Pituitary responsiveness to LH-RH, the occurrence of oestradiol-17 β -induced LH-positive feedback and the resumption of oestrous cycles in ewes *post partum*

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Summary. The relationship between pituitary responsiveness to exogenous LH-RH, return of oestradiol-17 β -induced positive-feedback effect on LH release and the resumption of oestrous cycles were studied in Merino ewes lambing in the breeding season. The mean ($\pm$ s.e.m.) increase in plasma LH 2 h after 50 μ g LH-RH i.v. was reduced ($P < 0.001$) in ewes tested in the last 4 weeks of pregnancy (8.6 ± 2.1 ng/ml, $N = 29$) but was similar ($P > 0.05$) for non-parturient dioestrous ewes ($N = 31$) and for anoestrous ewes tested around 14 ($N = 50$) and 28 ($N = 51$) days *post partum* (30 ± 3.9 , 37.8 ± 4.0 , 39.0 ± 3.2 ng/ml, respectively). Oestradiol-positive feedback was absent in 68.6% (35/51) and 54% (27/50) of ewes tested around 14 and 28 days *post partum*, respectively. Neither LH-RH responsiveness nor the presence of oestradiol-positive feedback was related to the incidence of (61.9%, 166/268) or interval to (62 ± 2.2 days, $N = 99$) first oestrus *post partum*.

We suggest that these endocrine responses do not represent factors limiting the resumption of ovarian cyclicity *post partum*.

Introduction

The resumption of ovulation and oestrus in the post-partum ewe can be influenced by season, lactation, nutrition and breed (Mauléon & Dauzier, 1965; Hunter, 1968; Restall, 1971; Mallampati, Pope & Casida, 1971; Shevah, Black, Carr & Land, 1974; Restall & Starr, 1977). However, the endocrine basis of post-partum ovarian acyclicity is not understood. It could be due to changes in pituitary responsiveness to LH-RH. During pregnancy the pituitary content of LH is depleted to less than 20% of that found in anoestrous ewes (Chamley, Jonas & Parr, 1976; Jenkin, Heap & Symons, 1977). This was reflected by the amount of LH released by the pituitary in response to exogenous LH-RH (Chamley, Findlay, Cumming, Buckmaster & Goding, 1974; Jenkin *et al.*, 1977). In Clun Forest and Finnish Landrace ewes lambing in the anoestrous season normal responsiveness to LH-RH had returned within 6–8 weeks of parturition (Jenkin *et al.*, 1977), whereas Romney ewes lambing in the anoestrous season had only partial restoration of LH-RH responsiveness by 6 weeks *post partum* (Chamley *et al.*, 1974).

The endocrine basis of post-partum ovarian acyclicity could also involve changes in responsiveness of the hypothalamic–pituitary axis to oestradiol-17 β . By 30 days *post partum* only 50% of Corriedale ewes lambing during the anoestrous season showed a positive feedback

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effect of oestradiol on LH release, whereas LH-RH responsiveness was normal and similar to that in non-parturient anoestrous ewes (Wright & Findlay, 1977).

Earlier studies were unable to relate the endocrine parameters to the resumption of ovulation and oestrus *post partum* because the experiments were conducted during the anoestrous season. Furthermore, restoration of normal endocrine function *post partum* could have been influenced by season. Therefore we studied LH-RH responsiveness and the occurrence of oestradiol positive feedback in Merino ewes lambing during the breeding season, and the results were related to the incidence of, and interval to, the first oestrus after parturition.

Materials and Methods

The experiment was undertaken at Mortlock Experimental Station near Clare, South Australia, from April (autumn) to October (spring) 1978. Mature Merino ewes (Bungaree strain) lambing from mid-April to mid-May and non-parturient cyclic ewes were studied (day of oestrus = Day 0). The post-partum ewes were allocated to Flocks I and II, and within flocks to treatment groups that were balanced with respect to age, live weight and time of lambing. In Flock I, vasectomized rams fitted with Sire-Sine harnesses and raddles were run with the ewes from the start of lambing. In Flock II, because of management constraints, the rams were introduced 7 weeks after lambing had begun. Daily observations for oestrus were continued until 31 October 1978. Some ewes (37) from Flock I received no treatment and were observed for oestrus only (Group NT).

LH-RH responsiveness was determined in mid-dioestrous ewes, synchronized by i.m. injection of a prostaglandin analogue (125 µg Cloprostenol, I.C.I.) 12 days previously (Group C, N = 31), in pregnant ewes up to 4 weeks before parturition (Group P: 16 from Flock I, 13 from Flock II), and in ewes at 16.6 ± 0.5 (s.e.m.) days *post partum* (Group G14: 26 from Flock I, 24 from Flock II) or at 28.8 ± 0.5 days *post partum* (Group G28: 25 from Flock I, 26 from Flock II).

LH-RH responsiveness was assessed in ewes given 50 µg LH-RH (i.v. in 2 ml 0.154 M-NaCl, 0.1% w/v gelatin). Blood samples (8 ml) were taken at 0 and +2 h after LH-RH, chilled, centrifuged and the plasma stored at -15°C until assayed for LH. Preliminary studies on the same flock of Merino ewes at the previous lambing at the same time of year had shown that the increment in plasma LH at 2 h was highly correlated ($r = 0.956$, $n = 28$, $P < 0.001$) with the area under the plasma LH-time response curve constructed from samples taken each 30 min for 4.5 h.

Oestradiol-positive feedback was assessed in ewes at 15.5 ± 0.6 days *post partum* (Group E14: 25 from Flock I, 26 from Flock II) and in other ewes at 28.5 ± 0.5 days (Group E28: 25 from each of Flocks I and II). These ewes were different from those given LH-RH. The positive-feedback effect of oestradiol was assessed after an i.v. injection of 40 µg oestradiol-17β (Ikapharm) (in 2 ml ethanolic 0.154 M-NaCl, v/v 1:4). Blood samples (8 ml) were taken immediately before injection and at 3-h intervals from 10 to 28 h after injection, chilled, centrifuged and the plasma stored at -15°C until assayed for LH or progesterone. Oestradiol-positive feedback was considered to have returned if LH levels were > 10 ng/ml in at least 2 plasma samples after oestradiol injection, or if plasma progesterone was > 1 ng/ml at the time of test, indicating that ovulation had occurred previously.

Plasma LH was measured by a double-antibody radioimmunoassay, described by Stelmasiak & Galloway (1977), using horse anti-bovine LH (Snook, 1968), goat anti-horse gamma globulin, NIH-LH-S18 as standard, and Papkoff LH (G3222 B) for iodination. Low-gonadotrophin plasma (oestrogen-treated rams) was included in the standard curve. All samples for measuring LH-RH responsiveness were assayed in single assay in which the within-assay coefficient of variation was $< 20\%$ over the range of 1.5–54.0 ng/ml. The samples that fell outside this range were diluted 1 in 10 and re-assayed. All samples from the experiment assessing oestradiol-

positive feedback were assayed by a solid-phase radioimmunoassay (Goding *et al.*, 1969) using the same antibody, standards and tracer as for the double-antibody radioimmunoassay. The within-assay coefficient of variation was < 20% over the range 0.8–20 ng/ml. The between-assay coefficient of variation at 12.8 ng/ml was 15%.

To determine whether ovulation had occurred in post-partum ewes before or after treatment, plasma progesterone concentrations were determined in samples taken at the time of LH-RH or oestradiol injection and again 10 days later. The assay was that previously described by Hossian, Lee, Clarke & O'Shea (1979). Within-assay variation was < 20% over the range 0.5–4.0 ng/ml. Between-assay variation ($n = 9$) was assessed by repeated assay of plasma pools from one wether and 2 ewes; respective mean $\pm$ s.e.m. values were 0.07 ± 0.02 , 2.3 ± 0.29 and 5.3 ± 0.19 ng/ml. The assay sensitivity was 0.1 ng/ml.

For all assays, plasma hormone concentrations and assay quality control data were calculated using a computer programme (Burger, Lee & Rennie, 1972).

Standard statistical techniques, Student's *t* test, χ^2 test, analysis of variance and correlation analysis, were used (Steel & Torrie, 1960). When treatment means were compared individually with control values after analysis of variance, the residual mean square was used as the estimate of population variance. Flock II ewes were excluded from analyses relating to the interval from parturition to first oestrus because records of the occurrence of oestrus were not begun until 7 weeks after the start of lambing.

Results

The overall incidence of oestrus within 135 days of parturition for ewes in both flocks was 61.9% (166/268). Treatments had no significant effects ($P > 0.05$). The mean interval from parturition to first oestrus for Flock I ewes was 62 ± 2.2 days ($N = 99$). Treatments had no significant effects.

Table 1. Concentrations of LH in response to LH-RH in ewes that did (+) or did not (–) show oestrus when tested in the last 4 weeks of pregnancy (Group P), ~14 (Group G14) or ~28 (Group G28) days *post partum*, and in dioestrus (Group C)

Occurrence of oestrus	LH conc. (ng/ml)			
	Group P	Group G14	Group G28	Group C
+	10.0 ± 2.9 (20)	38.3 ± 5.0 (31)	41.4 ± 4.6 (31)	30.0 ± 3.9 (31)
–	5.5 ± 1.4 (9)	37.0 ± 5.9 (19)	35.0 ± 3.8 (20)	—
Combined values	8.6 ± 2.1 (29)	37.8 ± 4.0 (50)	39.0 ± 3.2 (51)	30.0 ± 3.9 (31)

Values are mean $\pm$ s.e.m. for the no. of ewes indicated in parentheses.

Summary of analyses of variance			
Source of variation	d.f.	Mean Square	Variance ratio
Treatment group (P, G14, G28, C)	3	6667	13.24 ($P < 0.001$)
Residual	157	503	
Comparison of means	<i>t</i>	Significance	
C versus P	3.7	$P < 0.001$	
C versus G14	1.5	N.S.	
C versus G28	1.7	N.S.	
Treatment group (P, G14, G28)	2	9244	18.6 ($P < 0.001$)
Oestrus versus no oestrus	1	588	1.1
Interaction	2	8	0.16
Residual	124	495	

LH-RH responsiveness was less ($P < 0.001$) in pregnant ewes than in dioestrous and post-partum ewes, but was similar for dioestrous and post-partum ewes (Table 1). There was no significant relationship between LH-RH responsiveness and the occurrence of oestrus *post partum* (Table 1). There was no significant correlation between LH-RH responsiveness and the interval to first post-partum oestrus for the ewes in Groups P, G14 and G28 ($r = -0.35, 0.11$ and -0.4 , $N = 13, 16$ and 16 respectively; Flock I ewes only).

More ewes showed oestradiol-positive feedback around 28 than around 14 days *post partum* (46% in Group E28, 31.4% in Group E14), but the difference was not significant ($\chi^2 = 2.3$, $P > 0.05$). There was no significant ($P > 0.05$) relationship between the occurrence of oestradiol-positive feedback and either the incidence of, or interval to, first post-partum oestrus (Table 2).

Table 2. The occurrence of oestradiol-positive feedback in ewes tested about 14 (Group E14) and 28 (Group E28) days *post partum* and the incidence of, and interval to, first oestrus within 135 days of parturition

Group	Oestradiol-positive feedback (%)	Ewes showing oestrus (%)	Mean $\pm$ s.e.m. time between parturition and first oestrus (days)*
E14	Present 31.4 (16/51)	68.8 (11/16)	71.8 $\pm$ 11.9 (5)
	Absent 68.6 (35/51)	65.7 (23/35)	66.7 $\pm$ 6.4 (14)
E28	Present 46 (23/50)	60.9 (14/23)	72.8 $\pm$ 9.3 (9)
	Absent 54 (27/50)	66.7 (18/27)	66.6 $\pm$ 5.4 (5)

Values in parentheses are numbers of ewes.

* Flock I ewes only (see 'Materials and Methods').

No plasma progesterone was detected (< 0.1 ng/ml) in treated ewes around 14 days *post partum*, but 10 days later 6 ewes treated with LH-RH had detectable plasma progesterone (> 1.5 ng/ml). In treated ewes around 28 days *post partum* plasma progesterone was detected in 3 ewes (2 in Group E28, 1 in Group G28), and 10 days later in 13 different ewes treated with LH-RH and in 3 different ewes treated with oestradiol. The occurrence of detectable plasma progesterone was significantly greater after treatment with LH-RH than after oestradiol at both stages *post partum* ($P < 0.05$), using an exact test of independence for a 2×2 contingency table.

Discussion

This is the first report of a decreased LH-RH responsiveness occurring in late pregnancy in Merino ewes during the breeding season. Earlier reports were for other breeds lambing in the anoestrous season (Chamley *et al.*, 1974; Jenkin *et al.*, 1977). The apparent recovery of normal LH-RH responsiveness by 14 days *post partum* was sooner than that reported by these authors. This may reflect the breed and strain of ewe or the dose or mode (infusion compared with bolus injection) of administration of LH-RH. Alternatively, an effect of season of lambing is suggested by the higher plasma LH levels found in Merino ewes after lambing in winter compared to spring (Restall & Starr, 1977).

No relationship was found between LH-RH responsiveness and either the occurrence of, or interval to first post-partum oestrus. This suggests that LH-RH responsiveness did not reflect factor(s) limiting the onset of post-partum ovarian cyclicity, and that LH-RH responsiveness is of no value for the prediction of the time of resumption of breeding *post partum*. This suggestion agrees with data for women in which post-partum acyclicity can continue for 12 months in association with normal pituitary responsiveness to exogenous LH-RH (Delvoye, Marcellis, L'Hermite, Michaux-Duchene & Robyn, 1977).

The results relating to LH-RH responsiveness and oestradiol-positive feedback in post-partum ewes lambing in the breeding season were similar to those of Wright & Findlay (1977) who studied Corriedale ewes lambing in the anoestrous season. In both studies LH-RH responsiveness by 30 days *post partum* was similar to that in non-parturient ewes, although only 46% (this study) and 50% (Wright & Findlay, 1977) of ewes showed oestradiol-positive feedback by 30 days *post partum*, compared with 100% of non-parturient anoestrous ewes (5 ewes tested on 2–3 occasions by Wright & Findlay (1977)). A similar high incidence of oestradiol-positive feedback was observed in non-parturient anoestrous ewes tested with 10 or 50 µg oestradiol-17β (7/8 ewes; Goding *et al.*, 1969). The reason(s) for the failure of oestradiol-positive feedback is not known; it was not due to an inhibitory effect of progesterone (Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971; Cumming, Brown, Blockey & Goding, 1971) because plasma progesterone levels were < 0.1 ng/ml in those ewes failing to show positive feedback.

The failure of oestradiol-positive feedback has also been reported in post-partum monkeys (Yamaji *et al.*, 1971), cows (Radford, Nancarrow & Mattner, 1978) and women (Baird *et al.*, 1979). This failure or a reduction in the amount of LH released occurs in other circumstances also involving hyperprolactinaemia—in women with non-parturient amenorrhoea (Glass, Shaw, Butt, Logan-Edwards & London, 1975; Aono *et al.*, 1976), in TRF-treated ewes (Kann, Martinet & Schirar, 1976), in PMSG-treated anoestrous lactating ewes (Pelletier & Thimonier, 1973), and in sulpiride-treated women (L'Hermite, Delogne-Desnoeck, Michaux-Duchene & Robyn, 1978). The association between hyperprolactinaemia and hypothalamic dysfunction in rats (Grandison *et al.*, 1977; Gil-ad *et al.*, 1978; McNeilly, Sharpe, Davidson & Fraser, 1978) suggests that the basis of failure of oestradiol-positive feedback may be in the hypothalamus.

Although lowered LH-RH responsiveness before 14 days *post partum* and failure of oestradiol-positive feedback before 30 days *post partum* could represent factors limiting the resumption of ovarian cyclicity, it is probable that other factor(s) are involved. A causal relationship between ovarian acyclicity and an increase in the negative-feedback effect of oestrogen on LH secretion has been postulated from studies in ewes (Legan, Karsch & Foster, 1977) and in amenorrhoeic women (Santen *et al.*, 1978). The positive relationship between increasing plasma LH levels and the onset of post-partum breeding of Merino ewes (Restall & Starr, 1977) is consistent with this hypothesis. Changes in basal plasma LH levels and in the negative-feedback responses to oestrogen in post-partum ewes are now being studied.

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