Effect of prolonged exposure to oestradiol on subsequent LH secretion in ewes

M. Ozturk, R. F. Smith and H. Dobson*

Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool, Leahurst, Neston, South Wirral L64 7TE, UK

Abnormal follicles can produce oestradiol continuously for up to 20 days and this inhibits the hypothalamic-pituitary axis. The present experimental series was designed to determine the minimum exposure to high or low follicular phase concentrations of oestradiol that were required to exert inhibitory effects on LH surge secretion induced by additional oestradiol administered at the end of continuous exposure to oestradiol. Experiments were also included to establish whether the inhibitory effects of prolonged oestradiol were mediated at the pituitary, and whether the failure of response to the oestradiol challenge could be corrected by exposure to normal luteal phase patterns of progesterone. Treatment of ewes between 2 and 12 days with 1, 2 or 4 oestradiol implants (3 cm) totally blocked the subsequent normal LH surge in response to an oestradiol challenge in 45 of 52 ewes pretreated with oestradiol. In the seven ewes that did have an increase in LH, the response occurred at the expected time but was greatly reduced (14 versus 40 ng ml⁻¹), and occurred only in ewes pretreated with oestradiol implants for 2 or 4 days. We were unable to establish a robust linear time-dose relationship, i.e., when ewes were treated with lower doses of oestradiol (one or two implants) for a reduced time (2, 4 or 8 days), there was random distribution of the 7 of 32 animals that had a reduced LH surge after oestradiol challenge (with four implants or 50 µg injection). The present study is the first to show that exposure for only 2–4 days to continuous oestradiol at late follicular phase concentrations can disrupt LH surge release. However, in oestradiol-treated ewes, LH secretion was provoked by high or low doses (0.5 mg or 0.5 µg) of GnRH, although it was reduced by 50%, and a GnRH self-priming effect was still evident. All of these results suggest that inhibitory effects occur at the pituitary and hypothalamus. It remains to be confirmed whether the major effect is at the pituitary by reducing GnRH receptor or LH synthesis, or at the hypothalamus via inhibition of GnRH secretion.

Introduction

A condition of abnormal follicular development, referred to by veterinarians as ovarian cysts, occurs in the post-partum period of sheep and cattle. These abnormal follicles produce normal follicular phase concentrations of oestradiol (5–30 pg ml⁻¹) continuously for up to 20 days compared with a normal duration of 1–2 days (Savio et al., 1990; Dobson et al., 1997). Although there is then cessation of endocrine function, the cyst can remain as a structural entity for twice as long. Whatever the original cause of cyst formation, it is possible that prolonged oestradiol secretion, itself, becomes inhibitory to the hypothalamic-pituitary axis and further induction of an LH surge by oestradiol is inhibited. Preliminary evidence for this phenomenon has been provided by Dobson and Nanda (1992) who gave an oestradiol challenge to cows with cystic structures on the ovary but 50% did not respond with an LH surge. It is assumed that those that did respond had structures on the ovary which were not producing oestradiol, whereas the non-responders had cysts in a state of prolonged oestradiol production.

Other workers have investigated the effects of very high concentrations of oestradiol on LH synthesis and secretion in relation to the inhibitory effects exerted during, and immediately after, pregnancy (Herring et al., 1991). In contrast, the present experimental series was designed to determine the minimum period for which physiological follicular phase concentrations of oestradiol could exert inhibitory effects on LH surge secretion. Ovariectomized ewes were treated with steroids either to mimic the normal follicular phase (to act as controls), or to create a steroid environment similar to that occurring in ewes with prolonged follicular phases (cysts). At the end of each treatment, the ewes were challenged with oestradiol to examine the effects of prior exposure to steroid on surge release of LH. It was considered necessary to administer an increasing oestradiol stimulus at the end of continuous exposure, as there is evidence to show that

*Correspondence.
Received 19 November 1997.
increments in oestradiol are important for generating an LH surge (Goodman et al., 1981). Experiments were also included to establish whether the failure of response could be corrected by exposure to normal luteal phase patterns of progesterone, and whether the inhibitory effects were mediated at the pituitary (by administering GnRH at the time of the otherwise expected LH surge).

Materials and Methods

Animals and collection of blood samples

The study was carried out with mature Cheviot ewes and cross-bred Suffolk ewes weighing between 50 and 85 kg in experimental series during two consecutive breeding seasons. The ewes were ovariectomized 1 month before the start of Expt 1. Control treatments were included within each experiment to minimize the influence of time after ovariectomy. All ewes were loosely penned indoors, and provided with hay and water ad libitum. One day before each experiment, indwelling catheters were inserted into the jugular vein under local anaesthesia in all ewes and catheter patency was maintained with heparinized saline (100 IU ml⁻¹). Blood samples (4 ml) were collected via jugular catheters and immediately centrifuged at 1000 g for 20 min. Plasma was stored at −15°C till analysed.

Hormone treatment

Small oestradiol implants (1 cm length) remained subcutaneous immediately after ovariectomy and throughout all experiments. At the end of each experimental period, ewes were challenged with oestradiol either as an i.m. injection of 50 µg oestradiol benzoate (Intervet, Cambridge, UK) diluted in 2 ml arachis oil, or by subcutaneous implants of 3 cm length. All oestradiol implants were made from crystalline oestradiol packed into medical grade silicone tubing (ID: 3.3 mm × OD: 4.6 mm; Degania Silicone, Degania Bet, Israel) following Legan et al. (1977). Treatment with 1 or 3 cm oestradiol implants produced physiological concentrations of oestradiol in the peripheral circulation (1–2 or 8 pg ml⁻¹, respectively; Karsch et al., 1980; Goodman et al., 1981). Before insertion, the implants were soaked overnight in water to prevent an initial peak of steroid release, and then inserted subcutaneously under local anaesthesia in the axillary region of each sheep. Artificial oestrous cycles were imposed between each experiment with intravaginal progesterone (CIDR-G; InterAg, Hamilton, N.Z.) inserted for 10 days (Wheaton et al., 1993). When GnRH (Fertagyl; Intervet) was given, it was administered through the indwelling i.v. catheter as a 0.5 µg or 0.5 mg injection diluted in 2 ml normal saline.

Experimental design

A summary of the experimental design is given in Fig. 1 and Table 1. Experiment 1 was designed to examine the inhibitory effect of 12 days exposure to medium or high dose of oestradiol, as well as the subsequent corrective effect of progesterone. Pituitary sensitivity to low or high doses of GnRH was also assessed after exposure to oestradiol for 12 days. The low dose of GnRH was administered twice with an interval of 2 h to quantify changes in GnRH self-priming. Experiment 2 was designed to establish a time–dose response relationship for the inhibitory effect of oestradiol. Having shown that effects occurred after exposure for only 2 days the pituitary sensitivity to GnRH was again examined.

Experiment 1

In Expt 1, the oestradiol challenge consisted of step-wise insertion of two 3 cm oestradiol implants followed 10 h later by another two 3 cm oestradiol implants. Blood samples were collected from all ewes every 2 h for 32 h after insertion of the final two implants.

Experiment 1a: 12 days oestradiol exposure at high or medium doses. Ten ovariectomized ewes in group 1 (controls) were challenged with step-wise oestradiol implants as described above, beginning 22 h after progesterone withdrawal. Groups of ten ewes had two (group 2) or four (group 3) 3 cm oestradiol implants inserted for 12 days (in the absence of progesterone), followed by an oestradiol challenge.

Experiment 1b: corrective effect of progesterone. Group 4 ewes were initially treated with four 3 cm oestradiol implants for 12 days followed by progesterone for 10 days, to test whether the lesion could be reversed by progesterone treatment. Twenty-two hours after progesterone withdrawal, the ewes were challenged with oestradiol.

Experiment 1c: pituitary sensitivity to GnRH after 12 days oestradiol exposure at a high dose. Five ovariectomized ewes were treated with progesterone for 10 days followed by a step-wise oestradiol challenge 22 h after progesterone withdrawal (control group 5). A further ten ovariectomized ewes were treated with four oestradiol implants for 12 days and then challenged with oestradiol. At a time just before the expected LH surge (determined from group 1 results), injections of low dose GnRH (0.5 µg in 2 ml saline) were administered 10 and 12 h after oestradiol challenge to all control ewes, and to five ewes previously treated with oestradiol for 12 days (group 6a). In the event that the pituitary sensitivity may have been very markedly reduced, the remaining five long-term oestradiol-treated ewes (group 6b) received a very high dose of GnRH (0.5 mg in 2 ml saline) 10 h after oestradiol challenge. Additional blood samples were taken 15 and 30 min before, and 0, 15, 30 and 60 min after each GnRH injection.

Experiment 2

Having established in Expt 1 that there was an inhibitory effect of prolonged oestradiol exposure on subsequent challenges with oestradiol, it was no longer considered necessary to submit the animals to the discomfort of
Fig. 1. Design of experiments showing schematic representation of treatment and blood sampling regimens. Shaded box depicts additional oestradiol treatment with two or four implants for 8 h in Expt 2(b). Open and closed arrows represent oestradiol injections and GnRH injections, respectively.

* Oestradiol challenge in groups 11a and c, but not 11b.
repeated subcutaneous administration. Hence, in subsequent experiments, the post-treatment oestradiol challenge consisted of one i.m. injection of 50 µg oestradiol benzoate. The timing of this injection after progesterone withdrawal was altered so that the LH surge would occur at the same time after progesterone as that after the use of implants. There were four ewes in each group. Blood samples were collected every 4 h for selected periods following oestradiol implant insertion, and then every 2 h for 16 h from 10 h after the post-treatment oestradiol challenge.

Experiment 2a: 8 or 4 days oestradiol exposure at low or medium doses. All ewes in experiment 2a received 50 µg oestradiol implant at 16 h after progesterone withdrawal. Eight hours after insertion, implants were removed so that the remaining implants numbered one, or two in groups 7a, 9a, b or c, respectively. Ewes in group 10 were similarly treated except that four implants were initially inserted but the remaining implants still numbered none, one or two in groups 10a, b or c, respectively. All ewes in groups 9 and 10 were challenged with an oestradiol injection 40 h after the initial implant insertion, or at an equivalent time in the control ewes.

Table 1. Summary of experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Ewe (n)</th>
<th>Progesterone pretreatment (10 days)</th>
<th>Oestradiol pretreatment Duration</th>
<th>Number of implants</th>
<th>Oestradiol challenge Four implants</th>
<th>Injection</th>
<th>GnRH challenge (0.5 µg)</th>
<th>0.5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>10</td>
<td></td>
<td>12 days</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>10</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>10</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 1b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>10</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 1c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>5</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 6a</td>
<td>5</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 6b</td>
<td>5</td>
<td></td>
<td>12 days</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 2a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 7a</td>
<td>4</td>
<td></td>
<td>8 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 7b</td>
<td>4</td>
<td></td>
<td>8 days</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 7c</td>
<td>4</td>
<td></td>
<td>4 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 7d</td>
<td>4</td>
<td></td>
<td>4 days</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 2b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 9a</td>
<td>4</td>
<td></td>
<td>40 h</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 9b</td>
<td>4</td>
<td></td>
<td>40 h</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 9c</td>
<td>4</td>
<td></td>
<td>40 h</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 9d</td>
<td>4</td>
<td></td>
<td>40 h</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 2c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 11a</td>
<td>4</td>
<td></td>
<td>48 h</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 11b</td>
<td>4</td>
<td></td>
<td>48 h</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 11c</td>
<td>4</td>
<td></td>
<td>48 h</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

✓, treatment.

In groups 9 and 10, ewes had two or four oestradiol implants inserted for 8 h from 16 h after progesterone withdrawal. Group 11a received 50 µg oestradiol injection 16 h after progesterone withdrawal.
Experiment 2c: pituitary sensitivity to GnRH after 2 days oestradiol exposure at a medium dose. Each ewe in groups 11b and 11c received two 3 cm oestradiol implants 16 h after progesterone removal; group 11a ewes did not receive an implant. Ewes in all groups were injected with 50 μg oestradiol benzoate at the time of implant insertion, and all ewes in this experiment received 0.5 μg GnRH in 2 ml saline at 44, 46, 64 and 66 h after the first oestradiol injection. A post-treatment oestradiol challenge was administered 48 h after the initial oestradiol injection in groups 11a and 11c. Blood samples were taken every 2 h for 20 h beginning 10 h after the initial oestradiol injection, with an increased frequency near the time of each GnRH injection at -30, -15, 0, 15, 30 and 60 min.

Hormone analysis

Samples obtained within each experiment were analysed in one assay. Plasma LH was measured by a method characterized and verified in this laboratory (Dobson and Ward, 1977). Inter- and intra-assay coefficients of variation for LH were less than 5%. The results were expressed as nanogram equivalents of NIAMDD ovine LH 21 per millilitre of plasma.

Statistical analysis

The results are expressed as mean ± SE. Data obtained from control and treated animals (within the same experiments) were compared by Student’s two-tail paired t test. The effects of between animal variation were minimized by using the same ewes as control and oestradiol-treated within each experiment and the results were compared on a within-ewe basis, each sheep serving as its own control between groups. However, comparisons between the subgroups within or between Expts 1 and 2 were made by Student’s unpaired t test.

The occurrence of an LH surge was defined as at least two consecutive samples (at 2 h intervals) containing LH > 5 ng ml⁻¹, and the time of the first sample > 5 ng ml⁻¹ was designated as the onset time of the LH surge. The highest value during the LH surge was defined as the surge maximum, and the time between the onset of the surge and the last sample > 5 ng ml⁻¹ was used to define the duration of the LH surge.

In Expts 1b and 2c, the area under the LH profile was calculated after each GnRH challenge. Data after GnRH injection obtained from control and oestradiol-treated groups were compared by analysis of variance for repeated measures, followed by Student’s paired t test on a within ewe basis.

Results

Experiment 1a: 12 days exposure to oestradiol at high or medium doses. Two or four oestradiol implants inserted for 12 days completely inhibited the surge of LH in response to the post-treatment oestradiol challenge in all ewes (Table 2).

Experiment 1b: corrective effect of progesterone. Treatment with progesterone for 10 days following the insertion of four implants for 12 days normalised the LH response to oestradiol challenge (group 4, Table 2). Duration and maximum concentrations were similar for those groups that had an LH surge.

Experiment 1c: pituitary sensitivity to GnRH after oestradiol exposure for 12 days at a high dose. The response to the first low dose of GnRH was lower (Fig. 2a) in the implant-treated ewes (group 6a) than in the ovariectomized controls (group 5; comparison of areas under the curve, P < 0.05). Comparison within groups revealed no difference between the response after the first and second GnRH injection in the control animals not exposed to 12 days of oestradiol treatment (group 5), but the second response was greater than the first in ewes treated with implants for 12 days (within group 6a, P < 0.04). There was no difference between groups in the responses to the second low dose GnRH injection (P > 0.05). Treatment with the high dose of GnRH caused a much greater release of LH (Fig. 2b).

Experiment 2a: 8 or 4 days oestradiol exposure at low or medium doses. Blood samples were not collected from ewes in group 7 immediately after implant insertion. There was no LH surge in control group 8a for the first 2 days after progesterone withdrawal. Over the same period, an LH surge was observed in all ewes in groups 8b or 8c after insertion of one or two implants. The characteristics of these surges are shown in Table 3. There were no differences between groups 8b or 8c (one or two implants).

Table 2. Characteristics (mean ± SE) of the LH surge after an oestradiol challenge following steroid pretreatment in Expts 1(a) and (b)

<table>
<thead>
<tr>
<th>Ewe (n)</th>
<th>Time to LH surge onset (h)</th>
<th>Duration (h)</th>
<th>Maximum value (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post-progesterone</td>
<td>Post-oestradiol</td>
</tr>
<tr>
<td>Expt 1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>10</td>
<td>38.4 ± 1.2</td>
<td>16.4 ± 1.2</td>
</tr>
<tr>
<td>Group 2</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group 3</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Expt 1b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>10</td>
<td>42.8 ± 1.3</td>
<td>20.8 ± 1.3</td>
</tr>
</tbody>
</table>

—, no surge detected.
When the ewes in groups 7 and 8 were challenged with oestradiol 4 or 8 days after progesterone withdrawal, all ewes in control groups 7a and 8a had an earlier LH surge of shorter duration, but similar maximum value, to that immediately after implant insertion (in groups 8b and c). After 4 or 8 days treatment with oestradiol implants in groups 7b, c, or 8b, c, there were no normal LH surges after the challenge injection of oestradiol. However, two ewes in group 8b and one in group 8c had truncated LH increases of 4 h duration and less than 15 ng ml⁻¹ maximum value, although the increments occurred at the expected time.

Experiment 2b: 2 days oestradiol exposure at low or medium doses. The insertion of two or four implants for only 8 h in groups 9a and 10a was insufficient to cause the release of a normal LH surge except in three animals that had a surge with the correct timing but of reduced magnitude. However, when one or two implants remained after the eighth hour in groups 9b, c, and 10b, c, LH surges occurred but the onset was later, the duration shorter, and the maximum values lower, than if the multiple implants had remained in situ for longer (Table 3; for example, compare groups 9b, c, 10b, c with groups 8b, c; \( P < 0.05 \)).

The oestradiol challenge 40 h after progesterone withdrawal caused a normal LH surge in all control ewes (groups 9a and 10a). In contrast, when one or two implants remained in situ for 40 h, no normal LH surges occurred, although four of the 16 animals in groups 9b, c, and 10b, c did have a subnormal increase in LH.

Experiment 2c: pituitary sensitivity to GnRH after exposure to oestradiol at a medium dose for 2 days. The addition of an oestradiol injection at the time of oestradiol implant insertion 16 h after progesterone withdrawal resulted in a normal LH surge 25–30 h later in all ewes (Table 3, groups 11a–c).

There was a differential release of LH (represented by area under the curve) in response to GnRH, depending on when the GnRH was administered relative to previous GnRH or oestradiol administration. The response to the first GnRH injection before oestradiol challenge was not different between implant-treated groups and the control group (Fig. 3), but after the second GnRH injection, the ewes treated with oestradiol implants had a greater LH increase than the controls (within a group, Student’s paired \( t \) test comparison of areas under the curve, \( P < 0.05 \)).

Compared with the response after the first GnRH injection, approximately 50% less LH was released when the third GnRH injection was given at 64 h during the time of the expected LH surge after the second oestradiol injection (indicating that oestradiol had a negative feedback effect on the pituitary). However, after the fourth GnRH injection self-priming was again evident in all groups.

**Discussion**

We have shown for the first time that treatment of ewes between 2 and 12 days with one to four oestradiol implants totally blocked the subsequent normal LH surge in response to an oestradiol challenge in most ewes (45 of 52 animals). In the seven ewes in which there was an increase in LH, the response occurred at the expected time but was greatly reduced (14 versus 40 ng ml⁻¹), and occurred only in ewes pretreated with oestradiol implants for 2 or 4 days. However, we were unable to establish a robust linear time-dose relationship, i.e., when ewes were treated with lower doses of oestradiol (one or two implants) for a reduced time (4 or 8 days), there was a random distribution of the seven of 32 animals that had a reduced LH surge after oestradiol challenge. These results are compatible with the disrupted ovarian cyclicity observed by Bilfiar et al. (1989) who treated primates with high doses of oestradiol for 4 years and Hemmings et al. (1983) who similarly treated rats for 9 weeks. The present study enhances this information by being the first to show that continuous administration of physiological doses of oestradiol for only 2–4 days can inhibit surge release of LH. After high or low doses (0.5 mg or 0.5 \( \mu \)g) of GnRH, LH was secreted, albeit reduced by 50%; however, the self-priming effect was still evident in ewes treated with oestradiol implants. This finding suggests that there may be a partial effect at the pituitary, but to abolish the surge completely after oestradiol injection there must also have been an effect at the hypothalamus.
Table 3. Characteristics (mean ± se) of the LH surge after initial implant insertion and after oestradiol challenge at the end of steroid pretreatment in Expt 2

<table>
<thead>
<tr>
<th>Post-implant</th>
<th>Post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to LH surge onset (h)</td>
<td>Duration (h)</td>
</tr>
<tr>
<td>Expt 2a</td>
<td></td>
</tr>
<tr>
<td>Group 7a (8 days)</td>
<td>NBS</td>
</tr>
<tr>
<td>Group 7b</td>
<td>NBS</td>
</tr>
<tr>
<td>Group 7c</td>
<td>NBS</td>
</tr>
<tr>
<td>Group 8a (4 days)</td>
<td>—</td>
</tr>
<tr>
<td>Group 8b</td>
<td>21.5 ± 1.5⁴</td>
</tr>
<tr>
<td>Group 8c</td>
<td>19.8 ± 1.0⁴</td>
</tr>
<tr>
<td>Expt 2b</td>
<td></td>
</tr>
<tr>
<td>Group 9a (2 days)</td>
<td>30.25</td>
</tr>
<tr>
<td>Group 9b</td>
<td>25.8 ± 1.6⁶</td>
</tr>
<tr>
<td>Group 9c</td>
<td>26.5 ± 1.5⁶</td>
</tr>
<tr>
<td>Group 10a</td>
<td>29</td>
</tr>
<tr>
<td>Group 10b</td>
<td>30.8 ± 1.7⁶</td>
</tr>
<tr>
<td>Group 10c</td>
<td>31.8 ± 1.3⁶</td>
</tr>
<tr>
<td>Expt 2c</td>
<td></td>
</tr>
<tr>
<td>Group 11a</td>
<td>25.5 ± 1.9⁷</td>
</tr>
<tr>
<td>Group 11b</td>
<td>25.5 ± 0.9⁷</td>
</tr>
<tr>
<td>Group 11c</td>
<td>28.5 ± 1.6⁷</td>
</tr>
</tbody>
</table>

Values in italics represent results of individual animals. NBS, blood samples not taken from ewes; —, no surge detected. Within a column, values with different superscripts are significantly different (P < 0.05).

The failure of the control ewes in group 9 and 10 to have an LH surge after implantation of oestradiol for 8 h reflects previous observations that a minimum duration of oestradiol stimulus is required to promote an LH surge (12 h mon-

Fig. 3. Area under the LH curve (± SE) after each GnRH challenge at different times after the first oestadiol injection in four ewes per group. □, no implant plus oestradiol challenge (group 11a); ■, oestadiol implant, no oestradiol challenge (group 11b); □, oestadiol implant plus oestradiol challenge (group 11c). Values with different superscripts are significantly different (P < 0.05).
however, could also represent a reduction in the amount of GnRH secreted from the hypothalamus. Similarly, a reduction in LH synthesis could be a reflection of the negative feedback effect of oestradiol on the hypothalamus which again would reduce GnRH secretion. A decrease in pulsatile GnRH stimulation of the pituitary is known to inhibit LH synthesis (Kaiser et al., 1997). All of these mechanisms could have a role in the inhibition of the LH surge observed in the present study.

In the hypothalamus, GnRH neurones do not have oestradiol receptors but are indirectly controlled by interneurones sensitive to catecholamines or opioids (Herbison, 1995). Noradrenaline is excitatory to GnRH secretion whereas gamma amino-butyric acid and opioids have inhibitory effects (Robinson, 1995; Herbison, 1997). Most of these interneurones are influenced by oestradiol, and the effects of prolonged oestradiol treatment observed in the present study may be mediated via disruption of neurotransmitter activity. For example, chronic anovulation and polycystic ovaries have been observed 60 days after injection of oestradiol valerate (Brawer et al., 1978). Brawer et al. (1993) proposed that oestradiol is selectively toxic to β-endorphin neurones in the hypothalamic arcuate nucleus. The mechanism underlying this neurotoxic action appeared to involve the conversion of oestradiol to catechol oestrogen. The oestradiol-induced loss of β-endorphin neurones may generate a compensatory increment in µ-opioid binding in the medial preoptic area rendering this region supersensitive to residual β-endorphin or to other endogenous opioids. This persistent opioid inhibition may result in a cascade of catecholamine deficits that were ultimately expressed as a chronically attenuated plasma LH pattern (Brawer et al., 1993). Such opioid-mediated effects of prolonged oestradiol treatment remain to be investigated in sheep.

In conclusion, we have shown that continuous exposure to oestradiol at physiological values for 2–12 days will disrupt the LH surge. It remains to be confirmed whether this is mainly caused by effects at the pituitary reducing GnRH receptor or LH synthesis, or by effects at the hypothalamus via reduced GnRH secretion. The results may, however, explain the lack of the response to oestradiol injection previously observed in cattle with follicular cysts (Dobson and Nanda, 1992). The maximum concentration of oestradiol in peripheral plasma of animals with spontaneously occurring cysts is similar to that of the normal late follicular phase. By using physiological concentrations in the present experiment, we have shown that it is the duration of exposure to oestradiol which may cause endocrine lesions in the hypothalamus–pituitary axis rather than excessive concentrations. Evidence for correction of this endocrine lesion by administration of physiological doses of progesterone for 12 days strengthens the previously empirical choice of a similar treatment for clinical cases of bovine follicular cysts (Nanda et al., 1989).

M. Ozturk was supported by The Ministry of Agriculture and Rural Affairs, Turkey, by a scholarship from AFC, Ankara, and R. F. Smith was supported by a Veterinary Fellowship from The Wellcome Trust. The authors are also grateful to Jean Tebble, Hilary Purcell, Thelma Roscoe, Jean Sullivan and Rick Hollis for technical help; to Nigel Jones and his staff for animal care; and to NIAMDD, Bethesda, MD, for gonadotrophin assay reagents.

References

Billiar RB, Richardson DW and Little B (1989) Escape from chronic estrogenic suppression of ovarian function in the adult rhesus monkey: evidence for changing sensitivity of gonadotropin secretion to estrogen inhibition Endocrinology 124 2373–2382


Karsch FJ, Legan SJ, Ryan KD and Foster DL (1980) Importance of estradiol and progesterone in regulating LH secretion and estrous behavior during the sheep estrous cycle Biology of Reproduction 23 404–413

Legan SJ, Karsch FJ and Foster DL (1977) The endocrine control of seasonal reproductive function in the ewe: a marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion Endocrinology 101 818–824


