STUDIES ON THE RELATIONSHIP OF SERUM AND PITUITARY GONADOTROPHIN LEVELS TO OVULATION IN THE EWE

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Observed reductions in the pituitary content of follicle stimulating hormone (FSH) and luteinizing hormone (LH) preceding ovulation in sheep (Santolucito, Clegg & Cole, 1960; Robertson & Hutchinson, 1962; Dierschke & Clegg, 1965; Robertson & Rakha, 1966) clearly imply that both hormones are involved in bringing about this event. These observations regarding pituitary content would seem to provide acceptable criteria for hormone release, but determination of the precise time and interval of gonadotrophin release must be confirmed by quantitative analysis of blood FSH and LH. This communication reports an attempt to accomplish that confirmation utilizing a technique for collecting pituitary effluent blood from the cavernous sinus of sheep (McFarland, Clegg & Ganong, 1960) and the highly sensitive biological assay of Parlow (1961) for LH. Assay of pituitary FSH was done by the method of Steelman & Pohley (1953).

Forty-eight mature White-face ewes were used in a total of five experiments during two different breeding seasons. The protocol for each experiment was essentially the same, the difference being in time and interval of sampling and tissues collected for gonadotrophic assay. Onset of oestrus, defined as the time when the ewe would first accept mounting by an aproned ram, was determined by constant 24-hr observation. Ovarian status at autopsy was confirmed by gross and histological examination.

Cavernous sinus blood samples, ranging from 25 to 500 ml/animal, were collected at the designated times: series of samples from the same animal were included as well as those obtained with the pituitary tissue at the time of slaughter. The serum was separated and either lyophilized (in the initial experiments) or stored frozen until assay. The anterior pituitaries were rapidly removed after death by exsanguination, immediately frozen on dry ice and subsequently lyophilized. All assays were conducted with one dose level of the unknown (2 ml in the case of serum) and two to four doses of the reference preparation. Normally five rats were used per dose. Values for the slope, standard deviation and λ (index of precision) of the dose-response curves were calculated according to Bliss (1952).

In two separate experiments conducted during successive years, pituitary LH concentration as well as total content/gland was shown to become significantly depleted ($P < 0.01$) either between first acceptance (onset of oestrus)

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and 16 hr thereafter (Exp. 2) or between 8 and 18 hr following first acceptance (Exp. 5) with the levels remaining low at the later times of sampling (Table 1). The relative amounts of depletion in concentration were 67% and 62% respectively in the two experiments, or 68% and 48% on the basis of total content.

The pituitary FSH data were not as striking, but a consistent reduction was evident (25% and 27% depletion when expressed as concentration) during the same time interval as for maximal depletion of LH. In Exp. 5, this reduction in concentration was statistically significant ($P < 0.05$) and, in Exp. 2, the same level of significance could be shown by comparing ovarian weight responses

TABLE 1

LH AND FSH ACTIVITY IN ANTERIOR PITUITARY GLANDS AT VARIOUS STAGES OF THE OESTROUS CYCLE IN THE EWE

Time after onset of oestrus (hr)	No. of ewes	LH content ¹		FSH content ²	
		Mean $\pm$ S.E.		Mean $\pm$ S.E.	
		Experiment 2	Experiment 5	Experiment 2	Experiment 5
At first acceptance	5	30.7 $\pm$ 4.8*** (5989 $\pm$ 717)***		5.4 $\pm$ 0.6 (1156 $\pm$ 259)	
8	6	20.0 $\pm$ 3.6** (5794 $\pm$ 732)**		3.7 $\pm$ 0.3* (1122 $\pm$ 106)	
16	4	9.9 $\pm$ 2.3 (1913 $\pm$ 519)		4.0 $\pm$ 0.3 (771 $\pm$ 83)	
18	6	7.6 $\pm$ 0.7 (3009 $\pm$ 312)		2.7 $\pm$ 0.1 (1070 $\pm$ 82)	
32	4	10.9 $\pm$ 2.6 (1746 $\pm$ 461)		4.1 $\pm$ 0.4 (641 $\pm$ 24)	
35	6	8.3 $\pm$ 1.2 (3095 $\pm$ 447)		2.9 $\pm$ 0.1 (1085 $\pm$ 87)	

¹ $\lambda = 0.33, 0.19$ and 0.12 .

² $\lambda = 0.24$ and 0.19 .

³ Hormone concentration ($\mu\text{g NIH-LH-s}_7$ or $\text{NIH-FSH-s}_1/\text{mg dry weight}$).

⁴ Total hormone content ($\mu\text{g NIH-LH-s}_7$ or $\text{NIH-FSH-s}_1/\text{gland}$).

* $P < 0.05$ (comparing times of sampling in this experiment by analysis of variance).

** $P < 0.01$ (comparing times of sampling in this experiment by analysis of variance).

in the recipient assay animals. When analysing the latter data in quantitative terms with respect to the reference standard, however, this degree of difference is obscured, probably due to a rather flat dose-response curve in this particular assay. Total FSH content/gland dropped 33% in Exp. 2, which is consistent with the change in concentration. In Exp. 5, on the other hand, the significant reduction in concentration is obscured by expressing the results as total content. Thus, even though pituitary weights did not differ significantly between groups in either experiment, the group averages did vary enough to alter the final data, depending on the method of evaluation.

A summary of cavernous sinus serum LH assays from the final and most extensive experiment, as an example of the kind of results obtained, is given in Table 2. Assay results are presented in terms of ovarian ascorbic acid so that the statistical analysis could include those values where the quantitative amount of LH in the serum sample was considered to be non-detectable in this

assay system. The values may be compared to an average ovarian ascorbic acid in saline controls of 82.6 mg%. In this particular experiment, an elevated LH level in those samples collected during the early oestrous period (5 to 8 hr after the onset of oestrus) is indicated by the reduced ovarian ascorbic acid associated with those samples. In other experiments, there were trends toward increased levels at 8 to 16 hr, 6 to 14 hr, and 16½ to 17 hr, but none of the differences was statistically significant. Levels of FSH in these same samples were too low to be detected in the Steelman-Pohley system.

The positive aspect of this study is the demonstration of maximal release of

TABLE 2
LH ACTIVITY IN CAVERNOUS SINUS SERUM AT VARIOUS STAGES OF THE
OESTROUS CYCLE IN THE EWE. EXPERIMENT 5¹

Stage of cycle		No. of samples ²	Ovarian ascorbic acid	
Period	Specific times of sampling (hr after onset of oestrus)		mg% ± S.E.	Range
I	5, 5½, 6, 6½, 7, 7½, 8	38	68.0 ± 1.1**	45.2 to 85.3
II	14, 15½, 16, 16½, 17, 18, 20, 22	42	77.5 ± 0.9	59.3 to 100.6
III	32, 32½, 33, 33½, 34, 34½, 35	35	74.7 ± 1.2	57.9 to 92.3

¹ Average λ for assays in this series = 0.25 ± 0.4 (twenty-one assays).

² Usually seven samples were collected at different times from each animal and all samples from one animal were analysed in the same assay.

** $P < 0.01$ (comparing periods of sampling by analysis of variance).

pituitary LH and FSH during the interval of 8 to 16 hr after the onset of oestrus. This would seem to differ somewhat from the recent report by Robertson & Rakha (1966) who showed that minimal pituitary levels were attained by 6 hr and that this process began some 8 hr before oestrus for FSH and between the onset of oestrus and 4 hr thereafter for LH. Unfortunately, our experimental design does not allow a critical evaluation of pituitary levels during that part of the cycle. Another aspect of possible difference is that our data strongly suggest the relatively greater involvement of LH in this pre-ovulatory hormone release while their results indicate that both LH and FSH are equally involved over the total time. The present serum LH data tend to support the possibility of a major release occurring from 5 to 8 hr: this would not necessarily require pituitary depletion to begin then since previous reports on the rat suggest that the initial release is offset by increased LH synthesis (review by Lloyd & Weisz, 1966).

On the other hand, because of inconsistencies among times of peak activity in the different experiments, variations in serum results between experimental animals and the difficulty of consistently detecting increased levels of serum LH during pre-ovulatory periods, these serum data provide only speculative evidence of hormone release to compare with the clear-cut pituitary data. Other reports (for example: Frankel, Gibson, Graber, Nelson, Reichert & Nalbandov, 1965; Nallar & McCann, 1965; Pelletier, 1965) have indicated

that such inconsistent results may be due to factors other than LH in serum assayed by the Parlow method. This theory is supported by preliminary data from the radio-immunoassay of some of the serum samples from the present study (I. I. Geschwind, personal communication). The LH values obtained by the latter method were usually much lower (as much as a hundred-fold in some cases) and there was a general lack of correlation in relative amounts as determined by the two methods, thus supporting the suggestion that this assay may not be appropriate for quantitation of LH in sheep blood.

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