestradiol oestrus CIDR treatment had 20-4 treated Basal oestradiol + oestradiol h s.e.m.) Mean (± s.e.m.) peak (h) Treatment of oestradiol benzoate (mg) | Mean (± s.e.m.) time to onset of oestrus (h) | Mean (± s.e.m.) duration of oestrus (h) | Mean (± s.e.m.) time to LH peak (h) | Incidence of oestrus |
---|---|---|---|---|
1 | 24·0 ± 1·4 | 12·8 ± 2·2 | 20·4 ± 0·4 | 5/5 |
0·1 | 20·0 ± 0·9 | 9·6 ± 1·8 | 19·0 ± 0·4 | 6/6 |
0·05 | 18·7 ± 1·7 | 4·3 ± 1·5 | — | 6/6 |
0·01 | 24·8 ± 1·9 | 2·0 ± 0·0 | 21·7 ± 1·4 | 5/12 |
0·002 | — | — | — | 0/6 |
Total | 21·6 ± 0·9 | 7·2 ± 1·2 | 20·4 ± 0·6 | 22/35 |

Oestradiol benzoate was 3·7 ± 0·1 ng/ml then declined to 1·09 ± 0·05 ng/ml (P < 0·01) during the 10 h after administration. This was followed by an increase in plasma LH, that emulated a pre-ovulatory LH surge. In most instances, the peak plasma LH was observed at the onset of oestrus. The overall mean ± s.e.m. times to onset of oestrus and peak LH secretion were 21·6 ± 0·9 and 20·4 ± 0·6 h, respectively, after administration of oestradiol benzoate. The dose administered had a significant effect on the peak LH concentration (P < 0·01, Fig. 1a). Mean (± s.e.m.) peak concentrations of plasma LH of 27·7 ± 2·3, 25·9 ± 1·8 and 18·6 ± 3·4 ng/ml were recorded after treatment with 1, 0·1 and 0·01 mg oestradiol benzoate, respectively.

Experiment 3

Progestosterone treatment (0 vs. 6–12 days) had a significant effect on the incidence of oestrus (0/6 vs. 10/12, P < 0·05, Table 2), but the duration of progesterone treatment (12 vs. 6 days) had no effect on the incidence, time to onset, or duration of oestrus (Table 2). One doe treated with a CIDR device for 6 days showed no increase in plasma LH concentrations, and did not exhibit oestrus after administration of oestradiol benzoate and was excluded from the analyses of the data on plasma LH. Progesterone treatment (0 vs. 6–12 days) had no effect on plasma LH concentrations and the data for all individual profiles were pooled (Fig. 1b). The does treated with oestradiol benzoate only (i.e. no progesterone treatment) exhibited LH surges, but did not exhibit oestrus. The mean concentration of plasma LH before administration of oestradiol benzoate was 4·2 ± 0·2 ng/ml and then declined to 0·9 ± 0·09 ng/ml (P < 0·01) during the 10 h after administration of oestradiol benzoate. This was followed by a surge in plasma LH with a mean (± s.e.m.) peak of 22·4 ± 2·3 ng/ml at a mean (± s.e.m.) time of 17·5 ± 0·9 h after administration of oestradiol benzoate.

Experiment 4

Melatonin treatment significantly increased the proportion of does that exhibited oestrus during the nonbreeding season (7/8 vs. 1/8, P < 0·05, Table 3). There was a significant seasonal × melatonin effect on the time to onset of oestrus, melatonin-treated does in the nonbreeding season exhibiting oestrus significantly later (P < 0·01) than does in Expts 1, 2 and 3. The overall mean times to onset of oestrus for melatonin-treated does in February (Expt 4) and nonimplanted does in August–September (Expts 1, 2 and 3) were 29·4 ± 0·8 (n = 7) and 21·1 ± 0·7 h (n = 32), respectively.

Melatonin treatment significantly increased plasma concentrations of LH (P < 0·01, Fig. 1c). Basal concentrations of LH before administration of oestradiol benzoate were higher in melatonin-treated does than in control does (5·9 ± 0·5 vs. 2·1 ± 0·4 ng/ml, P < 0·01). After administration of
Fig. 1. Profiles of mean (± s.e.m.) plasma luteinizing hormone (LH) normalized around removal of Controlled Internal Drug Release (CIDR) device and peak LH surge concentration for ovariectomized fallow deer, after treatment with CIDR devices for (a) 12 days and 1 (●, n = 5), 0·1 (△, n = 6) or 0·01 (☐, n = 6) mg oestradiol benzoate in the breeding season; (b) 12, 6 or 0 days and 0·05 mg oestradiol benzoate (n = 8) in the breeding season; or (c) 6 days and 0·1 mg oestradiol benzoate (●, n = 8), or without (○, n = 1 and 7) prior melatonin treatment in the nonbreeding season; arrows designate the time of administration of oestradiol benzoate.
Table 2. Effect of duration of progesterone treatment on oestrous behaviour and time to peak in plasma luteinizing hormone (LH) in ovariectomized fallow deer during the breeding season (Expt 3)

<table>
<thead>
<tr>
<th>Duration of progesterone treatment (days)</th>
<th>Incidence of oestrus</th>
<th>Mean (± s.e.m.) time to onset of oestrus (h)</th>
<th>Mean (± s.e.m.) duration of oestrus (h)</th>
<th>Mean (± s.e.m.) time to LH peak (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5/6</td>
<td>20·0 ± 0·6</td>
<td>2·8 ± 0·5</td>
<td>18·0 ± 1·1 (n = 3)</td>
</tr>
<tr>
<td>6</td>
<td>5/6</td>
<td>20·0 ± 1·6</td>
<td>2·8 ± 0·5</td>
<td>15·0 ± 1·0 (n = 2)</td>
</tr>
<tr>
<td>0</td>
<td>0/6</td>
<td>—</td>
<td>—</td>
<td>18·7 ± 1·8 (n = 3)</td>
</tr>
<tr>
<td>Total</td>
<td>10/18</td>
<td>20·0 ± 0·7</td>
<td>2·8 ± 0·3</td>
<td>17·5 ± 0·9</td>
</tr>
</tbody>
</table>

Table 3. Effect of melatonin treatment on oestrous behaviour and time to peak in plasma luteinizing hormone (LH) in ovariectomized fallow deer during the nonbreeding season (Expt 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence of oestrus</th>
<th>Mean (± s.e.m.) time to onset of oestrus (h)</th>
<th>Mean (± s.e.m.) duration of oestrus (h)</th>
<th>Mean (± s.e.m.) time to LH peak (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin-treated does</td>
<td>7/8</td>
<td>29·4 ± 0·8</td>
<td>8·6 ± 2·1</td>
<td>27·5 ± 0·5</td>
</tr>
<tr>
<td>Control does</td>
<td>1/8</td>
<td>36·0</td>
<td>2·0</td>
<td>38·0</td>
</tr>
<tr>
<td>Total</td>
<td>8/16</td>
<td>30·2 ± 1·1</td>
<td>7·8 ± 2·0</td>
<td>28·6 ± 1·2</td>
</tr>
</tbody>
</table>

Oestradiol benzoate, concentrations of plasma LH declined significantly (P < 0·05) over 10 h to 1·9 ± 0·1 and 1·1 ± 0·1 ng/ml in each group, respectively. Only one control doe, which failed to show oestrous behaviour, exhibited an LH surge with a peak of 13·8 ng/ml at 36 h after administration of oestradiol benzoate. All melatonin-treated does exhibited surges in plasma LH, with an overall mean (± s.e.m.) peak concentration of plasma LH of 58·0 ± 8·4 ng/ml at a mean (± s.e.m.) time of 27·5 ± 0·5 h after administration of oestradiol benzoate. In most instances, the peak of the LH surge was observed at the onset of oestrus.

Discussion

It has been demonstrated that overt oestrus observed at the beginning of the breeding season in deer (fallow: Asher, 1985; wapiti: Morrison, 1960; moose: Simkin, 1965; black-tailed deer: Thomas & Cowan, 1975) is preceded by ‘silent ovulations’ and a period of progesterone secretion for several days. In ovariectomized sheep, treatment with large pharmacological doses of oestradiol alone can induce oestrous behaviour (Robinson, 1954; Moore & Robinson, 1957; Brander & Robinson, 1962). However, the present study on fallow deer has shown that a period of progesterone sensitization is essential for the expression of oestrous behaviour with physiological doses of oestradiol. This is in agreement with reports on ovariectomized sheep (Karsch et al., 1980) and red deer (Meikle & Fisher, 1990). Progesterone is believed to act on the central nervous system to condition the ovariectomized female to exhibit oestrous behaviour after the administration of low doses of oestradiol (Robinson et al., 1956; Scaramuzzi et al., 1972). Progesterone presumably lowers the hypothalamic-pituitary threshold to oestrogen stimulation; ovariectomized red deer hinds treated
late administered. Although no differences in oestrous behaviour were detected in this study after treatment with progesterone for 6 or 12 days, the duration of progesterone treatment has been shown to affect the sensitivity to oestrogen in other species. The latency between oestradiol injection and onset of oestrus was reduced as the period of progesterone treatment was increased from 3 to 12 days in ovariectomized sheep (Robinson et al., 1956) and from 4 to 8 or 12 days in red deer (Meikle & Fisher, 1990).

Behavioural oestrus in cervids is associated with an increase in concentration of plasma oestradiol-17β (fallow deer: Asher et al., 1986; white-tailed deer: Plotka et al., 1977; red deer: Kelly et al., 1982; Eld’s deer: Monfort et al., 1990). In entire fallow deer, oestrous behaviour is usually terminated at copulation (Asher, 1986). This renders observations on the potential duration of natural oestrus possible only in the absence of copulation. After continuous observations, Asher (1986) reported one incident in which the duration of oestrus for a fallow doe, as determined from repeated noncopulatory mounting by the buck, was at least 8 h. However, in ovariectomized fallow deer, multiple copulations were observed after the administration of as little as 0.05 mg oestradiol benzoate, and the duration of oestrus increased in a linear fashion with an increase in the dose administered. This variation in the duration of oestrus may be due to the slow release and subsequent longer period of activity of increasing doses of oestradiol benzoate.

There was a seasonal × melatonin effect on the incidence of oestrus after administration of oestradiol benzoate. Ovariectomized fallow deer treated with progesterone and oestradiol benzoate late in the nonbreeding season (Expt 4; control does) were largely unresponsive to treatment. Similar observations have been reported in sheep (Goodman et al., 1981). However, contemporary melatonin-treated does did exhibit oestrous behaviour. This suggests seasonal variation in the expression of oestrous behaviour and may be attributed to changes in the sensitivity of the animals to oestriadiol. This may be a consequence of an increase in the minimum threshold of oestradiol required to elicit oestrous behaviour and an LH response, and may even represent complete lack of responsiveness of the hypothalamic–pituitary axis to administered oestradiol benzoate mediated by the changing photoperiod. Moreover, there was a seasonal × melatonin effect on the time to onset of oestrus. The latency period between administration of oestradiol benzoate and the time to onset of oestrus was longer in melatonin-treated does during the nonbreeding season than in control does treated during the breeding season. Subsequent studies have revealed that the latency period in melatonin-treated does decreased progressively as the treatments with oestradiol benzoate and exogenous progesterone were applied later in the season (H. N. Jabbour, unpublished data). This is similar to observations in entire fallow deer, where the latency between removal of the CIDR device and the time to onset of oestrus was reduced progressively between March and May, the period spanning the natural rut (C. J. Morrow, unpublished data). This may imply that melatonin treatment of ovariectomized fallow deer does advances the period of responsiveness to oestradiol. As oestradiol is believed to regulate oestrous behaviour by acting on the hypothalamus (Clegg et al., 1958; Radford, 1967), the seasonal variation in the expression of oestrus may be due to a variation in the action of oestradiol on the hypothalamic–pituitary axis. In this study, the melatonin-treated deer were responsive in late February, some 8 weeks before the onset of the natural rut (the first natural mating in 1990 was recorded on 21 April); and contrary to other methods of advancing the onset of oestrus or ovulation in intact deer (pregnant mares’ serum gonadotrophin: Adam et al., 1985; Asher & Smith, 1987; gonadotrophin-releasing hormone (GnRH): Asher & Macmillan, 1986) melatonin treatment has been shown to induce recurring reproductive activity in the nonbreeding season (Adam & Atkinson, 1984; Adam et al., 1986; Asher et al., 1988b). Melatonin treatment appeared to affect the secretion of LH directly in female fallow deer; both tonic and surge concentrations were higher than those observed during the breeding season. Recent work on intact Père David’s deer has demonstrated that the pattern of LH secretion differs during different stages of anoestrus; the mean LH concentrations and LH pulse frequency of hinds were significantly higher in mid-anoestrus than in early anoestrus and the magnitude of the
LH response to administration of GnRH was significantly greater in mid- and late, than in early anoestrus (Curlewis et al., 1991; McLeod et al., 1991).

As expected, the plasma concentrations of LH increased after ovariectomy of fallow does. However, the administration of oestradiol benzoate produced two effects on LH secretion. Initially, a depression in concentrations of plasma LH (0–10 h after injection) was observed followed by an LH surge and peak, which, in most instances, coincided with the onset of oestrus. As demonstrated in sheep (Scaramuzzi et al., 1971), there can be little doubt that oestrogen provides the primary stimulus for LH release in ovariectomized fallow deer. In both magnitude and duration, this surge appears to be identical to the pre-ovulatory surge observed in intact animals during a synchronized oestrous cycle (Asher & Thompson, 1989). The results of Expt 3 revealed that immediate pre-treatment with progesterone is not essential for oestradiol benzoate to elicit an LH response during the breeding season, although oestrus was not induced.

In contrast to reports in sheep (Radford et al., 1971; Goodman et al., 1981) increasing doses of oestradiol benzoate had no effect on the time to onset of the LH surge. However, the magnitude of the LH surge increased with increasing doses. Similar observations have been reported in sheep after the administration of oestradiol benzoate by injection (Radford et al., 1971) but not by an implant (Goodman et al., 1981). This may be a result of a direct action of oestradiol benzoate on the hypothalamus to increase GnRH secretion. This is supported by findings that sodium phenobarbitone (Radford & Wallace, 1974), passive immunization against GnRH (Fraser & McNeilly, 1982) and transection of the pituitary stalk (Clarke et al., 1983) block or delay the oestrogen-induced LH surge in ewes. Furthermore, increases in GnRH secretion have now been directly measured during oestrogen-induced LH surges in ovariectomized ewes (Cummins & Clarke, 1985).

Oestrous behaviour in the ovariectomised fallow doe can be induced by treatment with progesterone and oestradiol benzoate. The animals undergo a period of complete refractoriness during the nonbreeding season, but treatment with melatonin advances the period of sensitivity to the exogenous steroids.

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