

Pituitary Responsiveness to LH-RH in Post-partum Ewes Treated with Oestradiol-17 β and Failing to Show a Plasma LH Surge

P. J. Wright,^A T. Stelmasiak^A and W. A. Chamley^B

^A Department of Veterinary Clinical Sciences, University of Melbourne, Veterinary Clinical Centre, Princes Highway, Werribee, Vic. 3030.

^B Animal Research Institute, Department of Agriculture, Princes Highway, Werribee, Vic. 3030.

Abstract

Oestradiol-17 β (40 μ g intravenously) failed to elicit a surge in plasma LH levels by 13 h after administration in 64% (16 out of 25) Merino ewes about 30 days post partum in the anoestrous season. LH-RH responsiveness and LH-RH priming effect were significantly greater in these ewes than in similar post-partum ($n = 9$) and non-parturient ewes ($n = 3$) not treated with oestradiol. This suggests that the failure of the oestrogen-positive feedback effect on LH release in post-partum ewes is not due to a failure of oestradiol action on the pituitary increasing pituitary responsiveness to LH-RH and LH-RH priming effect, but could be due to inadequate release of LH-RH from the hypothalamus.

Introduction

Failure of the positive feedback effect of oestrogens on LH release has been associated with ovarian acyclicity in post-partum ewes (Wright and Findlay 1977), women (Baird *et al.* 1979), monkeys (Yamaji *et al.* 1971) and cows (Radford *et al.* 1978), and in non-parturient amenorrhoeic women (Glass *et al.* 1975; Aono *et al.* 1976).

Increased release of LH-RH occurred during oestrogen-induced plasma LH surges in monkeys (Neill *et al.* 1977) and rats (Sarkar *et al.* 1976). Pituitary responsiveness to LH-RH and LH-RH priming effect—which results in increasing pituitary responsiveness to successive small doses of LH-RH—are both increased at oestrogen-dominated phases of the ovarian cycle of women (Wang *et al.* 1976), rats (Aiyer *et al.* 1974), and ewes (Stelmasiak and Cumming 1977) and by oestrogen treatment in these species (Lasley *et al.* 1975; Aiyer *et al.* 1976; Stelmasiak and Cumming, unpublished observations).

Failure of oestrogen-positive feedback could therefore involve inadequate release of LH-RH from the hypothalamus and/or a failure of oestrogen to increase pituitary responsiveness to LH-RH. To clarify the basis of failure of oestrogen-positive feedback we studied the effect of oestradiol-17 β on pituitary LH-RH responsiveness in post-partum Merino ewes that failed to show oestrogen-positive feedback. LH-RH responsiveness and LH-RH priming effect were assessed in post-partum anoestrous Merino ewes 13 h after intravenous administration of 40 μ g oestradiol-17 β and in similar post-partum and non-parturient anoestrous ewes not treated with oestradiol.

The positive feedback effect of 40 µg oestradiol-17β i.v. was manifested by raised plasma LH levels by 8–13 h after administration in both non-parturient (Goding *et al.* 1969) and post-partum ewes (Wright and Findlay 1977) in the anoestrous season. We were therefore able to identify ewes showing no positive feedback from their low plasma LH levels at the start of LH responsiveness assessment.

Materials and Methods

The experiment was performed at the Animal Research Institute, Werribee, Victoria, in the anoestrous season (December 1978).

Animals and Treatments

In December 1978, 37 mature Merino ewes between 27 and 33 days post partum and with single lambs (34) or that had not been pregnant for at least 6 months (3) were studied. Twenty-five post-partum ewes received 40 µg oestradiol-17β (in 2 ml 20% v/v ethanolic NaCl (0.154 M) solution at 2000 h. The remaining ewes received 2 ml ethanol-saline only i.v. The following day commencing at 0900 h LH-RH responsiveness and LH-RH priming effect were assessed. Each ewe received two i.v. injections each of 1 µg LH-RH (Lutal, Hoechst) in 2 ml 0.154 M NaCl solution at 0900 and 1000 h. Blood samples were collected via jugular vein cannulae at 0830, 0845, each 10 min from 0900 to 1100, then at 1120, 1140 and 1200 h. Blood was promptly centrifuged, plasma separated and stored at -20°C until assay.

Radioimmunoassays

Plasma LH was measured by double antibody radioimmunoassay described by Stelmasiak and Galloway (1977), using horse anti-bovine LH (Snook 1968), goat anti-horse gammaglobulin (NIH-LH-S18) as standard and Papkoff LH (G 3222B) for iodination. Low gonadotrophin plasma (oestrogen-treated rams) was included in the standard curve. All samples were measured in a single assay and the results and quality control data computed according to the methods and program of Burger *et al.* (1972). The within-assay coefficient of variation was <20% over the range 1.1–53.4 ng/ml. The few samples that fell outside this range were diluted 1:10 and reassayed. One sample from each ewe was assayed for progesterone using a radioimmunoassay as described by Hoppen *et al.* (1976). The sensitivity of the assay was 0.1 ng/ml.

Analysis of Results

Oestrogen-positive feedback was considered to have occurred if the plasma LH level was >8 ng/ml in any of the three samples taken before LH-RH administration.

LH-RH responsiveness could not be assessed in ewes showing positive feedback because the oestrogen-induced plasma LH surge swamped and confounded any response due to the exogenous LH-RH.

The response to the first injection of LH-RH was defined as the area under the plasma LH (ng/ml)-time (h) curve determined over 60 min from the time of injection (area 1). The response to the second injection (area 2) was similarly determined and corrected for LH released due to the first injection, assuming a half-life of 24 min (Akbar *et al.* 1974) for the plasma LH present at the time of the second injection. The priming effect ratio was area 2/area 1. The total LH-RH responsiveness was the area under the plasma LH concentration (ng/ml)-time (h) curve 0900–1200 h.

Significance of differences between means was assessed using students *t*-test (Steel and Torrie 1960).

Results

Of the post-partum ewes, 16 out of 25 (64%) failed to show oestrogen-positive feedback. The mean LH-RH responses and priming effect ratios were similar ($P>0.05$) for the post-partum and non-parturient ewes that received no oestradiol, but were significantly increased ($P<0.001$, $P<0.05$ respectively) in the oestradiol-treated ewes that failed to show oestrogen-positive feedback (Table 1, Fig. 1). No ewes had detectable (>0.1 ng/ml) levels of plasma progesterone.

Table 1. Responses of ewes to LH-RH and oestradiol injections

Responses to each of two 1- μ g LH-RH injections given 1 h apart and the priming effect ratio in ewes 27–33 days post partum treated with 40 μ g oestradiol-17 β i.v., in untreated ewes 27–33 days post partum, and in untreated, non-parturient anoestrous ewes. Values are means \pm s.e.m. A1, A2 denote responses to first and second LH-RH injections and correspond to areas 1 and 2 under the plasma LH-time curve respectively

No. of ewes	Treatment	Response (ng/ml \times h)	Priming effect ratio	Total response (ng/ml \times h)
16	Oestradiol, post-partum	(A1) 26.7 ± 2.9 (A2) 53.0 ± 7.0	2.0 ± 0.14	139.9 ± 14.4
3	Untreated, non-parturient	(A1) 8.9 ± 1.5 (A2) 13.2 ± 3.0	1.4 ± 0.13	32.6 ± 7.2
9	Untreated, post-partum	(A1) 12.0 ± 1.7 (A2) 19.6 ± 2.9	1.6 ± 0.12	43.5 ± 6.7
P^A		<0.001	<0.05	<0.001

^A Significance of difference between means for oestradiol-treated and non-treated ewes (pooled value).

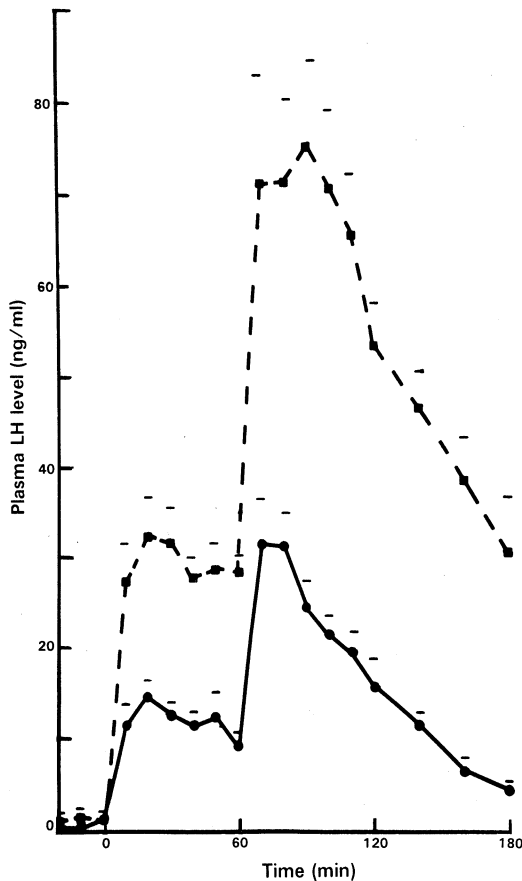


Fig. 1. Plasma LH levels (means \pm s.e.m.) in ewes injected with 1 μ g LH-RH at 0 and 60 min. ---- Oestradiol-treated ewes 27–33 days post partum ($n = 16$) that failed to show an oestrogen-positive feedback effect on plasma LH levels. — Untreated ewes, non-parturient ($n = 3$) or 27–33 days post partum ($n = 9$).

Discussion

The high incidence (64%) of failure of oestrogen-positive feedback in Merino ewes around 30 days post partum in the anoestrous season corroborates previous findings for Corriedale ewes lambing in the anoestrous season (Wright and Findlay 1977) and for Merino ewes lambing in the breeding season (Wright *et al.*, unpublished observations), when 50 and 54% failed to show oestrogen-positive feedback. While circulating progesterone can be associated with failure of oestrogen-positive feedback (Cumming *et al.* 1971; Scaramuzzi *et al.* 1971) the lack of detectable plasma progesterone suggests this was not the reason in these ewes.

In ovine pregnancy pituitary LH content and the amount of LH released in response to exogenous LH-RH is markedly decreased (Chamley *et al.* 1974, 1976; Jenkin *et al.* 1977). However, it is unlikely that failure of oestrogen-positive feedback in ewes around 30 days post partum was due to depletion of pituitary LH, as in this and in other studies LH-RH responsiveness at this stage was similar to that in non-parturient ewes (Wright and Findlay 1977; unpublished observations).

Failure of oestrogen to increase pituitary responsiveness to LH-RH has been described in some non-parturient amenorrhoeic women (Glass *et al.* 1976; Rebar *et al.* 1978; Shaw 1979). However, this was not the cause of failure of oestrogen-positive feedback in the post-partum ewes. Oestradiol markedly increased both pituitary LH-RH responsiveness and LH-RH priming effect. This suggests that failure of oestrogen-positive feedback in post-partum ewes may be due to a failure of adequate release of LH-RH from the hypothalamus rather than a failure of oestradiol to increase pituitary responsiveness to LH-RH or to increase LH-RH priming effect.

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