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Effects of Progesterone on the Responses of Merino Ewes to the Introduction of Rams during Anoestrus

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Abstract

The effects of progesterone on the responses of Merino ewes to the introduction of rams during anoestrus were investigated in two experiments. In the first experiment, the introduction of rams induced an increase in the levels of LH in entire ewes. The mean levels increased from $0{\cdot}68 \pm 0{\cdot}04$ ng/ml (mean $\pm$ s.e.m.) to $4{\cdot}49 \pm 1{\cdot}32$ ng/ml within 20 min in ewes not treated with progesterone ($n = 10$). In ewes bearing progesterone implants that provided a peripheral concentration of about 1·5 ng progesterone per millilitre plasma, the LH response to the introduction of rams was not prevented, but was reduced in size so that the concentration was $1{\cdot}38 \pm 0{\cdot}15$ ng/ml after 20 min ($n = 5$). Progesterone treatment begun either 2 days before or 6 h after the introduction of rams and maintained for 4 days prevented ovulation.

In the second experiment ovariectomized ewes were used to investigate further the mechanism by which the ram evoked increases in tonic LH secretion. In ovariectomized ewes treated with oestradiol implants, the introduction of rams increased the frequency of the LH pulses and the basal level of LH. In the absence of oestradiol there was no significant change in pulse frequency but a small increase in basal levels. Progesterone again did not prevent but reduced the responses in ewes treated with oestradiol.

It is suggested that following the withdrawal of progesterone treatment, the secretion of LH pulses in response to the ram effect would be dampened. This effect could be a component of the reported long delay between the introduction of rams and the preovulatory surge of LH in ewes treated with progesterone. Continued progesterone treatment prevented ovulation, probably by blocking positive feedback by oestradiol.

Introduction

Introducing rams will induce ovulation in seasonally anoestrous Merino ewes if the ewes have been conditioned by a period of isolation from the rams (Underwood *et al.* 1944; Schinckel 1954). The first known endocrine response to this ram stimulus is an increase in the frequency of luteinizing hormone (LH) pulses (Martin *et al.* 1980*a*, 1980*b*; Poindron *et al.* 1980). This is followed about 20 h later by a preovulatory surge of LH (Oldman *et al.* 1978; Knight *et al.* 1978; Martin *et al.* 1980*a*) and ovulation. This ovulation is not usually accompanied by oestrus (Schinckel 1954) and a large proportion of the corpora lutea formed regress prematurely so that the first ovulatory cycle is short and a second ovulation is observed within 10 days of the introduction of rams (Oldham and Martin 1978; Martin 1979). If the ewes are treated with progesterone prior to the introduction of rams, they exhibit oestrus with the first ovulation (Hunter *et al.* 1971) and all of

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the corpora lutea are maintained for the normal period of time (Oldham *et al.* 1980; Martin *et al.* 1981). Progestogen treatment with intravaginal sponges or subcutaneous implants must be withdrawn on the day of ram introduction if the ewes are to exhibit normal oestrus (Hunter *et al.* 1971; Oldham *et al.* 1980; Martin *et al.* 1981). If intramuscular injections of progesterone in oil are used, the last injection of a series is also given at the same time as the rams are introduced (Cognié *et al.* 1980), as is the single injection which prevents short cycles but does not ensure oestrus (Cognié *et al.* 1982; Lindsay *et al.* 1982). Progesterone suppresses the tonic secretion of LH in ewes during the breeding season (Hauger *et al.* 1977) and, since up to 6 h is required for the absorption and clearance of the steroid after an injection (Shelton *et al.* 1972), the progesterone in the peripheral circulation during this period may inhibit the increase in pulsatile secretion of LH that would be induced by the introduction of rams. However, ewes pretreated with progesterone have a normal ovulatory response to the introduction of rams (Oldham *et al.* 1980), inferring either that the increase in pulsatile secretion of LH is not essential if the ewe is to ovulate or that progesterone does not completely inhibit the increase in pulsatile secretion of LH in response to the introduction of rams.

The events between the initial increase in the frequency of the LH pulses and the onset of the preovulatory surge of LH are still not entirely clear. Following our initial studies, we suggested that the LH surge could be the result of direct action of the ram stimulus on LH release (Oldham *et al.* 1978; Knight *et al.* 1978). However, we were unable to show that the introduction of rams could evoke an LH surge in ovariectomized ewes, even in the presence of low concentrations of oestrogen (Martin *et al.* 1983*b*). It therefore seemed plausible that the surge resulted from the positive feedback exerted by oestrogen that was secreted in response to the increased frequency of LH pulses, as happens in the cyclic ewes (Baird 1978). Positive feedback by oestradiol can be inhibited by progesterone (Pelletier and Signoret 1969; Scaramuzzi *et al.* 1971). Therefore, maintenance of high progesterone levels around the time at which rams are introduced could inhibit ovulation if positive feedback by oestradiol is an important component of the mechanism of the ram effect.

It was therefore proposed to test whether progesterone could inhibit either the increase in the tonic secretion of LH or ovulation induced in anoestrous ewes by the introduction of rams. Ovariectomized ewes also respond to the introduction of rams with an increase in the frequency of their LH pulses (Martin *et al.* 1983*b*), so it was proposed to test further the effects produced by the introduction of rams and progesterone in these ewes.

Materials and Methods

Experiment 1: *Entire Ewes*

From a flock of 51 mature Merino ewes which had been isolated from rams for 6 weeks during November and December 1979 (mid to late anoestrus, Underwood *et al.* 1944), 13 were chosen at random and each was given one subcutaneous progesterone implant ('Silestrus', Abbott Laboratories, Sydney). Two days later, the entire flock was subjected to laparoscopy (Oldham *et al.* 1976) and nine (18%), including two of those with implants, were found to have corpora lutea or corpora albicantia on their ovaries. The 42 remaining anovulatory ewes were then divided into four groups:

Group A (n = 10) was kept in complete isolation from rams;
Group B (n = 11) was introduced to rams;
Group C (n = 10) was treated with progesterone starting 6 h after introduction of rams;
Group D (n = 11) was given progesterone implants two days prior to the introduction of rams.

Five ewes in each of groups B, C and D were catheterized without anaesthesia and the following day jugular blood (3 ml) was sampled at 15-min intervals from 2½ h before until 8 h after the introduction of eight vasectomized rams. The ewes were not heparinized but the catheters were flushed after each sample with 3 ml of heparinized (5 i.u./ml) saline. All of the ewes in group C received an intravenous injection of 10 mg of progesterone (Sigma) in 1 ml 50% (v/v) ethanol when they were implanted with progesterone, 6 h after the introduction of rams. This treatment was chosen on the following basis: the LH surge is observed in anoestrous ewes 20–30 h following the introduction of rams (Oldham *et al.* 1978; Knight *et al.* 1978; Poindron *et al.* 1980; Martin *et al.* 1980*a*); an LH surge is observed in ovariectomized or anoestrous ewes 12–15 h after an injection or infusion of oestrogen (Goding *et al.* 1969; Scaramuzzi *et al.* 1971); thus if oestradiol is mediating the effect of rams, sufficient must be secreted by the ovary in response to the pulses of LH induced by the rams in the first 6 h or so following their introduction. It was therefore proposed to avoid the suppressive effects of progesterone on tonic secretion of LH in this period, but then increase the peripheral levels precisely 6 h after the introduction of rams. Thus, if progesterone at this time failed to block ovulation, it would indicate that positive feedback by oestrogen was not involved. Conversely, if ovulation was blocked it would indicate that positive feedback was involved and that the progesterone had inhibited the action of oestrogen in this regard (Pelletier and Signoret 1969; Scaramuzzi *et al.* 1971).

Four days after the introduction of rams the ewes again underwent laparoscopy to determine the number which had ovulated. Upon inspection it was found that one of the ewes in group C had lost its implant, so its data for ovulation were omitted from the analysis.

The concentration of progesterone was measured in the first and last blood samples from each ewe, and also in the blood samples taken just before and 2 h after the injection of progesterone in the ewes in group C. The levels of LH were measured in all of the samples.

Experiment 2: Ovariectomized Ewes

Eighteen months after ovariectomy a flock of 16 ewes was isolated from rams for a period of 8 weeks, and then divided into four equal groups:

Group E—controls, no steroid treatment;
Group F—given subcutaneous implants containing oestradiol;
Group G—given progesterone (Silestrus) implants;
Group H—given both oestradiol and progesterone implants.

The construction and testing of the oestradiol implants has been described in detail elsewhere (Martin *et al.* 1983*a*). Briefly, they were made according to the method of Karsch *et al.* (1973) but using smaller-size silastic tubing (5 mm long, 2·41 mm o.d., 1·57 mm i.d., Dow Corning, Michigan, U.S.A). The rate of release into serum, measured *in vitro* at 37°C with implants containing labelled hormone, was 3·5±0·3 μg oestradiol-17β per day (mean ±s.e.m., $n = 4$).

Six days after implantation, the ewes were cannulated as described earlier and during the following day blood samples were drawn every 15 min for 12 h. Eight rams were placed among the ewes at noon, 6 h after sampling had commenced. All of the samples were assayed for LH and the first and last samples for each ewe were also assayed for progesterone.

The experiment was carried out in November, near the end of the anoestrous season for Merino ewes in Western Australia (Underwood *et al.* 1944).

Hormone Assays

Levels of LH were measured in duplicate aliquots of 0·1 ml plasma by double-antibody radioimmunoassay as described previously (Martin *et* al. 1980*b*). The activity of the LH standard (M3-CNRS) was 1·8 i.u./mg NIH-LH-S1 (M. Jutisz: College de France), the limit of detection of the standard curve was 42±14 pg/tube (mean±s.e.m.) and non-specific binding was always less than 5%. Six replicates of three pooled samples, containing 1·18±0·05 ng/ml, 2·96±0·11 ng/ml and 5·51±0·17 ng/ml, were included in each assay to allow estimation of variation within and between the eight assays used in this study. The coefficients of variation within assays were 16·3±2·3, 6·7±1·4 and 5·3±1·0% for the respective pools. The coefficients of variation between assays were 8·0, 7·6 and 6·1%. The antiserum (UWA.3B) exhibited minor cross-reactions with NIH-FSH-S12 (0·9%), NIH-P-S12 (0·2%), NIH-TSH-S8 (4·6%) and NIH-GH-S11 (1·3%).

The concentration of progesterone was measured in duplicate aliquots of 0·1 ml plasma with a double-antibody radioimmunoassay, as described in detail by Martin *et al.* (1983*a*). The limit of detection of the standard curve was 28±1 pg/tube and non-specific binding was 2·0±0·3% in the four assays used in this study. Hexane and water blanks read 22±9 pg/tube. A pooled sample of plasma containing 2·30±0·13 ng/ml of progesterone was used to measure the coefficients of variation within assays (17·3±5·0%) and between assays (10·9%). The principal cross-reactions exhibited by the antiserum were 5β-pregnan-3,20-dione (25%), 11β-hydroxyprogesterone (11·5%), 21-deoxycortisol (4%) and corticosterone (1%).

Analysis of Data

In experiment 1, it was not possible to determine accurately the effects of the rams, or the effects of the progesterone treatments on the frequency of the LH pulses due to the short sampling period prior to ram introduction and the low, variable frequency of LH pulses in anoestrous Merino ewes (Martin *et al.* 1980*b*). The analysis of effects of treatments was therefore restricted to comparison of mean levels of LH. In experiment 2, however, analysis of pulse profiles was possible. The basal levels of LH, over which the pulses are superimposed, were defined as the mean of the 10 lowest samples in the profile, and were estimated before and after ram introduction. The pulses were identified and counted, as previously described (Martin *et al.* 1983*a*). Briefly, a pulse was defined as any increase in the level of LH that was completed within 30 min (two sample intervals) and was followed by a decline that commenced within 30 min of the attainment of the peak concentration. The increases and decreases in concentration exceeded the combined assay errors, as estimated from the coefficients of variation, for the two samples at either end of the changes in concentration.

For experiment 1, the effects of progesterone on the change in the mean concentration of LH following ram introduction was tested by analysis of variance. In experiment 2, there was significant heterogeneity in the variance (F_{max} test, Sokal and Rohlf 1969) of the data for pulse frequency (variance inversely proportional to mean) and LH concentration (variance directly proportional to mean), so the data were transformed prior to analysis for the effects of the steroid treatments. The concentration data (basal levels, changes in LH levels, and the changes in basal levels induced by the rams) were transformed logarithmically, the frequency data were squared, and the effects of oestradiol and progesterone were tested by analysis of variance. Means and 95% confidence limits were calculated, back-transformed, and listed in Table 2. The effects of the introduction of rams were tested by paired comparison analysis of variance without transformation (Sokal and Rohlf 1969).

Results

Experiment 1

In the ewes without progesterone implants, the levels of progesterone were undetectable, being below the assay blank (0·65±0·07 ng/ml). In the ewes in group D, which had been implanted two days prior to the experiment, the concentration was 1·52±0·28 ng/ml at the time of the introduction of rams. In the ewes of group C, the concentration of progesterone increased from undetectable levels to 304±65 ng/ml by the second hour after the intravenous injection.

The effect of the introduction of rams on the mean levels of LH in ewes is shown in Fig. 1. The levels are plotted separately for the ewes which were pretreated with progesterone (group D). In these ewes, the increase in LH due to the introduction of rams was significantly ($P = 0·012$) smaller than for the other two groups, and the mean levels of LH were always lower in ewes treated with progesterone than in the untreated ewes (Fig. 1). In individual animals, these effects of the presence of progesterone were characterized by a small, transient increase in the secretion of LH, with pulses of low amplitude. Only one ewe from each group did not show an increase (greater than assay error) in its levels of LH within the first sampling period following the introduction of rams. In the ewes which were given progesterone 6 h after the introduction of rams, there was a fall in the mean levels of LH

immediately after the injection. However, the final concentration of LH was not significantly lower than the pre-injection level, and there were no significant differences between the mean LH levels of groups B, C and D at the end of the sampling period. There was considerable variability in the levels of LH in the ewes not treated with

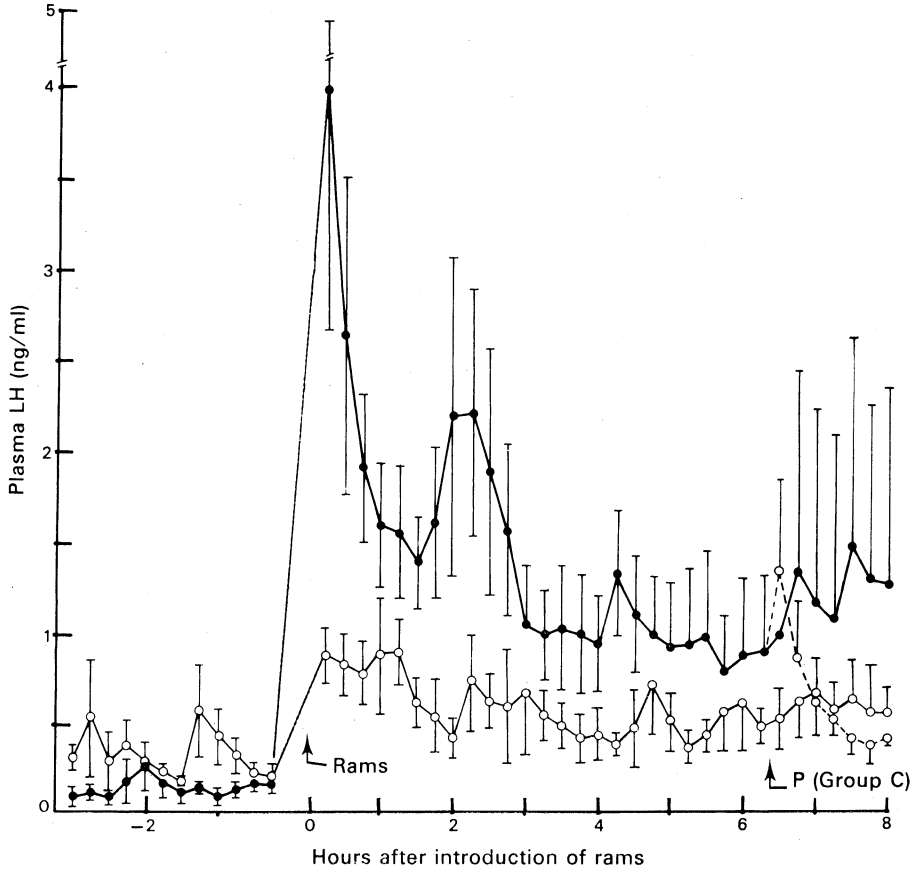

Fig. 1. Effects of introduction of rams and progesterone (P) on the basal levels of LH in anoestrous Merino ewes. Each point is the mean ±s.e.m. of all the ewes in the respective treatment groups. ●——● Group B (no progesterone treatment). ○---○ Group C (progesterone treatment 6 h after introduction of rams). ○——○ Group D (progesterone treatment 2 days prior to introduction of rams).

Table 1. Effects of progesterone (P) and the introduction of rams on ovulation in seasonally anovular ewes

Group	Treatment	*n*	Ovulated (%)
A	Control (−rams)	10	0 (0)
B	+Rams	11	5 (45)[B]
C	+Rams+P (6 h)	9[A]	0 (0)
D	+Rams+P (−2 days)	11	1 (9)

[A] One ewe lost implant and was omitted.
[B] Group B *v.* group A, $P = 0{\cdot}06$; group B *v.* (group C+group D), $P = 0{\cdot}025$.

Table 2. Effects of the introduction of rams on the tonic secretion of LH in ovariectomized ewes treated with oestradiol and progesterone

The means and 95% confidence limits (lower, upper) have been back-transformed after either logarithmic (basal level) or square (pulse frequency) transformation

Group	*n*	Basal LH level (ng/ml)			Pulse frequency (pulses/6 h)		
		Before rams	After rams	Change	Before rams	After rams	Change
E (control)	4	5·3 (2·3, 12·1)	7·2 (4·9, 10·6)	1·4 (0·5, 3·1)	5·6 (3·5, 7·2)	7·3 (6·5, 7·9)	1·6 (0·7, 3·2)
F (oestradiol)	4	4·5 (2·3, 8·7)	8·2 (5·0, 13·3)	6·7 (3·5, 8·9)	5·6 (1·2, 7·8)	7·6 (5·6, 9·2)	2·1 (1·0, 4·0)
G (progesterone)	4	4·0 (2·6, 6·2)	4·4 (2·8, 7·0)	0·4 (0, 1·2)	5·5 (4·7, 6·3)	6·6 (4·5, 8·2)	0·8 (0, 2·8)
H (oestradiol+ progesterone)	4	1·1 (0·4, 3·1)	1·4 (0·4, 4·8)	0·5 (0, 2·0)	2·8 (0, 4·6)	3·6 (0, 5·7)	0·6 (0, 1·9)

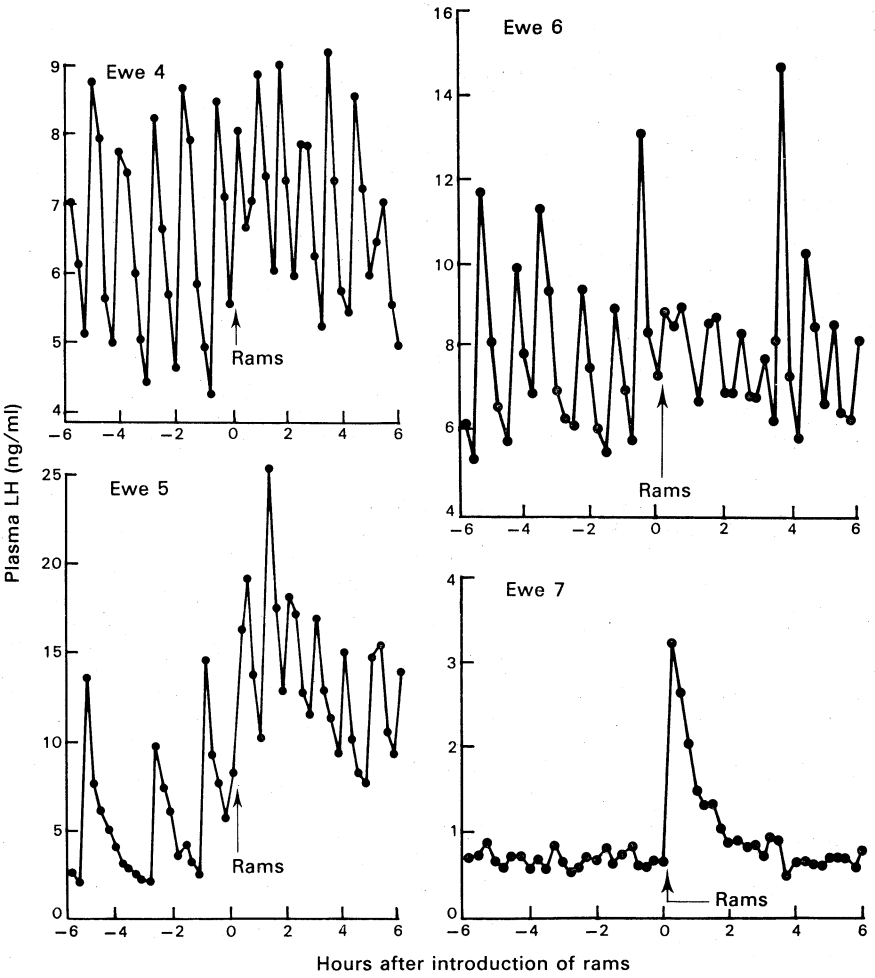

Fig. 2. Examples of the effect of the introduction of rams on the secretion of LH pulses in ovariectomized ewes treated with either no implant (ewe 4), an oestradiol implant (ewe 5), a progesterone implant (ewe 6) or both oestradiol and progesterone implants (ewe 7).

progesterone (Fig. 1) because most of them were experiencing frequent pulses of LH.

The introduction of rams induced a significant number of ewes to ovulate and, with the exception of one ewe in group D, corpora lutea were observed only among those not treated with progesterone (Table 1). There were no ovulations in the ewes which did not increase their secretion of LH immediately after the introduction of rams.

Experiment 2

The progesterone implants increased the plasma levels of progesterone from $0{\cdot}33 \pm 0{\cdot}02$ ng/ml (within the range of the assay blank) to $1{\cdot}28 \pm 0{\cdot}10$ ng/ml. Prior to the introduction of rams, both oestradiol and progesterone had lowered the basal levels of LH (Table 2; $P = 0{\cdot}01$, $P = 0{\cdot}005$). The effect of the interaction was also significant ($P = 0{\cdot}038$) with basal levels being lowest in ewes treated with both steroids. The combination of oestradiol and progesterone also appeared to reduce pulse frequency but none of the treatment effects were statistically significant ($P > 0{\cdot}12$, Table 2).

The introduction of rams increased the frequency of the LH pulses ($P = 0{\cdot}003$, paired comparison of all ewes, Table 2). There was a significant effect of progesterone ($P = 0{\cdot}028$) on this response since the increase in frequency induced by the rams (cf. change in frequency, Table 2) was large in the ewes treated only with oestradiol, but was small or undetectable in ewes treated with progesterone. This is illustrated in the examples of individual profiles shown in Fig. 2. The final frequency after the introduction of rams was significantly ($P = 0{\cdot}044$) lower in ewes treated with progesterone than in ewes not treated with progesterone.

The effects of the introduction of rams on pulse frequency were reflected by increases in basal level ($P = 0{\cdot}005$, Table 2). Again, the response was smallest in ewes treated with progesterone (cf. change in LH, Table 2: $P = 0{\cdot}005$) and this effect of progesterone was independent of oestradiol. The increases in basal levels were higher, but not significantly so, in the ewes treated with oestradiol alone than in the other ewes (Table 2).

Discussion

The study presented here shows that low levels of exogenous progesterone cannot prevent the stimulatory effect of the introduction of rams on the secretion of LH in the anoestrous ewe. However, the size of the response in LH was reduced by progesterone, and we would expect a similar dampening effect while progestogens are being cleared after treatments used to ensure oestrus (Hunter *et al.* 1971) or normal luteal function (Oldham *et al.* 1980; Cognié *et al.* 1982). At least 6 h is required for absorption and clearance of progesterone after an intramuscular injection in oil (Shelton *et al.* 1972) and the secretion of LH could be expected to increase once that process has been completed. The sequence of endocrine events which is presumed to lead to ovulation, namely increased secretion of oestradiol followed by positive feedback and the preovulatory surge of LH, could then continue uninhibited. Thus, the overall effect of an injection of progesterone should be to delay ovulation. If intravaginal sponges or implants are used instead of injections, the delay would probably be reduced, since little time will be required for the absorption of hormone remaining at the site of administration.

We have previously observed a delay of about 40 h in the attainment of the LH surge in anoestrous ewes injected with progesterone at the time they were introduced to rams (Martin *et al.* 1980*a*). This is apparently far greater than can be explained in terms of absorption and clearance of progesterone so it seems likely that, well after it has been withdrawn, progesterone still has a delaying effect on the hypothalamo-pituitary response to positive feedback by oestradiol. This effect of progesterone has been observed in ovariectomized ewes treated with oestradiol implants (Karsch *et al.* 1980) and is apparently important in the formation of functional corpora lutea (Pearce *et al.* (1982).

Our studies with ovariectomized ewes (Martin *et al.* 1983*b*) indicate that the LH surge is not evoked by a direct action of the ram stimulus on the hypothalamic centres controlling LH release in the ewe. We proposed that positive feedback by oestradiol is involved and this is supported by the results presented here. Progesterone treatment was able to block ovulation, even in the group of ewes treated 6 h after the introduction of rams and when the levels of oestrogen should have already increased. This suggests that progesterone inhibited the secretion of the preovulatory surge of LH by blocking positive feedback by oestradiol, an effect that has been demonstrated in ovariectomized ewes (Scaramuzzi *et al.* 1971; Karsch *et al.* 1980) and intact ewes (Baird and Scaramuzzi 1976). However, accurate measurements of the amount of oestradiol released in response to the ram stimulus are still required, as the delay of 6 h may have been too short to allow the secretion of sufficient oestradiol.

In the ovariectomized ewes prior to the introduction of rams, the frequency of the LH pulses was not affected by the oestradiol treatment. This contrasts with our previous studies using similar implants (Martin *et al.* 1983*a*, 1983*b*), in which oestradiol alone was able to reduce pulse frequency in the spring. If we accept responsiveness to negative feedback by oestradiol as a criterion for 'anoestrus' in ovariectomized ewes, then it seems likely that the ewes in the present experiment were in fact not anoestrous. Indeed, the deepest point of the anoestrous season of the Merino was previously shown to be September or October (Underwood *et al.* 1944) and our earlier experiments were carried out at this time. The experiment described here was carried out at the end of anoestrus, or perhaps in the transitional period leading to the breeding season and for this reason some of the ewes may have become insensitive to oestrogen. This is supported by the variability in pulse frequencies in the oestrogen-treated group (Table 2). In any case, the major interest of this study is that the introduction of rams was still able to elicit powerful responses in oestrogen-treated ewes and that the response was lower in the presence of progesterone.

As in our previous study (Martin *et al.* 1983*b*), the introduction of rams induced a small increase in the tonic secretion of LH in some of the ovariectomized ewes not treated with exogenous steroids (e.g. ewe 4, Fig. 2) but the overall effect was not significant. Again the response was greatly magnified, and statistically significant, in the presence of oestradiol (e.g. ewe 5, Fig. 2) so we must conclude that the ram effect acts primarily by inhibiting the negative feedback exerted by oestradiol, the major factor limiting the frequency of LH pulses during the anoestrous season (Goodman and Karsch 1981; Martin *et al.* 1983*a*).

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References

Baird, D. T. (1978). Pulsatile secretion of LH and ovarian estradiol during the follicular phase of the sheep estrous cycle. *Biol. Reprod.* **18**, 359–64.

Baird, D. T., and Scaramuzzi, R. J. (1976). Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the peri-ovulatory period in the ewe: effect of progesterone. *J. Endocrinol.* **70**, 237–45.

Cognié, Y., Gayerie, F., Oldham, C. M., and Poindron, P. (1980). Increased ovulation rate at the ram-induced ovulation and its commercial application. In 'Animal Production in Australia'. (Pergamon Press: London.) [*Proc. Aust. Soc. Anim. Prod.* **13**, 80–2.]

Cognié, Y., Gray, S. J., Lindsay, D. R., Oldham, C. M., Pearce, D. T., and Signoret, J-P. (1982). A new approach to controlled breeding in sheep using the 'ram effect'. *Proc. Aust. Soc. Anim. Prod.* **14**, 519–22.

Goding, J. R., Catt, K. J., Brown, J. M., Kaltenbach, C. C., Cumming, I. A., and Mole, B. J. (1969). Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. *Endocrinology* **86**, 133–42.

Goodman, R. L., and Karsch, F. J. (1981). The hypothalamic pulse generator: a key determinant of reproductive cycles in sheep. In 'Colson Papers No. 32, Biological Clocks in Seasonal Reproductive Cycles'. (Eds B. K. Follett and D. E. Follett.) pp. 223–236. (Wright: Bristol, U.K.)

Hauger, R. L., Karsch, F. J., and Foster, D. L. (1977). A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum, and evidence that progesterone inhibits tonic LH secretion. *Endocrinology* **101**, 807–17.

Hunter, G. L., Bolenje, P. C., and Van Niekerk, C. H. (1971). Synchronized mating and lambing in spring-bred Merino sheep: the use of progestagen-impregnated intra-vaginal sponges and teaser rams. *Agroanimalia* **3**, 133–40.

Karsch, F. J., Dierschke, D. J., Weick, R. F., Yamaji, T., Hotchkiss, J., and Knobil, E. (1973). Positive and negative feedback control by estrogen of luteinizing hormone secretion in the Rhesus monkey. *Endocrinology* **92**, 799–804.

Karsch, F. J., Legan, S. J., Ryan, K. D., and Foster, D. L. (1980). Importance of estradiol and progesterone in regulating LH secretion and estrous behaviour during the sheep estrous cycle. *Biol. Reprod.* **24**, 404–13.

Knight, T. W., Peterson, A. J., and Payne, E. (1978). The ovarian and hormonal response of the ewe to stimulation by the ram early in the breeding season. *Theriogenology* **10**, 343–8.

Lindsay, D. R., Cognié, Y., and Signoret, J. P. (1982). Méthode simplifiée de maitrise de l'oestrus chez la brebis. *Ann. Zootech.* **31**, 77–82.

Martin, G. B. (1979). Ram-induced ovulation in seasonally anovular Merino ewes: effect of oestradiol on the frequency of ovulation, oestrus and short cycles. *Theriogenology* **12**, 283–7.

Martin, G. B., Cognié, Y., Gayerie, F., Oldham, C. M., Poindron, P., Scaramuzzi, R. J., and Thiery, J. C. (1980*a*). The hormonal responses to teasing. In 'Animal Production in Australia'. (Pergamon Press: London.) [*Proc. Aust. Soc. Anim. Prod.* **13**, 77–9.]

Martin, G. B., Oldham, C. M., and Lindsay, D. R. (1980*b*). Increased plasma LH levels in seasonally anovular Merino ewes following the introduction of rams. *Anim. Reprod. Sci.* **3**, 125–32.

Martin, G. B., Scaramuzzi, R. J., and Henstridge, J. D. (1983*a*). Effects of oestradiol, progesterone and androstenedione on the pulsatile secretion of luteinizing hormone in ovariectomized ewes during spring and autumn. *J. Endocrinol.* **96**, 181–93.

Martin, G. B., Scaramuzzi, R. J., and Lindsay, D. R. (1981). Induction of ovulation in seasonally anovular ewes by the introduction of rams: effects of progesterone and active immunization against androstenedione. *Aust. J. Biol. Sci.* **34**, 569–75.

Martin, G. B., Scaramuzzi, R. J., and Lindsay, D. R. (1983*b*). Effect of the introduction of rams on the pulsatile secretion of luteinizing hormone in ovariectomized ewes. *J. Reprod. Fertil.* **67**, 47–55.

Oldham, C. M., Cognié, Y., Poindron, P., and Gayerie, F. (1980). The influence of progesterone or FGA priming on the ovarian function of seasonally anovular ewes induced to ovulate by their re-introduction to rams, teasing. Proc. 9th Int. Congr. Anim. Reprod. and A.I. Vol. 3, p. 50.

Oldham, C. M., and Martin, G. B. (1978). Stimulation of seasonally anovular Merino ewes by rams. II. Premature regression of ram-induced corpora lutea. *Anim. Reprod. Sci.* **1**, 291–5.

Oldham, C. M., Knight, T. W., and Lindsay, D. R. (1976). A comparison of the effects on reproductive performance in sheep, of two methods of estimation of ovulation rate. *Aust. J. Exp. Agric. Anim. Husb.* **16**, 24–7.

Oldham, C. M., Martin, G. B., and Knight, T. W. (1978). Stimulation of seasonally anovular Merino ewes by rams. I. Time from introduction of rams to the preovulatory LH surge and ovulation. *Anim. Reprod. Sci.* **1**, 283–90.

Pelletier, J., and Signoret, J-P. (1969). Contrôle de la decharge de LH dans le sang par la progestérone et le benzoate d'oestradiol chez la brebis castrée. *C.R. Acad. Sci. Paris*, Series D **269**, 2595–8.

Pearce, D. T., Martin, G. B., and Oldham C. M. (1982). Function of corpora lutea induced in ewes by rams: importance of timing of the LH surge. *Proc. Aust. Soc. Reprod. Biol.* **14**, 91.

Poindron, P., Cognié, Y., Gayerie, F., Orgeur, P., Oldham, C. M., and Revault, J-P. (1980). Changes in gonadotrophins and prolactin levels in isolated (seasonally or lactationally anovular ewes associated with ovulation caused by the introduction of rams. *Physiol. Behav.* **25**, 227–36.

Reeves, J. J., Arimura, A., and Schally, A. V. (1970). Studies on dose-response relationships of luteinizing hormone-releasing hormone (LH-RH) in sheep. *J. Anim. Sci.* **31**, 933–6.

Scaramuzzi, R. J., Tillson, S. A., Thorneycroft, I. H., and Caldwell, B. V. (1971). Action of exogenous progesterone and estrogen on behavioural estrus and luteinizing hormone levels in the ovariectomized ewe. *Endocrinology* **88**, 1184–9.

Schinckel, P. G. (1954). The effect of the presence of the ram on the ovarian activity of the ewe. *Aust. J. Agric. Res.* **5**, 465–9.

Shelton, J. N., Eggleton, S. C., and Wilkins, J. F. (1972). Effect of injection vehicle of progesterone on peripheral progesterone levels and its relationship to fertility at controlled oestrus. *J. Reprod. Fertil.* **28**, 152–3.

Sokal, R. R., and Rohlf, F. J. (1969). 'Biometry.' pp. 328–32. (Freeman: San Francisco.)

Underwood, E. J., Shier, F. L., and Davenport, N. (1944). Studies in sheep husbandry in W.A. V. The breeding season in Merino, crossbred and British breed ewes in the agricultural districts. *J. Agric.* (*Western Australia*) (2) **11**, 135–43.

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