Effects of time after ovariectomy, season and oestradiol on luteinizing hormone and follicle-stimulating hormone secretion in ovariectomized ewes

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Summary. During the breeding season, five groups of three ewes were implanted at ovariectomy with 0.36, 0.5, 1.0 and 6.0 cm oestradiol implants or implants containing no steroid. Eleven days after receiving implants, blood samples were taken every 10 min for 6 h; implants were then removed. Treatments were repeated three times during each of two consecutive breeding seasons and four times during the intervening anoestrus. In ovariectomized ewes without steroid treatment, luteinizing hormone (LH) pulse frequency increased from early to mid-breeding season, decreased to a minimum at mid-anoestrus and increased to reach a maximum at the mid-point of the second breeding season, subsequently declining. LH pulse amplitude was inversely related to frequency. Basal serum LH concentrations decreased gradually from the first breeding season to reach a minimum at mid-anoestrus and gradually increased to reach a maximum at the end of the second breeding season. Mean serum LH and follicle-stimulating hormone (FSH) concentrations were higher at the end of the second breeding season compared with the beginning of the first breeding season.

All parameters of gonadotrophin secretion were decreased much more by oestradiol during the anoestrus than during the breeding season. LH pulse frequency was decreased during anoestrus and at high oestradiol concentrations during the first breeding season. Apart from LH pulse amplitude, the decreases in all parameters of gonadotrophin secretion were less during the second compared with the first breeding season. The minimum effective dose of oestradiol required to decrease mean and basal serum concentrations of LH during anoestrus was lower than in the breeding season. The minimum effective dose of oestradiol required to decrease mean serum concentrations of FSH was lower in the first compared with the second breeding season. Oestradiol depression of LH pulse amplitude and mean serum concentrations of LH and FSH showed a dose dependency during the breeding season. During anoestrus dose dependency was seen for basal concentrations of LH and mean serum concentrations of LH and FSH. We conclude that significant chronic changes in gonadotrophin secretion occur in the ewe with time after ovariectomy. Sensitivity to oestradiol also changes, and the effects of oestradiol are not always dose dependent. We suggest that the circannual pattern of LH pulse frequency and basal LH secretion are directly linked to the circannual cycle of photoperiod. Enhanced negative feedback depression of tonic gonadotrophin secretion by oestradiol during anoestrus may be symptomatic or result from the LH pulse generating systems being depressed below a certain threshold by a steroid independent mechanism, directly linked to the circannual cycle of photoperiod.

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Introduction

Withdrawal of ovarian steroids by ovariectomy leads to rapid and possibly long-term changes in the tonic secretion of gonadotrophins (Martin, 1984; Webb *et al.*, 1985). However, the reported changes in mean and basal serum concentrations of LH and LH pulse frequency and amplitude differ between studies; there is little information about FSH (Karsch *et al.*, 1980; Goodman *et al.*, 1982; Montgomery *et al.*, 1985, 1987; Thomas *et al.*, 1988). Secretory patterns for gonadotrophins in ovariectomized ewes, without steroid treatment, do show some loose linkage to the annual cycle of photoperiod, but this often appears to be lost in ovariectomized ewes that have been ovariectomized for longer (Karsch *et al.*, 1980; Goodman *et al.*, 1982; Montgomery *et al.*, 1985, 1987; Robinson *et al.*, 1985; Thomas *et al.*, 1988). A clear understanding of the changes of tonic gonadotrophin secretion after ovariectomy and with season would seem essential to make full use of the ovariectomized ewe as a model for studies on gonadotrophin secretion and seasonal breeding.

Oestradiol has been shown to depress tonic gonadotrophin secretion during the breeding season, with a marked enhancement of this effect during anoestrus (Pant *et al.*, 1978; Goodman & Karsch, 1980; Goodman *et al.*, 1980; Karsch *et al.*, 1980; Goodman *et al.*, 1981a; Rawlings *et al.*, 1984). However, when mean and basal serum LH concentrations and LH pulse frequency and amplitude are considered, many disagreements are found (Goodman *et al.*, 1982; Martin *et al.*, 1983; Montgomery *et al.*, 1985, 1987; Thomas *et al.*, 1988). In the few studies on the effects of oestradiol on FSH secretion, depression was suggested (Goodman *et al.*, 1981b; Rawlings *et al.*, 1984; Webb *et al.*, 1985). It is unclear whether the effects of oestradiol on gonadotrophin secretion are dose related, although this has been suggested in anoestrus ewes (Goodman *et al.*, 1980; Thomas *et al.*, 1988). It has been suggested that the annual cycle of photoperiod regulates tonic gonadotrophin secretion primarily by an oestradiol-dependent mechanism, while a steroid-independent mechanism is equivocal (Robinson *et al.*, 1985; Goodman, 1988).

The present study was therefore designed to (1) characterize the tonic gonadotrophin secretory patterns in the ovariectomized ewe with time after ovariectomy, (2) detect the lowest effective dose of serum oestradiol that would depress basal gonadotrophin secretion during the breeding season and anoestrus and to test for a dose-response and (3) examine the pattern and interrelationship of steroid-independent and oestrogen-dependent photoperiodic regulation of tonic gonadotrophin secretion during the breeding season and anoestrus.

Materials and Methods

Experimental animals

Fifteen sexually mature Rambouillet $\times$ Columbia crossbred ewes were kept outdoors in dry lots, except during periods of experimentation. During the periods of treatment the ewes were moved indoors and kept under fluorescent lighting, with light exposure set to simulate normal day length. The ewes were fed rations of alfalfa pellets supplemented with hay. Cobalt iodized salt and water were freely available. Oestrus was synchronized using a 12 day treatment of intravaginal sponges containing 60 mg medroxyprogesterone acetate (Veramix: Tuco Products Company, Orangeville, Ontario, Canada). Six days after oestrus, ovariectomy was performed at mid-ventral laparotomy under general anaesthesia. The presence of corpora lutea in all ewes at ovariectomy confirmed that they were cycling at the onset of the experiment.

Implants

The steroid implants were prepared by mixing 10% (w/w) steroid with liquid medical grade elastomer (Silastic, 382 Medical grade elastomer: Dow Corning, Midland, MI, USA) and a curing catalyst (Catalyst: Dow Corning, Midland,

MI, USA). The oestradiol (Sigma Chemical Company, St Louis, MO, USA) elastomer mixture was injected into Silastic tubing, 0.22 cm i.d. $\times$ 0.24 cm o.d. or 0.32 cm i.d. $\times$ 0.48 cm o.d. (Dow Corning, Midland, MI, USA). The oestradiol implants were cut into appropriate lengths (Table 1).

Table 1. Dimensions of oestradiol implants, mean *in vitro* concentrations of oestradiol released per day over 19 days ($n = 57$ in all cases) and mean serum oestradiol concentrations created in ovariectomized ewes during all three seasons ($n = 30$ in all cases)

Dimension of oestradiol implant (diameter $\times$ length) (cm $\times$ cm)	<i>In vitro</i> concentration of oestradiol (ng ml ⁻¹ saline day ⁻¹)	Serum oestradiol concentration (pg ml ⁻¹)
Blank (0.32 $\times$ 1.0)	—	3.19 $\pm$ 0.14 ^a
0.22 $\times$ 0.36	61 $\pm$ 6 ^a	5.07 $\pm$ 0.15 ^b
0.32 $\times$ 0.50	180 $\pm$ 13 ^b	7.09 $\pm$ 0.14 ^c
0.32 $\pm$ 1.0	259 $\pm$ 14 ^c	8.96 $\pm$ 0.20 ^d
0.32 $\pm$ 6.0	818 $\pm$ 46 ^d	15.8 $\pm$ 0.99 ^e

Numbers in columns with different superscripts are significantly different ($P < 0.05$).

The implants were incubated in sterile 0.9% (w/v) NaCl (saline) for 24 h, at 37°C, under constant agitation, before insertion. Implants were introduced subcutaneously in the axillary region under local anaesthesia (Duracaine: Roger/STB Inc., Montreal, Quebec, Canada).

The release pattern of steroid from the oestradiol implants were checked *in vitro*. The implants were placed in 16 cm $\times$ 150 cm borosilicate glass tubes ($n = 3$) and 18 ml of 0.1% phosphate-buffered saline (PBS) was added to each tube and incubated at 37°C in a water bath under constant agitation. At the end of 24 h, a 5 ml sample of buffer was withdrawn from each tube and the remainder was discarded and replaced with 18 ml of fresh 0.1% PBS. The procedure was repeated for 19 days and the samples were frozen until assayed for oestradiol.

Experimental protocol

Five groups of three ewes were implanted at ovariectomy in late October (early breeding season) with four different sizes of Silastic rubber implants containing oestradiol, or with blank implants containing no steroid (Table 1). Implants were left in place for 12 days and blood samples (10 ml) were collected by jugular venepuncture every other day for measurement of serum oestradiol concentrations. Ten days after implantation (early November), indwelling jugular catheters (i.d. 1.00 mm; o.d. 1.50 mm, SV-70, Dural Plastics and Engineering, Dural, NSW) were inserted and on day 11 blood samples were collected every 10 min for 6 h, for characterization of tonic LH and FSH secretion. All serum samples were stored at -20°C until assayed for oestradiol, FSH and LH.

At six week intervals, the same group of ewes was randomly redistributed to the implant treatment groups described in Table 1. Ewes were treated three times during each of two consecutive breeding seasons and four times during the intervening single anoestrus, with the last treatment in mid-January, that is, late in the second breeding season, to give a total of ten periods of treatment. Demarcation of breeding season was based on previous observations (Jeffcoate *et al.*, 1984).

Hormone assays

Serum concentrations of FSH and LH were determined in validated radioimmunoassays (Rawlings *et al.*, 1984; Currie & Rawlings, 1989). Concentrations of FSH and LH are expressed in terms of NIDDK-oFSH-RP1 and NIDDK-oLH-24, respectively. The sensitivity of the gonadotrophin and oestradiol assays is expressed as the lowest standard different from zero, using an unpaired *t* test. The limit of detection was 0.25 ng ml⁻¹ and 0.06 ng ml⁻¹ for FSH and LH, respectively. Intra- and inter-assay coefficients of variation (CVs) for ovine reference sera with mean FSH concentrations of 14.1 or 1.13 ng ml⁻¹ were 6.6% ($n = 8$) and 9.6% ($n = 87$) or 7.6% ($n = 11$) and 9.7% ($n = 110$) respectively. Intra- and inter-assay CVs for ovine reference sera with mean LH concentration of 3.01 or 0.15 ng ml⁻¹ were 5.0% ($n = 18$) and 9.9% ($n = 110$) or 9.6% ($n = 18$) and 14.9% ($n = 110$), respectively.

Oestradiol was measured using a modification of an assay previously validated and published (Rawlings *et al.*, 1984; Currie & Rawlings, 1989). The changes were that ether extracts of serum were assayed without the previous partition step and the assay standards were prepared in charcoal stripped serum and extracted with ether before assay, rather than being put directly into assay tubes. Extraction efficiency was $87 \pm 0.5\%$ ($n = 96$; $\bar{x} \pm \text{SD}$). Sensitivity was 1 pg ml⁻¹ serum. When extracts of charcoal stripped serum were analysed, values did not differ significantly from zero ($P > 0.10$). When 2 or 10 pg of oestradiol was added to 1 ml of serum from ewe, the values obtained after correction for endogenous oestradiol were 2.6 ± 0.25 pg ($n = 96$) ($\bar{x} \pm \text{SD}$) and 11.2 ± 0.34 pg ($n = 97$) respectively. Serum

concentrations of oestradiol measured in this assay in ovariectomized ewes, were 3.2 ± 0.1 pg ml⁻¹ ($\bar{x} \pm$ SD) and, in intact ewes, were 4.7 ± 0.7 pg ml⁻¹ and 12.7 ± 3.3 pg ml⁻¹ in the midluteal and follicular phases of their oestrous cycles, respectively. These values are similar to those previously published (Pant *et al.*, 1977; Rawlings *et al.*, 1977; Herriman *et al.*, 1979; Hauger *et al.*, 1977). For sera with oestradiol concentrations of 23 pg ml⁻¹, 11.3 pg ml⁻¹ or 8.8 pg ml⁻¹, intra- and inter-assay CVs were 7.3% ($n = 2$) and 9.1% ($n = 10$), 11% ($n = 2$) and 11.6% ($n = 10$) or 9.4% ($n = 2$) and 12.5% ($n = 11$), respectively.

Statistical analysis

The PULSAR program (Merriam & Wachter, 1982) was used to identify LH pulse frequency in pulses h⁻¹, amplitude in ng ml⁻¹, basal serum LH and mean serum LH concentrations (ng ml⁻¹ serum). Basal serum LH values are those after termination of pulses. According to this method serum FSH concentrations did not show evidence of pulsatility and are expressed in ng ml⁻¹ serum.

Concentrations of oestradiol produced by the implants *in vitro*, over a 19 day period, were analysed using a two-way analysis of variance (ANOVA) and least squares difference (LSD) test (Steel & Torrie, 1980). The data for the first day of *in vitro* incubation were excluded from analysis because they were equivalent to the preincubation of the implants used *in vivo*. A correlation analysis was run between concentrations of oestradiol produced by implants *in vitro* and *in vivo* and these values and implant size.

Serum oestradiol concentrations for samples taken every other day during implant treatment of ewes and the serum LH and FSH data collected from the frequent bleeding at the end of each implant treatment period, were initially analysed using 5×10 factorial ANOVAs. Implant type or size (blank and various sizes of oestradiol implants) and period of treatment were the main effects. To isolate an interaction between oestradiol implant size and period of treatment, were analysed LH and FSH data for the first and second breeding seasons and the anoestrus, in separate 5×6 and 5×4 factorial ANOVAs, respectively. As serum oestradiol concentrations did not differ between periods of treatment, data for all periods of treatment were combined to correlate with the *in vitro* data for oestradiol release from the various sizes of implants.

The LH and FSH data from control ewes were re-analysed in a one-way ANOVA to show steroid independent effects of season and/or time after ovariectomy.

The percentage depression by oestradiol (difference between treatment and control, expressed as a percentage of control for the respective period of treatment) of each parameter of serum LH and FSH data was calculated to demonstrate steroid-dependent depression of tonic LH and FSH secretion. The percentage depression data, from animals treated with 0.5, 1 and 6 cm implants, were analysed as a 3×10 factorial ANOVA. The data from the animals treated with the 0.36 cm implant size were excluded from this analysis as the initial statistical analysis showed that this implant had little influence on tonic gonadotrophin secretion. A chi-square goodness-of-fit test was performed to determine the significance of the number of ovariectomized ewes that demonstrated LH pulses across all three seasons, when treated with 0.5, 1.0 and 6.0 cm sizes of oestradiol implants.

Results

In vitro and *in vivo* oestradiol release pattern

The concentrations of oestradiol produced *in vitro* and *in vivo* (Table 1) were positively correlated with the implant size, $r = 0.99$ ($P < 0.05$) and $r = 0.95$ ($P < 0.05$), respectively, and with each other, $r = 0.99$ ($P < 0.05$). Concentrations of oestradiol created *in vitro* or *in vivo* by oestradiol implants were slightly increased for the first 3 days and then remained stable for the next 17 or 9 days, respectively. The concentrations of oestradiol created by each size of oestradiol implant differed from all other implant sizes *in vitro* and *in vivo*. *In vivo* and *in vitro*, the oestradiol concentrations created by the smallest oestradiol implant (0.36 cm) differed from that recorded for the control implant.

Effects of time after ovariectomy and season on gonadotrophin secretion in ovariectomized ewes with no oestradiol treatment

LH pulse amplitude was highest at mid-anoestrus (Fig. 1), with gradual transitions to and from the low points seen at the middle of each breeding season ($P < 0.05$). Pulse frequency of LH showed the opposite trend and was negatively correlated with amplitude ($r = -0.91$, $P < 0.05$).

Mean serum LH concentrations were stable during the first breeding season and early anoestrus, rising subsequently to a peak late in the second breeding season ($P < 0.05$). Basal serum LH concentrations fell from the middle of the first breeding season to mid-anoestrus and then rose to their highest concentration late in the second breeding season ($P < 0.05$). Mean serum FSH concentration increased ($P < 0.05$) from the first (11.6 ± 1.12 ng ml⁻¹) to the second (24.3 ± 2.59 ng ml⁻¹) breeding season, no seasonal pattern was noted (Fig. 1).

Fig. 1. Changes in LH pulse amplitude (a), pulse frequency (b), mean serum LH (c), basal serum LH (d) and mean serum FSH (e) concentrations in ovariectomized ewes without oestradiol treatment. Blood was sampled every 10 min for 6 h on 10 occasions ($n = 3$ in all cases). The first blood sampling was in the early breeding season (mid-October), 11 days after ovariectomy, with subsequent samplings at 6-week intervals through anoestrus and to the latter part of a second breeding season (mid-January). Different letter superscripts indicate significant differences ($P < 0.05$).

Depression of gonadotrophin secretion by oestradiol and the effects of size of oestradiol implant, time after ovariectomy and season

The effects of oestradiol implant size and period of treatment were shown to be significant in an initial 5×10 factorial analysis ($P < 0.05$). The interaction between implant size and period of treatment was significant ($P < 0.05$) for mean and basal serum concentrations of LH; these interactions disappeared when the data for anoestrus were removed from the analysis.

(a) Effects of size of oestradiol implant. Serum gonadotrophin data for ewes with implants are summarized by implant type and size and by season (Figs 2–4). This simplification of the data presentation was justified because there were few individual treatment period effects within season but there were seasonal effects and an interaction between season and implant size. Serum oestradiol concentrations created by the 0.36 cm oestradiol implant depressed mean serum FSH concentrations during the first breeding season and mean and basal serum concentrations of LH during anoestrus only. The serum oestradiol concentrations created by the 0.5, 1.0 and 6.0 cm oestradiol implants depressed LH pulse amplitude and mean serum concentrations of LH and FSH during all three seasons of study (Figs 2–4). Dose dependency was apparent for mean serum concentrations of LH and FSH and LH pulse amplitude during all three seasons with the exception of LH pulse amplitude during anoestrus. Dose dependency during the breeding season is illustrated for representative ewes in Fig. 5. The serum oestradiol concentrations created by the 0.5, 1.0 and 6.0 cm implants depressed LH pulse frequency during anoestrus, but during the breeding season, only the oestradiol concentrations created by the 6 cm implant decreased LH pulse frequency and then only during the first breeding season. Basal serum concentrations of LH were depressed at all seasons, but in the later breeding season only the 6 cm implant was effective (Figs 2–4); dose dependency was indicated only during anoestrus.

(b) Effects of season and time after ovariectomy. The variation in the percentage depression of gonadotrophin secretion by oestradiol with season and time after ovariectomy and the data for ewes treated with the 0.5, 1.0 and 6.0 cm implants were combined for summarization (Fig. 6). Data for ewes treated with the 0.36 cm implant were excluded as this implant produced only limited effects on parameters of gonadotrophin secretion. This data presentation is an attempt to simplify and illustrate the seasonal pattern of oestradiol effects on gonadotrophin secretion separately from trends in gonadotrophin secretion that are independent of oestradiol negative feedback. Depression of all parameters of gonadotrophin secretion by the serum oestradiol concentrations created by the 0.5, 1.0 and 6.0 cm oestradiol implants was greatest during anoestrus compared with either breeding season ($P < 0.05$). Depression of all parameters of gonadotrophin secretion, except for tonic LH pulse amplitude, was greater in the first compared with the second breeding season ($P < 0.05$). The transition in the depression of parameters of gonadotrophin secretion from breeding season to anoestrus was abrupt, except for LH pulse amplitude and mean serum concentrations of FSH, where the degree of depression changed gradually from mid-breeding season to mid-anoestrus. A similar gradual transition was also seen for LH pulse frequency from anoestrus to the second breeding season (Fig. 6). During anoestrus, from the earliest to latest treatment period, 3/9, 0/9, 1/9 and 6/9 ewes had pulses respectively. During the breeding seasons, no fewer than 8/9 ewes had pulses at the six treatment periods. The calculated χ^2 statistic ($\chi^2 = 18.8$) was significant ($P < 0.05$), indicating a significant difference between breeding season and anoestrus.

Discussion

These results show that ovariectomized ewes not treated with oestradiol have a circannual pattern of LH pulse frequency apparently linked to the annual pattern of photoperiod. Pulse frequency of LH was at its peak at mid-breeding season in December and lowest in mid-anoestrus in June–July. In intact ewes ovulation rate is at its peak in mid-breeding season (Ortavant *et al.*, 1988). These photoperiod-linked patterns in LH pulse frequency have not always been noticed in the past. In Ile-de-France ewes, ovariectomized during the breeding season and followed for one year, LH pulse frequency and amplitude both appeared to peak in the middle of the first breeding season of the study, but no such pattern was seen in the subsequent breeding season (Montgomery *et al.*, 1985). In ovariectomized Suffolk ewes studied for one year, LH pulse frequency was low in the initial anoestrous period of the study (Robinson *et al.*, 1985), rose during the early breeding season but then remained fairly constant, with a limited decline during the ensuing seasonal anoestrus. In

Fig. 2. LH pulse amplitude (a), LH pulse frequency (b), mean serum LH (c), basal serum LH (d) and mean serum FSH (e) concentrations in ovariectomized ewes treated with different sizes of oestradiol implants during the first breeding season of the study ($n = 12$ in all cases). Data are based on blood samples taken every 10 min for 6 h, 11 days after insertion of implants; implants were inserted at ovariectomy and again 6 and 12 weeks later. Different letter superscripts indicate significant differences ($P < 0.05$).

the present study, LH pulse amplitude was inversely related to frequency; this relationship has been seen before in some (Karsch *et al.*, 1980) but not all studies (Rawlings *et al.*, 1984; Montgomery *et al.*, 1985). This suggests that as LH pulse frequency increases, there is less LH available for each pulse (Clarke & Cummins, 1985).

The marked annual cycle of basal serum LH concentrations in our untreated ovariectomized ewes was not found by Montgomery *et al.* (1985), but in both cases an increase in basal LH secretion occurred with time after ovariectomy. This emphasizes the possible existence of basal secretion of LH as an entity unrelated to LH pulse frequency. The increase in basal serum LH concentrations, with time after ovariectomy, caused the increase in mean serum LH concentrations seen in the second breeding season of the present study. These long-term shifts in basal and mean serum LH concentrations after ovariectomy have not always been evident in the past (Goodman & Karsch, 1981).

Fig. 3. LH pulse amplitude (a), LH pulse frequency (b), mean serum LH (c), basal serum LH (d) and mean serum FSH (e) concentrations in ovariectomized ewes treated with different sizes of oestradiol implants during anoestrus ($n = 12$ in all cases). Data is based on blood samples taken every 10 min for 6 h, 11 days after insertion of implants. Implants were inserted at 18, 24, 30 and 36 weeks after ovariectomy. Different letter superscripts indicate significant differences ($P < 0.05$).

The lack of variation in mean serum concentrations of LH with season in our untreated ovariectomized ewes agrees with previous findings (Goodman & Karsch, 1981). The fairly constant mean serum LH concentration seen over most of the present study reflect the inverse relationship between LH pulse frequency and both basal LH concentrations and LH pulse amplitude.

In studies on the control of gonadotrophin secretion in ewes less consideration has been given to FSH. Our results and those of Montgomery *et al.* (1987) show that mean serum concentrations of FSH rise with time after ovariectomy, with little apparent influence of season.

The serum concentrations of oestradiol produced by the implants in the present study encompassed a similar range to those for intact ewes. The reports of effects of oestrogen treatment on LH pulse amplitude in ovariectomized ewes are inconsistent. There are reports ranging from a small increase (Goodman *et al.*, 1982; Martin *et al.*, 1983; Thomas *et al.*, 1988), or no effect (Thomas *et*

Fig. 4. LH pulse amplitude (a), LH pulse frequency (b), mean serum LH (c), basal serum LH (d) and mean serum FSH (e) concentrations in ovariectomized ewes treated with different sizes of oestradiol implants during the second breeding season of the study ($n = 12$ in all cases). Data are based on blood samples taken every 10 min for 6 h, 11 days after insertion of implants. Implants were inserted at 42, 48 and 54 weeks after ovariectomy. Different letter superscripts indicate significant differences ($P < 0.05$).

al., 1988), to a marked reduction (Pant *et al.*, 1978; Goodman & Karsch, 1980; Goodman *et al.*, 1981b; Rawlings *et al.*, 1984), both during the breeding season and seasonal anoestrus. These conflicting results are probably due to variation in the doses of oestrogens used and, perhaps, the breed of ewe. The influence of oestradiol on LH pulse amplitude in our work indicated a hypersensitivity of the hypothalamic-pituitary axis to the negative feedback effect of oestradiol during anoestrus, but a similar threshold for effect.

Previous studies have not revealed a reduction in the frequency of LH pulses by oestradiol during the breeding season (Goodman & Karsch, 1980; Goodman *et al.*, 1981b; Thomas *et al.*, 1988). This was largely confirmed in our study. The ability of oestradiol to depress LH pulse

Fig. 5. Serum LH profile of an ovariectomized ewe (control) ($\square$) with no oestradiol treatment and representative ovariectomized ewes ($\blacklozenge$) treated with 0.36 cm (a), 0.5 cm (b), 1.0 cm (c) and 6.0 cm (d) of oestradiol implants during the first breeding season of the study (treatment period 2). Implants were in place for 11 days before blood was sampled every 10 min for 6 h. Ovariectomy was performed approximately 7 weeks prior to this blood sampling period.

amplitude, but not frequency, in the ovariectomized ewe, during the breeding season, is in agreement with previous reports (Goodman & Karsch, 1980; Goodman *et al.*, 1981b, 1982). Reduction of LH pulse frequency by oestradiol during anoestrus has been well documented (Goodman & Karsch, 1980; Goodman *et al.*, 1981b, 1982). However, our observations did not reveal dose dependency, in contrast with other reports (Thomas *et al.*, 1988), where a marked dose dependency was noted during anoestrus.

Reports on the effect of oestradiol on mean and basal serum LH concentrations and mean serum FSH concentrations in ovariectomized ewes are sparse. Oestradiol has been shown to depress mean serum concentrations of LH during the breeding season and anoestrus in ovariectomized ewes, with greater depression seen during anoestrus (Goodman *et al.*, 1980; Karsch *et al.*, 1980; Goodman *et al.*, 1981a). However, in previous studies a dose response was not evident (Goodman *et al.*, 1980). Our data show that depression of mean serum LH concentrations by oestradiol during the breeding season reflects depression of LH pulse amplitude and basal serum LH concentrations. The greater depression of mean serum LH concentrations by oestradiol during anoestrus would appear to reflect the additional reduction of LH pulse frequency, as well as greater depression of LH pulse amplitude and basal serum concentrations of LH. Oestradiol administration during the breeding season has been found to have no effect on basal LH in ovariectomized Suffolk ewes and actually caused an increase in Merinos (Thomas *et al.*, 1988).

Oestradiol can suppress FSH concentrations in ovariectomized ewes during the breeding season (Goodman *et al.*, 1981a) and anoestrus (Webb *et al.*, 1985). In addition to this, data from the present study demonstrated a clear dose-response relationship during the breeding season and anoestrus with a threshold for effect, and a greater depression during anoestrus. In general, the ability of oestradiol to depress gonadotrophin secretion decreased with time in our ovariectomized

Fig. 6. Depression of LH pulse amplitude (a), pulse frequency (b), mean serum LH (c), basal serum LH (d) and mean serum FSH (e) concentrations in ovariectomized ewes, by serum oestradiol concentrations created by 0.5, 1.0 and 6.0 cm implants. Depression is expressed as a percentage of control values (see text for details). Data points are based on ten separate intensive bleedings, where samples were taken every 10 min for 6 h, 11 days after implant insertion. Implants were first inserted at ovariectomy early in the breeding season (October) and treatments were repeated every 6 weeks, three times in breeding season 1, four times in anoestrus and three more times in a second breeding season, with the final treatment late in the season (January). Different letter superscripts indicate significant differences ($P < 0.05$). No pulses were recorded at 24 weeks and only one at 30 weeks.

ewes, indicating, as for progesterone, that steroid feedback sensitivity declines with time after ovariectomy (Goodman, 1988).

Measurable serum concentrations of oestradiol were shown to be present in nonoestradiol-treated ovariectomized ewes. Charcoal-stripped serum blanks in the assay never gave recorded

values in terms of the oestradiol standards used. This suggests that some measurable oestradiol is present in ovariectomized ewes, an adrenal source is possible (Montgomery *et al.*, 1985). Oestradiol concentrations in ovariectomized ewes not treated with oestradiol were lower than in those fitted with the smallest oestradiol implant (0.36 cm). This small implant was, in most instances, ineffective in depressing gonadotrophin secretion allowing the delineation of a threshold concentration of oestradiol for depression of gonadotrophin secretion. The very low concentrations of oestradiol seen in control ewes and ewes treated with the smallest oestradiol implant did not produce significant depression of gonadotrophin secretion. We have assumed that the control ewes in the present study show steroid-independent effects of photoperiod changes. The difference in gonadotrophin values between steroid treated and nonoestradiol-treated ewes should reveal oestradiol-dependent influences.

It has been suggested that steroid-dependent and -independent photoperiod driven control of tonic gonadotrophin secretion in the ovariectomized ewe are different, mainly because their seasonal patterns are quite distinct (Robinson *et al.*, 1985). Steroid-dependent effects changed markedly at the onset and end of anoestrus, but steroid-independent effects followed a less distinct profile (Robinson *et al.*, 1985). In our data a clear annual cycle in basal serum LH concentration and LH pulse frequency and amplitude was seen, which appeared clearly linked to the annual cycle of photoperiod. It could be argued that enhanced negative feedback depression of gonadotrophin secretion by oestradiol is manifest when tonic LH pulse frequency, basal LH secretion or both, fall below a set point and that the depression is removed when the setpoint is reached at the end of anoestrus. This suggests that photoperiod directly influences tonic LH pulse frequency as originally postulated (Goodman & Karsch, 1981) and that negative feedback sensitivity to oestradiol is not a separate mechanism independently linked to photoperiodic patterns. The superimposition of enhanced negative-feedback depression of gonadotrophin secretion by oestradiol during anoestrus may act as a failsafe mechanism to delimit anoestrus more precisely. Clearly, the enhanced effectiveness of oestradiol in negative-feedback depression of gonadotrophin secretion during anoestrus is dramatic, reversing the steroid-independent trend in tonic LH pulse amplitude and imposing a marked seasonal trend on mean serum concentrations of LH and FSH.

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