Suppressed release of LH in ovariectomized post-partum ewes

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Summary. The patterns of LH release were studied in 5 ewes, ovariectomized when 3 days *post partum*, at 4, 14, 33, 47 and 61 days after ovariectomy. These patterns were compared with those in 4 seasonally anoestrous (control) ewes ovariectomized and sampled at the same times. In the control ewes regular pulsatile release of LH was apparent 4 days after ovariectomy: plasma LH concentrations and LH pulse frequencies, but not pulse amplitudes, then declined. In the post-partum ewes all indices of LH release were lower than those in the control ewes at 4 days after ovariectomy, but then they increased: pulse amplitude was greater ($P < 0.05$) at 14 days after ovariectomy, and plasma LH concentrations were greater ($P < 0.05$, 0.01) at 14 and 33 days than in control ewes. These results indicate that the hypothalamo-pituitary axis of lactating ewes has a reduced capacity to release LH for only a short period after ovariectomy early in the post-partum period. This probably reflects the time needed for recovery from the inhibitory effects of ovarian and placental hormones, and may reflect reduced secretion of LH-RH.

Introduction

The frequency of secretory episodes (pulses) of LH in post-partum ewes was less than in cyclic ewes, 7 days after ovariectomy (Wright, Geytenbeek, Clarke & Findlay, 1981a), indicating that impaired hypothalamic function could be a causal factor in ovarian acyclicity *post partum*. These previous studies were performed on ewes about 29 days *post partum* and time-related changes in hypothalamo-pituitary function during the post-partum period were not defined. It was therefore unclear whether the reduced frequency of LH pulses in the post-partum ewes after ovariectomy reflected a slower rate of recovery from the inhibitory effects of ovarian hormones, or reduced function of the hypothalamo-pituitary unit similar to that associated with lactation in ovariectomized sows (Parvizi, Elsaesser, Smidt & Ellendorff, 1976) and in ovariectomized rats (Smith & Neill, 1977; Smith, 1978). Hypothalamo-pituitary function in post-partum ewes has been studied further by examining the patterns of LH release, at various stages during the post-partum period, in ewes ovariectomized 3 days after parturition. The patterns of hormone release were compared with those in seasonally anoestrous ewes ovariectomized and sampled at the same times.

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Materials and Methods

The experiment was undertaken at the Veterinary Clinical Centre, Werribee, Victoria, from August to October (anoestrous season) 1979, using mature cross-bred (Border Leicester $\times$ Merino) ewes. Five ewes at 3 days *post partum*, each suckling one lamb, and 4 control anoestrous ewes that had not been pregnant for at least 6 months were ovariectomized.

The patterns of LH release were assessed at 4, 14, 33, 47 and 61 days after ovariectomy, from blood samples taken via jugular venous cannulae every 20 min for 6 h on each of these days. Plasma LH levels were determined by a double-antibody assay (Wright *et al.*, 1980) using horse anti-bovine LH (Snook, 1968), goat anti-horse gamma globulin, NIH-LH-S18 (biopotency $1.02 \times$ NIH-LH-S1) as standard and Papkoff LH (G3222B; biopotency $2.69 \times$ NIH-LH-S1) for iodination by the chloramine T method (Greenwood, Hunter & Glover, 1963). Plasma LH concentrations and assay quality control data were calculated using the methods and computer programme of Burger, Lee & Rennie (1972). All samples were assayed in the one assay. The assay sensitivity was 0.4 ng/ml and the intra-assay coefficient of variation was $<20\%$ over the range of 0.8–37.1 ng/ml.

Mean plasma LH concentrations and LH pulse frequencies and amplitudes were defined as stated by Wright *et al.* (1981a). An LH pulse was identified when the plasma LH concentration in one or in a series of samples was at least 1 ng/ml and greater than the concentration in preceding and/or following samples by a factor of at least 4 times the coefficient of variation of the assay. Pulse amplitude was the maximum plasma LH concentration associated with the pulse minus the concentration before the pulse. The effects of reproductive status and of time after ovariectomy on these variables were assessed by two-way analyses of variance. Mean values were compared using Duncan's new multiple-range test or Student's *t* test taking the residual mean square from the analyses of variance as an estimate of population variance (Steel & Torrie, 1960).

Results

Marked differences in the patterns of LH release were associated with differences in reproductive status up to 33 days after ovariectomy (Text-fig. 1; Tables 1 & 2). At 47 days after ovariectomy the patterns of LH release in control and in post-partum ewes were similar. In the control ewes regular pulsatile release of LH was apparent at 4 days after ovariectomy. Subsequently, mean LH concentrations, LH pulse amplitudes and frequencies declined. In the post-partum ewes the changes in patterns of LH release were more marked. Initially, all indices of LH output were very low. At 14 days after ovariectomy LH pulses were of similar ($P > 0.05$) frequency to, but of higher ($P < 0.05$) amplitude than, those seen in control ewes. Plasma LH concentration was still greater ($P < 0.01$) in post-partum ewes at 33 days after ovariectomy, but was similar ($P > 0.05$) to that in control ewes thereafter.

Discussion

As found by others (Butler, Malven, Willett & Bolt, 1972; Reeves, O'Donnell & Denorscia, 1972; Diekman & Malven, 1973), shortly after ovariectomy the control anoestrous ewes had plasma LH concentrations, pulse amplitudes and frequencies higher than those reported in entire anoestrous ewes (Martensz, Baird, Scaramuzzi & Van Look, 1976; Scaramuzzi & Baird, 1977). If the pulsatile release of LH reflects the pulsatile release of LH-RH (Lincoln & Short, 1980), in the control anoestrous ewes just after ovariectomy the pulsatile release of LH-RH was frequent and regular. The subsequent reduction of the frequency and regularity of LH pulses may have been an effect of season or of time after ovariectomy. A season effect would suggest that the inadequate release of LH, considered to be the basis of ovarian acyclicity in seasonally anoestrous ewes (Karsch,

Text-fig. 1. Plasma LH concentrations, at various times after ovariectomy (ovx.) in (a) ewes ovariectomized when seasonally anoestrous and (b) ewes ovariectomized 3 days *post partum*. The arrows indicate LH pulses.

Goodman & Legan, 1980), could in part reflect a reduction in hypothalamo-pituitary activity independent of the influence of ovarian hormones, as well as an increased inhibitory effect of

Table 1. Plasma LH concentration, pulse frequencies and pulse amplitudes in 5 ewes ovariectomized on Day 3 *post partum* and in 4 control anoestrous ewes ovariectomized during the anoestrous season

		Days after ovariectomy				
Group		4 (a)†	14 (b)†	33 (c)†	47 (d)†	61 (e)†
Plasma LH (ng/ml)	Post-partum	1.4 ± 0.29 (e, <i>P</i> < 0.05)* (b, c, d, <i>P</i> < 0.01)	8.1 ± 1.55 (c, e, <i>P</i> < 0.05) (a, <i>P</i> < 0.01)	12.0 ± 1.66 (b, <i>P</i> < 0.05) (a, d, e, <i>P</i> < 0.01)	6.2 ± 0.60 (a, c, <i>P</i> < 0.01)	4.9 ± 0.57 (a, b, <i>P</i> < 0.05) (c, <i>P</i> < 0.01)
	Control	7.1 ± 1.09 (e, <i>P</i> < 0.05)*	4.7 ± 1.26	6.3 ± 1.24	4.7 ± 0.82	3.7 ± 1.12 (a, <i>P</i> < 0.05)
	<i>P</i> †(<i>t</i>)	<0.01 (3.6)	<0.05 (2.13)	<0.01 (3.7)	>0.05 (0.9)	>0.05 (0.8)
Pulse frequency (pulses/6 h)	Post-partum	1.2 ± 0.58 (b, c, <i>P</i> < 0.01)	4.4 ± 0.24 (a, e, <i>P</i> < 0.01)	4.8 ± 0.66 (a, e, <i>P</i> < 0.01)	3.0 ± 0.89	1.0 ± 0.31 (b, c, <i>P</i> < 0.01)
	Control	5.5 ± 0.64 (d, <i>P</i> < 0.05)	3.8 ± 1.10	5.0 ± 0.7	2.5 ± 0.95 (a, <i>P</i> < 0.05)	3.5 ± 1.55
	<i>P</i> †(<i>t</i>)	<0.01 (3.7)	>0.05 (0.6)	>0.05 (0.2)	>0.05 (0.4)	>0.05, <0.1 (2.2)
Pulse amplitude (ng/ml)	Post-partum	3.7 ± 0.84 (b, c, <i>P</i> < 0.01)*	13.2 ± 3.3 (a, e, d, <i>P</i> < 0.01)	10.9 ± 1.90 (a, e, d, <i>P</i> < 0.01)	6.1 ± 1.0 (b, c, <i>P</i> < 0.01)	4.6 ± 0.67 (b, c, <i>P</i> < 0.01)
	Control	9.1 ± 1.65	6.1 ± 1.42	5.2 ± 1.02	6.1 ± 1.76	4.5 ± 0.6
	<i>P</i> †(<i>t</i>)	<0.1 (2.2)	<0.05 (2.8)	>0.05, <0.1 (2.3)	>0.05 (0.03)	>0.05 (0.04)

Values are mean ± s.e.m.

* Significance of differences between means for examination times within reproductive status group using Duncan's new multiple range test.

† Significance of differences between means for control and post-partum ewes, using Student's *t* test.

‡ Designation of times of examination for within group, between-mean statistical comparison using Duncan's new multiple range test.

oestrogen on LH release (Legan, Karsch & Foster, 1977). A time effect would suggest that ovarian hormones have a role in the control of the neuroendocrine mechanisms responsible for the rhythmic release of LH.

The low LH output in post-partum ewes relative to control ewes 4 days after ovariectomy probably reflected both reduced release of LH-RH and reduced pituitary responsiveness to LH-RH. The occurrence of a few LH pulses suggests that some LH-RH was released and that the pituitary was capable of some response, albeit small. The results of other studies suggest that pituitary responsiveness to LH-RH would also have been impaired (Chamley, Findlay, Cumming, Buckmaster & Goding, 1974; Wright, Jenkin & Heap, 1981b). Pituitary responsiveness to exogenous LH-RH (50 µg i.v.) in 8 entire ewes 10 days *post partum* was 48% that in 5 seasonally anoestrous control ewes (Wright & Findlay, 1977). Subsequently, the frequency of pulsatile

Table 2. Summary of 2-way analyses of variance of characters reflecting LH release patterns at various stages after ovariectomy (4, 14, 33, 47 and 61 days) in post-partum and seasonally anoestrous ewes

	Residual mean square	Mean square, variance ratio (<i>F</i>) and probability (<i>P</i>) for sources of variance		
		Reproductive status	Time	Interaction
Plasma LH (ng/ml)	5.5	16.8, 3.0, <i>P</i> > 0.05	44.0, 7.9, <i>P</i> < 0.001	39.9, 7.2, <i>P</i> < 0.001
LH pulse frequency (pulses/6 h)	2.9	15.2, 5.3, <i>P</i> < 0.05	10.9, 3.8, <i>P</i> < 0.05	10.3, 3.6, <i>P</i> < 0.05
LH pulse amplitude (ng/ml)	13.7	38.6, 2.8, <i>P</i> > 0.05	36.7, 2.7, <i>P</i> > 0.05	49.4, 3.6, <i>P</i> < 0.05

discharge of LH increased then diminished, suggesting that the neuroendocrine mechanisms responsible for the rhythmic release of LH were inhibited by hormones associated with pregnancy and the early post-partum period. Removal of these inhibitory effects resulted in the regular pulsatile release of LH after a short recovery period. The higher LH pulse amplitudes in post-partum ewes than in control ewes at 14 days after ovariectomy could have been due to a greater release of LH-RH, or to a greater pituitary sensitivity to LH-RH. There was no evidence of prolonged reduction in LH output associated with lactation as has been described for ovariectomized pigs (Parvizi *et al.*, 1976) and rats (Smith & Neill, 1977; Smith 1978). This may reflect the reduced intensity of suckling for one lamb compared with a litter of young, because in rats the degree of suppression of LH release was related to the number of young suckled (Smith & Neill, 1977).

The results of this study indicate that the reduced frequency of pulsatile release of LH in ovariectomized ewes 29 days *post partum* in comparison with ovariectomized cyclic ewes (Wright *et al.*, 1981a) was due to a slower recovery of the hypothalamo-pituitary axis in post-partum ewes from the inhibitory effects of ovarian hormones. An increased inhibitory effect of oestrogen on LH release has been postulated as the basis of ovarian acyclicity in both seasonally anoestrous (Legan *et al.*, 1979) and post-partum anoestrous (Wright *et al.*, 1981a) ewes. Data of Karsch *et al.* (1980) indicate that, at 10–11 days after ovariectomy, the LH pulse frequency in seasonally anoestrous ewes is less than that in cyclic ewes. This suggests that the increased inhibitory effect of oestrogen on LH release and a reduced LH pulse frequency shortly after ovariectomy are both manifestations of the same basic mechanism(s).

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