Studies of pituitary function in lactating ewes

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Summary. The release of LH from the pituitary of lactating ewes was studied. In Exp. 1, ewes were injected with 50 µg oestradiol benzoate (OB), 2.0 mg testosterone propionate (TP) or oil only (control) on Days 5, 10 or 20 after lambing. LH was measured in peripheral plasma samples obtained 20–38 h after treatment, and ovulations were recorded. The number of ewes in which an LH release was detected, and the amount released, declined between Days 5 and 20 after OB treatment but increased after TP treatment. The releases of LH were not always accompanied by ovulation and the incidence of ovulation was higher in ewes treated with TP. In Exp. 2, lactating ewes were injected with 1 or 5 (at 2-h intervals) doses of 50 µg Gn-RH, on Days 12 or 25 after lambing. LH was measured in peripheral plasma samples collected every 2 h for 10 h and every 3 h for a further 70 h. Release of LH occurred in all ewes, the amount being greater in ewes receiving multiple injections and in ewes treated on Day 25. The incidence of ovulation was higher after treatment on Day 25. Multiple injections of Gn-RH appeared to reduce the incidence of abnormal corpora lutea.

Introduction

When lactating ewes are naturally or artificially bred in the first 40 days after lambing, variable responses of oestrus, ovulation and fertility have been reported (see Foote, 1968; Hunter, 1968; Colas, Brice, Courot & Cottier, 1971; Restall, Kearins, Herdegen & Carberry, 1977). The endocrinological and physiological state of such ewes is poorly understood (Hunter, 1968). Pelletier & Thimonier (1973) concluded that the preovulatory release of LH was reduced during lactation in the ewe. Chamley *et al.* (1974a) suggested that the pituitary of lactating ewes was either depleted of LH and FSH or refractory to releasing hormone for at least 3 weeks after lambing, but Restall, Radford & Wallace (1972) were able to obtain release of LH in lactating ewes in the first 2 weeks after lambing. The level of LH in peripheral plasma gradually increases after lambing, and season and early weaning have been shown to affect the recovery of pituitary function (Restall & Starr, 1977).

Because of the importance of developing methods for inducing fertile matings in sheep, we investigated whether the pituitary of the lactating ewe can release LH in sufficient quantity to cause ovulation.

Materials and Methods

Experiments

Two experiments were conducted at the Agricultural Research Station, Condobolin, in the Central Western area of New South Wales.

In Exp. 1, the release of LH in response to treatment with steroids at various times after lambing was examined. Mature Merino ewes lambing during May 1972 were randomly assigned to treatments (5/group) in a 3 × 3 factorial experiment. For Factor 1, the steroid treatments, given i.m., were (1) 50 µg oestradiol benzoate in 1 ml peanut oil; (b) 2.0 mg testosterone propionate in 1 ml peanut oil; (c) 1 ml peanut oil (control). For Factor 2, the time of treatment after lambing was investigated for Days 5, 10 and 20. Peripheral plasma samples were obtained from all ewes by venepuncture at 20, 23,

26, 29, 32, 35 and 28 h after treatment as a preliminary experiment had indicated that lactating ewes responded during this period. Ovarian inspections were performed via midventral laparotomy 5 days after steroid treatment and recent ovulations were noted and estimates made of their age (Restall, 1964).

In Exp. 2, the effect of single or multiple injections of a gonadotrophin-releasing hormone (Gn-RH: Abbott) were investigated at two times after lambing. Mature Border Leicester $\times$ Merino and Dorset Horn $\times$ Merino ewes lambing in September 1973 were used in a 3×2 factorial experiment. For Factor 1, the 3 Gn-RH treatments were (a) a single s.c. injection of 50 μ g Gn-RH; (b) 5 s.c. injections of 50 μ g Gn-RH at 2-h intervals; and (c) no treatment (control). For Factor 2, the times of the treatment after lambing were Days 12 and 25. The two control groups contained 5 ewes, the other groups 10 ewes. Peripheral plasma samples were collected via jugular venepuncture before, at, and 2, 4, 6 and 8 h after Gn-RH injection and thence every 3 h for a further 70 h. Ovarian inspections were performed 5–6 days after treatment for determination of ovulation and assessment of age of the CL.

Assays

All plasma samples were stored at -15°C until assayed for LH by the method of Goding *et al.* (1969). Internal standards were included in all assays and the results were adjusted for inter-assay variations. The LH standard used was equipotent with NIH-LH-S17. Within- and between-assay coefficients of variation were 4–9% and 8–14%, respectively, and the sensitivity of the assay was approximately 0.5 ng/ml. This sensitivity is sufficient to measure basal LH levels and the precision is satisfactory for detecting large increases in plasma LH. The amount of LH released was calculated as the area under the plasma LH curves.

Table 1. The mean LH concentration (ng/ml) in the peripheral plasma of lactating ewes (5/group) treated with oestradiol benzoate (OB) or testosterone propionate (TP) at various times after lambing

Days after lambing	Treatment	Time after injection (h)							Mean $\pm$ S.E.M.
		20	23	26	29	32	35	38	
5	Control	3.08	2.64	3.02	2.60	3.28	3.88	3.68	3.17 $\pm$ 0.18
	OB	5.88	22.04	35.12	27.90	9.62	4.24	3.52	15.47 $\pm$ 3.78
	TP	5.00	4.32	14.36	9.12	7.36	4.48	2.58	6.60 $\pm$ 1.20
10	Control	3.28	2.74	3.18	2.54	4.88	2.12	3.24	3.14 $\pm$ 0.30
	OB	42.78	17.70	28.26	9.48	4.90	3.16	3.34	15.15 $\pm$ 4.29
	TP	34.36	32.22	39.12	29.80	15.32	5.92	3.72	22.92 $\pm$ 4.85
20	Control	3.84	3.54	3.48	2.70	4.84	2.66	3.10	3.45 $\pm$ 0.28
	OB	29.00	8.04	5.30	3.80	3.06	2.94	2.08	7.75 $\pm$ 2.23
	TP	42.28	60.78	30.86	11.10	11.32	8.32	5.24	24.27 $\pm$ 5.52

Summary of the analysis of variance

Source of variation	d.f.	Mean square	Variance ratio
Between treatments (A)	2	5852.32	16.20**
Between days (B)	2	807.18	2.23
A $\times$ B	4	1646.59	4.55**
Between ewes within A $\times$ B (error 2)	36	361.26	1.23
Between times after treatment (C)	6	1962.10	6.68**
C $\times$ A	12	614.94	2.09
C $\times$ B	12	519.48	1.77
C $\times$ A $\times$ B	24	379.60	1.29
Residual (error 1)	216	293.69	

** $P < 0.01$.

Table 2. The mean $\pm$ S.E.M. amount of LH released (in arbitrary units, area under curve) in lactating ewes (no. in parentheses) treated with steroids at various times after lambing

Treatment	Evidence of LH release	Days after lambing		
		5	10	20
Control	+	—	—	—
	—	53.5 $\pm$ 1.7 (5)	55.2 $\pm$ 5.9 (5)	58.4 $\pm$ 6.3 (5)
Oestradiol benzoate (50 μ g)	+	296.9 $\pm$ 31.3 (5)	271.9 $\pm$ 73.4 (4)	126.1 $\pm$ 18.0 (3)
	—	—	59.4 (1)	54.6 $\pm$ 20.5 (2)
Testosterone propionate (2 mg)	+	171.9 $\pm$ 50.4 (3)	470.2 $\pm$ 70.9 (4)	386.9 $\pm$ 75.7 (5)
	—	72.4 $\pm$ 0.5 (2)	73.9 (1)	—

Results

Experiment 1

The mean concentration of plasma LH in the ewes in each group is shown in Table 1. There were significant differences between the treatments and between times after treatment, the steroids resulting in elevations in LH concentrations between 20 and 30 h after treatment. Not all ewes given steroids had an LH release in the period studied, and there was a significant 'treatment $\times$ day of treatment' interaction, which was also significant for the amount of LH released ($P < 0.01$; Table 2). The number of ewes in which an LH release was detected after treatment with oestradiol benzoate declined from 5 ewes on Day 5 to 3 ewes on Day 20 while the reverse was evident for ewes treated with testosterone propionate. The amount of LH released declined with time after lambing in response to the oestradiol and increased up to Day 10 and was still at a high level on Day 20 after lambing in ewes receiving testosterone.

Elevations in plasma LH concentration were not always accompanied by ovulation: of the 12 ewes releasing LH in response to oestradiol benzoate, 0/5, 1/4 and 1/3 ovulated on Days 5, 10 and 20,

Table 3. Incidence of ovulation and mean $\pm$ S.E.M. amount of LH released (in arbitrary units, area under curve) for the first 14 h after injection of Gn-RH in lactating ewes treated on Days 12 or 25 after lambing

Treatment	Days after lambing							
	12				25			
	No. of ewes	LH released	No. of ovulations		No. of ewes	LH released	No. of ovulations	
			Normal	Abnormal			Normal	Abnormal
1 $\times$ 50 μ g Gn-RH	10	237.0 $\pm$ 40.5	5	—	10	300.0 $\pm$ 40.5	4	4
5 $\times$ 50 μ g Gn-RH at 2-h intervals	10	343.0 $\pm$ 62.3	5	—	10	484.0 $\pm$ 75.4	9	1
None (control)	5	8.6 $\pm$ 1.5	0	—	5	18.0 $\pm$ 3.1	0	0

Summary of analysis of variance

Source of variation	d.f.	Mean square	Variance ratio
Between treatments	2	82016.65	53.67**
Between days of treatment	1	7561.50	4.95*
Interaction	2	2202.02	1.44
Error	49	1528.10	

* $P < 0.05$; ** $P < 0.01$.

respectively, and release of LH in ewes treated with testosterone propionate was followed by ovulation in 1/3 (Day 3), 4/4 (Day 10) and 3/5 (Day 20) ewes.

Experiment 2

The plasma LH concentrations after treatment with Gn-RH are shown in Text-fig. 1. Release of LH was observed in all treated ewes, the amounts released being greater on Day 25 and after multiple injections of LH (Table 3). There were no further releases of LH in the 70 h following the initial response to the Gn-RH.

The number of ewes ovulating varied but the incidence was higher after treatment on Day 25. Some of the CL were classed as abnormal, being small and pale with undeveloped rupture points, and their incidence was lower in ewes receiving multiple injections of the Gn-RH (Table 3).

Text-fig. 1. Peripheral mean $\pm$ S.E.M. plasma concentrations of LH in lactating ewes treated with (a) a single (50 μ g) or (b) multiple (5 $\times$ 50 μ g, 2-h intervals) injections of Gn-RH at 12 (—) or 25 (---) days after lambing. The lower graphs show the basal LH levels in the treated ewes after single (a') and multiple (b') injections of Gn-RH from 15–77 h after Gn-RH treatment and in the untreated ewes (c). The results are for 10 treated ewes and 5 control ewes per group.

Discussion

These experiments show that the sheep pituitary is capable of releasing significant amounts of LH as early as 5 days after lambing, although Chamley *et al.* (1974 a,b) did not find significant LH or FSH

releases in lactating ewes infused with Gn-RH at parturition and 3 weeks after lambing. This disparity may be due to either the low dose of Gn-RH infused (1.5 µg/h) or the mode of administration used by Chamley *et al.* but is clearly not because the pituitary is depleted of LH or refractory to Gn-RH. The pituitary content of LH is low at parturition but does increase in the following 3 weeks (O'Reilly & Dziuk, 1973; Jaume cited by Chamley *et al.*, 1973), and these stores of LH are apparently sufficient for the significant releases reported here.

The reduction in the occurrence and amount of LH release after oestradiol benzoate treatment 20 days after lambing may be due to responses occurring before sampling began. The preliminary study which suggested the 20–38 h sampling period (Restall *et al.*, 1972) did not examine ewes beyond 11 days after lambing. In the present study plasma LH levels in 3 ewes were already elevated when sampling began, accounting in part for the apparently reduced release. We think it more likely that there has been a change in the time of response between 5 and 20 days after lambing rather than an absence of response. This implies a change in some part of the hypothalamo-hypophysial releasing mechanism, as yet unknown (McGuire & Lisk, 1969; Radford & Wallace, 1974).

A similar change in the time of LH response, in the reverse direction, occurred with testosterone treatment. Radford & Wallace (1971) showed that large doses of testosterone propionate caused ovarian stimulation and our results, with similarly large doses, suggest that this is due to release of LH. Testosterone may act directly on the pituitary (Sar & Stumpf, 1973) or a small part may be aromatized to oestrogen (McDonald, Grodin & Siiteri, 1971). In the latter case, a change in the rate of aromatization between Days 5 and 20, together with a change in the time of response to oestrogen, would account for the interaction between the effects of oestradiol and testosterone observed here.

The releases of LH observed in response to Gn-RH are in agreement with other studies (Foster & Crighton, 1973) and are similar to Gn-RH-evoked releases in anoestrous and spayed ewes (Reeves *et al.*, 1972; White, Hedlund, Rippel, Arnold & Flouret, 1973; Radford & Wallace, 1974; Symons, Cunningham & Saba, 1974). The pattern of release of LH was similar on Days 12 and 25 after lambing, but significantly more was released at the later time, indicating a change in pituitary sensitivity to Gn-RH, a change in pituitary content of LH, or increased synthesis. Despite the differences in response to the two steroids, the data from these two experiments indicate that the release mechanism for LH can function in the lactating ewe and that significant quantities of endogenous LH can be discharged shortly after lambing.

However, ovulation was not always a consequence of the LH releases, especially those after oestradiol treatment. The ovary is capable of responding to gonadotrophin as early as 15 days after lambing (Restall *et al.*, 1977) but oestradiol may have directly altered the ovarian responsiveness in our study. The improved ovulatory response to Gn-RH at 25 days after lambing is not considered to be due to follicle maturation as a result of FSH release by Gn-RH (Reeves *et al.*, 1972; White *et al.*, 1973) because this would be expected to lead to oestrogen secretion and, consequently, to a second ovulating release of LH and this was not observed. Another explanation is that follicular growth gradually recommences after parturition and, in ewes lambing together, follicular development will be relatively synchronized. With such animals, the ovulatory response would be expected to be more uniform than that observed in anoestrous ewes (Reeves *et al.*, 1972).

The abnormal CL observed after Gn-RH treatment on Day 25 after lambing (Table 3) are apparently similar to those observed by Restall (1971) and Hunter & Van Aarde (1973). No reasonable explanation of these CL has been advanced, but the ruptured follicle does not appear to luteinize. Multiple injections of Gn-RH, with the consequent significant increase in the amount of LH released, appeared to reduce the incidence of the abnormal CL suggesting that they are associated with a gonadotrophic insufficiency.

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