Serum Levels of Prolactin and Luteinizing Hormone (LH) in the Ewe at Various Stages of the Estrous Cycle[1] (34916)

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Since recent evidence indicates that prolactin may not be the luteotropic hormone of the sheep (1), the role of prolactin and its cyclic changes during the estrous cycle in the ewe remains to be clarified. For this reason studies on variations in blood prolactin and LH measured simultaneously during various stages of the estrous cycle in the ewe may be important.

The most common methods used for determining prolactin and LH levels in biological material have been the pigeon crop sac bioassay of Lyons (2) and the ovarian ascorbic acid depletion (OAAD) assay of Parlow (3). However, these assays are not sensitive enough to measure satisfactorily prolactin or LH in the blood of intact animals. Recent development of radioimmunoassays (RIA) for ovine prolactin (4) and ovine LH (5, 6) have made possible the quantitation of prolactin and LH levels in serum of intact sheep.

This study was designed to simultaneously determine serum prolactin and LH levels during the estrous cycle in the intact ewe using double antibody radioimmunoassays.

Materials and Methods. Three whitefaced, cross-bred, yearling ewes were used throughout the experiment and tested daily for estrus by a vasectomized ram. The estrous cycle of the sheep was divided into 4 periods as described by Cupps *et al.* (7). Estrus was defined as the period when the ewe accepts the male and was usually 2 days in length (Days 1 and 2). Metestrus was considered as Days 3 and 4 of the cycle. Diestrus was considered as Days 5 through 13 and proestrus as Days 14 until estrus. During the five estrous cycles studied in the ewes, jugular blood samples were collected daily at 8 a.m.

The double antibody radioimmunoassay of prolactin described by Arai and Lee (4) and modified by Arimura (unpublished data) was utilized in these studies. NIH-prolactin-S8 was used both as a standard and for labeling with ^{125}I. The prolactin ^{125}I was diluted with 1% bovine serum albumin (BSA) so that 100 μl gave 6000 to 7000 cpm in a liquid scintillation spectrometer. Guinea pig anti-ovine prolactin (GPAOP) antiserum was supplied by Drs. Arai and Lee and was used after a dilution of 1:20,000. An excellent standard curve was obtained within a range of 2 to 64 mμg/ml of prolactin (NIH-P-S8). In this assay there is no cross-reaction with ovine LH, FSH, or GH (Arimura, unpublished data). This assay is also supported by present findings that prolactin could not be detected in hypophysectomized ewe serum.

Plasma LH was determined by a double antibody radioimmunoassay. Purified ovine LH obtained from Dr. D. N. Ward was iodinated with ^{125}I according to the methods of Greenwood and Hunter (8). The LH-^{125}I was repurified and free ^{125}I was removed on a Sephadex G-50 column just before use. The repurified fraction of LH-^{125}I was then diluted with 0.1% egg white (EW) in 0.01 *M* phosphate, 0.15 *M* NaCl, 0.01% merthiolate, pH 7.6 (PBS), so that 100 μl contained 8000 to 9000 cpm. One percent EW-PBS was used as diluent for all reagents and serum samples in this assay. Dr. W. F. White supplied the first antibody used, which was guinea pig anti-ovine LH (GPAOLH) an-

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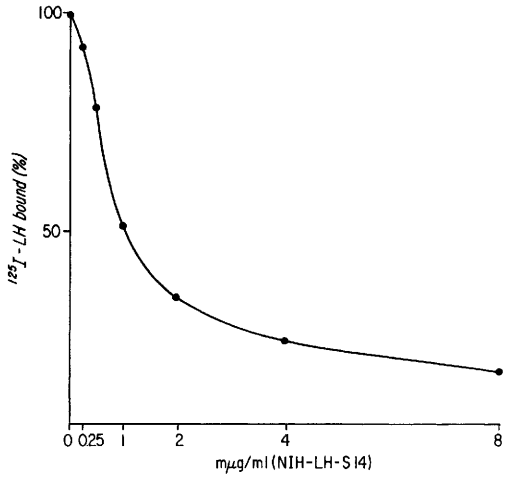

FIG. 1. A typical standard curve for the determination of ovine LH by radioimmunoassay. The amount of LH ^{125}I bound in the absence of unlabeled LH was set equal to 100%.

tiserum, diluted 1:80,000 in a 1:400 normal guinea pig serum EW-PBS solution. Five-hundred μl of standard NIH-LH-S14 solution or diluted serum samples were added in duplicate to 10 × 75-mm disposable culture tubes. Two-hundred μl of the first antibody was then added to the tubes and the mixture was incubated for 2 days at 4°. One-hundred μl of LH-^{125}I was then added, followed 24 hr later by 200 μl of goat antiguinea pig gamma globulin (GAGPGG) antiserum diluted 1:40. After the tubes had been incubated for an additional 3 days, 1 ml of PBS was added to each of the tubes which were then centrifuged at 1,465*g* for 15 min. The supernatant was decanted and the precipitate was counted in a liquid scintillation spectrometer.

Results. The standard curves obtained for LH (Fig. 1) appeared satisfactory over a range of 0.25 to 8 mμg/ml of NIH-LH-S14. The quantity of ovine LH-^{125}I bound to the antibody in the absence of unlabeled LH (EW-PBS Tubes) was considered to represent 100%. Dose–response curves demonstrating the decrease in antibody-bound ovine LH-^{125}I, due to addition of increased amounts of pituitary preparations are shown in Fig. 2. The area where 20 to 80% of the ovine LH-^{125}I was bound by the antibody was used to calculate relative potency estimates. In this area the curves for all preparations were similar in slope.

Table I shows the relative potencies of five pituitary preparations as estimated by OAAD and RIA. In all cases, except NIH-TSH-S5, the indices of discrimination (OAAD LH/RIA LH) approach equality. There was also no detectable LH in serum obtained from a hypophysectomized ewe.

TABLE I. Luteinizing Hormone Content of Ovine Pituitary Preparations.[a]

Preparation	LH OAAD (U/mg)	LH RIA[b] (U/mg)	OAAD-LH /RIA-LH
NIH-GH-S8	0.025	0.023	1.08
NIH-FSH-S4	0.017	0.015	1.13
NIH-LH-S14	0.98	0.98	1.00
Ovine pituitary homogenate	0.0028	0.0031	0.90
NIH-TSH-S5	0.0059	0.10	0.059[c]

[a] LH relative potencies are expressed in terms of NIH-LH-S1; 1 unit = 1 ng of the standard preparation.

[b] NIH-LH-S14 was used as standard for determination of relative potency of radioimmunoassays.

[c] See discussion of this hydrogen peroxide-treated preparation.

The possible interference with the determination of serum LH by the second antibody (GAGPGG) containing goat LH, which could possibly cross-react with sheep LH was also investigated. The test involved addition of three different levels of ovine LH (0, 2, and 4 mμg/ml) with the second antibody of Day

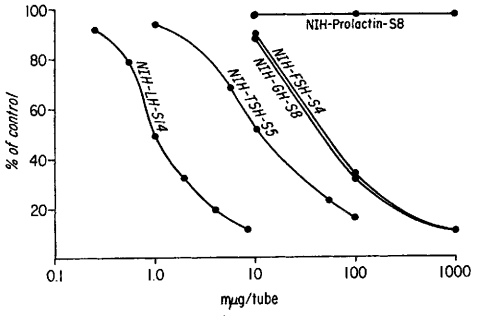

FIG. 2. Dose–response curves for ovine pituitary preparations. Radioactivity in all cases is expressed as the percentage of OLH-^{125}I found in the buffer control tubes.

TABLE II. Effect of Temperature and Time of Storage of Blood on Prolactin Content.

Ewe no.	Stored (hr)	Prolactin[a] (mμg/ml) Blood stored at 22°	Blood stored at 4°
1	6	72	80
2	6	50	61
3	6	0	0
1	12	90	85
2	12	70	65
3	12	15	0
1	24	85	85
2	24	68	70
3	24	0	5

[a] NIH-Prolactin-S8.

3 of the assay. There were no significant changes in labeled LH-^{125}I precipitated in samples with or without addition of LH to the second antibody. This indicates that goat LH present in GAGPGG serum does not interfere with the LH determination.

Table II shows the effect of time and temperature during storage of the blood before centrifugation on immunoassayable prolactin concentration in the serum. There were no significant differences in prolactin levels whether they were stored for 6, 12, or 24 hr. Subsequently, all blood samples were stored 24 hr at 4° before centrifugation and the resulting serum was stored at −25°. It has previously been demonstrated (9) that radioimmunoassayable LH activity in sheep blood is not altered by this type of handling.

Table III shows serum prolactin and LH levels during the five estrous cycles studied. Prolactin levels were significantly higher during proestrus (49 mμg/ml) and Day 1 estrus (40 mμg/ml) than during Day 2 estrus (11 mμg/ml), metestrus (15 mμg/ml) and diestrus (13 mμg/ml). Elevated LH values of 95 and 175 mμg/ml were detected in only two of the five serum samples collected on Day 1 of estrus. However, the mean LH value for all of the Day 1 values was 56 mμg/ml (Table III) which was greater ($p<.01$) than at any other stage of the estrous cycle. Thus, high prolactin levels are not correlated with the elevated LH values, even though the peak in LH was obtained while prolactin levels were high.

Discussion. A double antibody radioimmunoassay for ovine LH, which involves the use of guinea pig anti-ovine LH as a first antibody and goat antiguinea pig gamma globulin as a second antibody has been shown to be satisfactory for the measurement of ovine serum LH levels. Ovine prolactin, FSH, and GH exhibited no significant cross-reaction in the LH radioimmunoassay. However, some cross-reaction observed with TSH in this assay is similar to the finding previously reported (6, 10) by workers using hydrogen peroxide treated TSH. The slope of the curves given by LH and TSH standards (Fig. 2) were quite similar, indicating that contamination with LH was possibly responsible for the observed cross-reaction. However, the biological contamination of NIH-TSH-S5 with LH is much lower than that observed by the present radioimmunoassay observations. The NIH-TSH-S5 used in this study was treated with hydrogen peroxide to destroy the biological activity of LH present in the preparation (11). However, recently Reichert and Treadwell (12) reported that ovine LH treated in the same way retained its ability to react immunologically with antibovine LH

TABLE III. Serum Prolactin and LH Levels in Ewes at Various Stages of the Estrous Cycle.

Period of estrous cycle (days)	No. of observations	Prolactin (mμg/ml; mean ± SE)[a]	LH (mμg/ml; mean ± SE)[b]
Estrus			
(1)	5	40 ± 5.5[c]	56 ±27.9[d]
(2)	4	11 ± 2.6	1.9 ± 0.5
Metestrus			
(3–4)	6	15 ± 5.4	1.6 ± 0.2
Diestrus			
(5–13)	28	13 ± 2.0	1.1 ± 0.1
Proestrus			
(14–17)	14	49 ± 8.2[c]	1.4 ± 0.2

[a] NIH-P-S8.

[b] NIH-LH-S14.

[c] Significantly higher than day 2 estrus, metestrus, and diestrus ($p<.05$).

[d] Significantly higher than next ranked mean ($p<.01$).

serum. Therefore, the hydrogen peroxide treatment of NIH-TSH-S5 does not necessarily destroy the immunoreactivity of the LH contaminant in this preparation. These previous findings support the assumption that the cross-reactivity of TSH in this assay may be due to immunoreactive LH contaminating the TSH preparation rather than a cross-reaction with the TSH molecule. However, whatever the correct explanation may be, it is improbable that cross-reaction with TSH could account for the results observed with ewe serum, since the degree of cross-reaction was less than 10%.

Prolactin levels were shown to be significantly higher during proestrus and Day 1 of estrus than at any other stage of the estrous cycle (Table III). These observations are in agreement with the findings of Kwa and Verhofstad (13) which demonstrated that in mice a surge of prolactin starts at proestrus and extends through the first part of estrus followed by a decrease during the latter stages of estrus. Niswender *et al.* (14) also reported that in rats, the highest levels of prolactin in serum obtained during the estrous cycle occurred during the afternoon of proestrus. Previously, Santolucito *et al.* (15), using the crop sac response in pigeons, found that the prolactin content of sheep pituitaries did not vary significantly during the various stages of the estrous cycle.

Although the mean LH levels were found to be significantly higher on Day 1 of estrus, elevated values were only detected in two of the five observations on Day 1. The LH peak is thought to last only 10 hr (10), so that with daily sampling of serum the peak of LH may be theoretically missed more than 50% of the time.

It has been reported (16, 17) that plasma levels of estrogen in the sheep are at a minimum during metestrus with increasing levels thereafter and maximum levels being noted during proestrus and early estrus. Goding *et al.* (10) reported that 9 hr after injection of 50 μg of estradiol, an LH peak can be detected in the blood comparable to that occurring prior to ovulation. Prolactin levels have also been shown to be increased by injections of estradiol benzoate in the rat (18). Thus, it is possible that the endogenous rise in estrogen levels in blood during proestrus and prior to ovulation induces an increase in both prolactin and LH release by acting either directly via the pituitary or indirectly via the hypothalamus.

Summary. Estrus activity was checked daily in three whitefaced, cross-bred, yearling ewes and jugular blood samples were collected daily during five estrous cycles. Serum prolactin and LH levels were determined by double radioimmunoassays. Prolactin levels were significantly higher during proestrus, (49 mμg/ml) and Day 1 estrus (40 mμg/ml) than during Day 2 estrus (11 mμg/ml), metestrus (15 mμg/ml) and diestrus (14 mμg/ml). LH levels were elevated on Day 1 estrus in only two of the five estrous cycles observed. The mean LH level for all five of the Day 1 estrus observations was 56 mμg/ml. This level was significantly higher than that during Day 2 estrus (1.9 mμg/ml), metestrus (1.6 mμg/ml), diestrus (1.1 mμg/ml) and proestrus (1.4 mμg/ml). These data suggest that the duration of the rise in prolactin levels is longer and precedes the elevated LH value at estrus. Serum prolactin levels did not seem to be correlated with the rise in LH, even though the increase in LH occurs at a time when serum prolactin levels are still high.

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