DEVELOPMENT OF A HOMOLOGOUS RADIOIMMUNOASSAY FOR OVINE FOLLICLE STIMULATING HORMONE: STUDIES AFTER ESTRUS, OVARIECTOMY, ESTRADIOL AND RELEASING HORMONE\textsuperscript{1,2}

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Summary

The development of a specific, sensitive homologous ovine follicle stimulating hormone (FSH) radioimmunoassay (RIA) is described. The key procedural modifications of basic RIA methods were immunoabsorption of the anti-ovine FSH serum with luteinizing hormone (LH) to improve specificity and adsorption chromatography of the radioiodinated ovine FSH on hydroxyapatite (HTP) to improve immunoreactivity. Cross-reactivity of the immunoabsorbed FSH antisera (R5-41A) was less than .5\% with NIH-prolactin-S10, NIH-GH-S11, NIH-TSH-S5 and LER-LH-1374a. Sensitivity (5.5 ng of NIH-FSH-S10) of the assay was adequate for quantification of plasma FSH in all ewes studied. Accuracy of the assay was high. The recovery of FSH from ovine plasma was nearly quantitative ($y = -5.5 + 1.1x$, $r^2 = .999$), and the mean ratio of biological potency by bioassay to immunological potency by RIA of several FSH preparations was $1 \pm .2$. Intraassay and interassay coefficients of variation were both less than 15\%. Four studies with Rambouillet ewes were conducted. Study 1 was with ovariectomized ewes. FSH and LH both increased at a similar rate after surgery. Values for both hormones were four- to sixfold greater 15 days after surgery than before surgery. Study 2 was with ewes at estrous. Two peaks of similar magnitude were observed in plasma FSH. The first occurred about 10 hr after the onset of estrus and coincided with the large peak in plasma LH. The second occurred about 24 hr later, when plasma LH was low. Study 3 was with ovariectomized ewes that were injected with estradiol. Plasma concentration of both FSH and LH tended to decline for the first 6 hr postinjection. The large peak in plasma LH, which occurred about 14 hr postinjection, was similar in both height and duration to that observed in estrous ewes. The small increase in plasma FSH, which coincided with the LH peak, was not significant. Study 4 was conducted with ovariectomized ewes given single or multiple injections of LH-RH/FSH-RH. Both FSH and LH increased significantly, but the response was considerably greater for LH. These results confirm that the anti-ovine FSH serum after immunoabsorption and radioiodinated ovine FSH after absorption chromatography on HTP were reliable reagents for the development of a specific, sensitive homologous ovine FSH radioimmunoassay.

(Key Words: Ewes, Ovine, Follicle Stimulating Hormone, Luteinizing Hormone, Radioimmunoassay.)

Introduction

Several homologous luteinizing hormone (LH) radioimmunoassays (RIA) have been developed and used successfully for studying the fluctuation of LH in plasma of sheep (Geschwind and Dewey, 1968; Pelletier et al., 1968; Goding et al., 1969; Niswender et al., 1969; Wheatley and Radford, 1969; Scaramuzzi et al., 1970). However, a reliable, homologous ovine follicle stimulating hormone (FSH) RIA has been difficult to develop because ovine FSH tends to lose immunological potency after radioiodination and because antisera...
against ovine FSH, even highly purified FSH, usually show a high degree of cross-reactivity with LH and thyroid stimulating hormone (TSH).

Absorption of nonspecific anti-ovine FSH serum with TSH (Bailly du Bois et al., 1970) reduced the problem of cross-reactivity, but the antiserum was not suitable for measurement of FSH in plasma because sensitivity was inadequate. Others have unsuccessfully attempted to develop a reliable RIA for FSH by absorbing anti-ovine FSH serum with LH or TSH to remove excessive cross reaction (L'Hermite et al., 1972; Siddall and Crighton, 1977). In addition, albumin and other substances in ovine plasma have been reported to cause nonspecific interference in homologous ovine FSH RIA (Salamonsen et al., 1973a).

Because of these difficulties, several workers (L'Hermite et al., 1972; Salamonsen et al., 1973b; McNeilly et al., 1976; Sanford et al., 1977; Pant et al., 1977) have resorted to quantifying FSH in ovine plasma with assays in which nonovine FSH is used to radioiodinate and to generate antiserum. Although the sensitivity and specificity of the resulting heterologous FSH assays have been adequate, immunological differences in FSH among species (Cheng, 1978) suggest the need for an ovine FSH assay in which only ovine FSH is used for the assay tracer and the antigen in the preparation of the assay antiserum.

The present paper describes the development, characterization and use of a highly specific, sensitive, homologous RIA for ovine FSH. Also described are changes in plasma FSH and LH in ewes after: (1) ovariectomy, (2) estrus, (3) treatment with estradiol and (4) treatment with releasing hormone (LH-RH/FSH-RH).

Materials and Methods

Assay Buffers. The basic assay buffer was phosphate-bicarbonate-buffered saline (PBBS) that contained .135 M NaCl; .01 M sodium phosphate (NaPO₄), pH 7.5; .005 M sodium bicarbonate; 1:10,000 merthiolate, and 1:100,000 phenol red. The FSH antisera were diluted in PBBS that contained .05 M sodium ethylenediamine tetraacetate, pH 7.5 (EDTA-PBBS), and 1:300 normal rabbit serum (NRS). The anti-rabbit gamma globulin serum (DJB, 5X3) was diluted at 1:80 in EDTA-PBBS without NRS. The standards, unknown plasma and assay tracer (¹²⁵I-FSH) were diluted with PBBS that contained .1% gelatin (gel-PBBS)⁴. Albumin from various species was also tested because ovine albumin has been reported to interfere in ovine FSH RIA (Salamonsen et al., 1973a). The proteins tested included egg white albumin⁵, bovine serum albumin (fraction V⁶) and ovine serum albumin (fraction V⁶).

All buffers and reagents used during radioiodination of FSH were freshly prepared with water that had been purified by reverse osmosis involving resin deionization⁷, glass distillation and vacuum degassing. Buffers used in radioiodination of ovine FSH were also passed through .45-µm filters⁸.

Assay Tracer. The most successful of several radioiodination methods was a modification of the chloramine T method (Greenwood et al., 1963). Highly purified ovine FSH (HP-G4-150) with a biological potency of about 50 × NIH-FSH-SI was used. The following reagents were quickly added to a 1-ml stoppered borosilicate glass vial that contained 5 µg .5 M NaPO₄, pH 7.5; 250 µCi ¹²⁵I⁹, and 2.5 µg of chloramine T (freshly dissolved) in 5 µl .25 M NaPO₄.

The ¹²⁵I-Bound FSH was separated from free ¹²⁵I with an anion exchange resin (AGX10, chloride form, 50 mesh¹⁰) as suggested by Chard et al. (1970). The resin, approximately .5 ml in a 2.5-ml plastic syringe, was prewashed with 3 ml each of .5 M NaPO₄ (pH 7.5), 1% bovine serum albumin in H₂O and .05 M NaPO₄ (pH 7.5). The resin retained the free ¹²⁵I and, possibly, acidic fragments and aggregated forms of the radioiodinated hormone (Tower et al., 1978). The ¹²⁵I-FSH was eluted into a tube containing .5 ml of gel-PBBS with an additional 1.5 ml .05 M NaPO₄. The specific activity (microcuries/microgram) of the ¹²⁵I-FSH was estimated after anion

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⁴ Mann Research Laboratories, New York 10006.
⁵ Sigma Chemical Co., St. Louis, MO.
⁶ Miles Laboratories, Kankakee, IL 60901.
⁷ Culligan, Northbrook, IL 60062.
⁸ Millipore Corporation, Bedford, MA 01730.
⁹ Amersham Corporation, Arlington Heights, IL 60005.
¹⁰ Bio-Rad Laboratories, Richmond, CA 94804.
exchange chromatography. Calculations assumed that $^{125}$I-FSH was present only in the effluent from anion exchange and in the rinsed radioiodination vial. Any fragments, aggregates or other forms of $^{125}$I-FSH that were retained by the resin would cause the specific activity to be underestimated. The specific activity averaged $23 \pm 2 \mu$Ci/μg for the four preparations of $^{125}$I-FSH used for assaying the plasma samples described under “Animal Studies” (see below).

To improve the immunoreactivity of the assay tracer, the $^{125}$I-FSH was subjected to adsorption chromatography on hydroxyapatite (HTP) by a modification of the method described by Tiselius et al. (1956) and Hjerten (1959). Before adsorption chromatography, the $^{125}$I-FSH was desalted on a Sephadex G-25 column (PD 10). The column was equilibrated and eluted with .005 M NaPO₄, pH 6.8. Next, the $^{125}$I-FSH from G-25 was applied to HTP (7 x 10 cm column) and eluted batchwise at the rate of .5 ml/min with increasing concentrations of NaPO₄ buffer, pH 6.8. The concentrations of buffer were 20 ml .005 M, 20 ml .05 M and 15 ml .5 M. The entire .05 M effluent was diluted in gel-PBBS and used as assay tracer. The immunoreactivity (percentage of tracer bound by excess antibody) for each batch was determined after a 24-h incubation of 1 ng of $^{125}$I-FSH and anti-FSH serum, R5-4IA, at a final dilution of 1:1,200.

In an effort to describe more completely the chromatographic behavior of $^{125}$I-FSH on HTP, radioactivity and immunoreactivity were determined for each of the 1-ml fractions, as shown in figure 1. Subsequently, an aqueous extract of ovine pituitary glands was pooled with previously chromatographed $^{125}$I-FSH (fraction 33 to 38 of the .05 M effluent from HTP shown in figure 1) and eluted from a second HTP column as shown in figure 2. Each 1-ml fraction was assayed for FSH (as described under “Antisera”), LH (Echternkamp et al., 1976) and radioactivity.

Antisera. Six rabbits were injected eight times over a 6-month period with ovine FSH (HP-G4-50C, biological potency about 10 x NIH-FSH-S1, H. Papkoff, personal communication), by the multiple site injection technique (Vaitukaitis et al., 1971). Sixty sera samples from 10 blood collections were screened at a final dilution of 1:16,000 for specificity by their capacity to bind FSH and not LH. Screening for FSH binding was done with about 1 ng of $^{125}$I-FSH. Screening for LH binding was done with 1 μg of NIH-LH-S12 added to the $^{125}$I-FSH.

Because screening failed to identify an antiserum that bound FSH but not LH, two procedures for reducing LH crossreactivity were attempted. The first was to preabsorb the most promising antiserum, R5-4, with LH. A working dilution of R5-4 was incubated overnight at 4 C with LH (NIH-LH-S12) equivalent to 10 rig/assay tube. The resulting preabsorbed antiserum was centrifuged and designated as R5-4PA.

The R5-4 and R5-4PA antisera were compared at a final dilution of 1:16,000 for both cross reaction with LH (with up to 1,000 ng/tube, as shown in figure 3) and for parallel response between purified ovine FSH (HP-G4-96) and four dilutions of two ovine plasma samples.

Because responses of ovine plasma samples and purified ovine FSH were not parallel when the preabsorbed antiserum was used, a second absorption procedure involving immunoabsorption (Wosfy and Burr, 1969) was employed. Sepharose 4B, 1 g, was coupled with 1 mg of

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NIH-LH-S12 by previously described methods (Axen et al., 1967). The immunoabsorbant, LH-Sepharose, and 21 ml of a 1:3000 dilution of R5-4 were incubated overnight at 4 C. The immunoabsorbed antiserum was recovered after centrifugation at 2,500 × g and was designated as R5-4IA. The R4-4IA antiserum was used at a final dilution of 1:20,000.

Specificity of the R5-4IA antiserum was evaluated by cross reactions with NIH-prolactin-S10, NIH-GH-S11, NIH-TSH-S5 and LER-LH-1374a, with the amounts shown in figure 4. Cross-reactivity was calculated from the relative amounts of test hormone and FSH (HP-G4-150) that inhibited binding of the assay tracer by 50%. When a test hormone failed to inhibit binding by 50%, calculations for cross-reactivity were based on the largest amount assayed. Specificity of the R5-4IA antiserum was also tested in assays of plasma from ewes that were treated with thyrotropin releasing hormone (TRH) to cause the release of prolactin and TSH (Siddal and Crighton, 1977).

Accuracy of the assay was evaluated both by the recovery of NIH-FSH-S10 (10 to 640 ng) from .1 ml of three ovine plasma samples and by the ratio of biological potency to immunological potency of seven FSH preparations (four ovine, two bovine and one porcine). Immunological potencies of these preparations were determined by the amount required to inhibit binding in the RIA by 50%. The reference was NIH-FSH-S1. Biological potencies were also relative to NIH-FSH-S1 and were obtained from the following sources: NIH hormones (Reichert and Wilhelmi, 1973); HP-G4-150, (Papkoff, personal communication) and USDA-BPI (Grimek et al., 1979).

Assay Protocol. The routine protocol was designed to achieve equilibrium assay conditions (Rodbard et al., 1971) and was carried out by the simultaneous addition\(^{12}\) of the sample (.2 ml of the plasma or standard), assay diluent (.3 ml gel-PBBS), \(^{125}\)I-FSH assay tracer (.1 ml containing approximately 1 ng with specific activity of about 25 μCi/μg) and

Animal Studies

Mature Rambouillet ewes were used in each of the four studies. All plasma FSH and plasma LH values are reported in the text as nanograms/milliliter ± standard errors. The reference standards were NIH-FSH-S10 and NIH-LH-S12, respectively. Profiles of FSH and LH were plotted on the same log scale with appropriate exponents to facilitate comparisons of relative changes between these two hormones over time.

Study 1. Ovariectomy. Ewes were ovariectomized in late March when most had ceased showing behavioral estrus. At the time of surgery, six ewes were designated as anestrus because their ovaries contained no corpora lutea and no large corpora albicans. Six other ewes were designated as luteal because their ovaries contained large corpora lutea. Blood samples for the determination of plasma FSH and LH were obtained by jugular puncture daily for 1 week, then less frequently, as shown in figure 5.

Study 2. Estrus. Ewes were checked for behavioral estrus at 2-hr intervals with the aid of vasectomized rams. Blood samples for plasma FSH and LH analysis were obtained by jugular puncture at frequent intervals from the onset of estrus to 56 hr after estrus, as shown in figure 6a.
Study 3. Estradiol Treatment. Six ovariectomized ewes were treated IM with 50 μg of estradiol-17β (estradiol) approximately 2 months after surgery. Blood samples were obtained for plasma FSH and LH analysis by jugular puncture at frequent intervals, as shown in figure 6b.

Study 4. Releasing Hormone. Twenty-four ewes that had been ovariectomized about 2 months earlier were each assigned to one of four LH-RH/FSH-RH treatment groups. Ewes were injected IV with 0, 30 or 90 μg in 1 ml of saline or with 90 μg divided into three 30-μg injections at 1-hr intervals. Blood samples for plasma FSH and LH analysis were obtained by jugular puncture over a 7-hr period at the times indicated in figures 7a and 7b.

As mentioned previously, plasma samples from ewes treated with TRH were used to show the lack of cross-reactivity of the R5-41A antiserum with prolactin and, presumably, TSH. These samples were obtained from the six control ewes (0 μg of LH-RH/FSH-RH) after an IV injection of 300 μg of TRH as indicated in figure 7. Plasma prolactin was determined by a previously described prolactin RIA using antiprolactin serum (DJB 7-0330) and NIH-prolactin-S8 as a standard (Echtern-kamp et al., 1976).

Results and Discussion

Assay Buffers. The basic assay buffer, PBBS, was phosphate-buffered saline (PBS) with two modifications. The first was the addition of phenol red to provide a visual indicator of pH in all samples. The second was the addition of sodium bicarbonate to insure that samples containing ovine plasma and assay standards both incubated at the same pH. The pH of assay standards tended to decrease during incubation when PBS buffer was used.

Gelatin (1%) was added to all nonantibody-containing assay buffers. The effect of gelatin and albumin from three species on the binding of 125-I-FSH by anti-ovine FSH serum (RS-4IA) is shown in table 1. Although ovine serum albumin reduced binding, the quantity required was greater than that contained in the usual 0.2-ml plasma sample, assuming plasma contains about 3% albumin (Swenson, 1977). The possibility cannot be ruled out that ovine serum albumin may have inhibited binding to some extent because the FSH antiserum was nonspecific, as suggested by Salamonsen et al. (1973a), but a more likely explanation is that the preparation of ovine serum albumin was actually contaminated with a small amount of FSH. The apparent FSH contamination in the ovine serum albumin was only 0.0002% (w/w) compared to NIH-FSH-S10.

Submicron filtration of the buffers for radioiodination removed a noticeable amount of unidentified, gray-colored particulate matter.

Assay Tracer. Initial attempts to radioiodinate ovine FSH were made by various modifications of the chloramine T procedure...
TABLE 1. EFFECT OF SEVERAL PROTEINS ON THE PERCENTAGE OF BINDING$^a$ OF $^{125}$I-FSH BY R5-4IA ANTI-OVINE FOLLICLE STIMULATING HORMONE

<table>
<thead>
<tr>
<th>Protein in .5-ml sample (mg)</th>
<th>% of binding</th>
<th>Egg white albumin</th>
<th>Bovine serum albumin</th>
<th>Ovine serum albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gelatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.5</td>
<td>.1</td>
<td>105%</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>2.5</td>
<td>.5</td>
<td>NT$^b$</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>5.0</td>
<td>1.0</td>
<td>NT</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>25.0</td>
<td>5.0</td>
<td>NT</td>
<td>102</td>
<td>98</td>
</tr>
<tr>
<td>50.0</td>
<td>10.0</td>
<td>NT$^b$</td>
<td>NT</td>
<td>96</td>
</tr>
</tbody>
</table>

$^a$Percentage of binding (B/Bo) of duplicate protein samples (B) was compared with the mean for six samples with no protein added (Bo). Nonspecific binding with phosphate bicarbonate-buffered saline alone was 1.9% and that with Bo/T was 32%. The antiserum was R5-4IA at a final dilution of 1:20,000. The assay tracer was eluted from hydroxypapitite and was diluted to about 1 ng/.1 ml of gel-PBBS.

$^b$NT = not tested.

(Greenwood et al., 1963) followed by gel filtration on Sephadex G-100 (1 x 27 cm$^{11}$). Radioiodination reactions that used 5 to 25 $\mu$g of chloramine T, 1 mCi of $^{125}$I and 5 $\mu$g of FSH appeared to damage or aggregate the hormone, as indicated by two protein peaks in the elution profile from G-100. The elution profile after less vigorous iodination reactions (FSH:chloramine T, 1:1 w/w and 1 mCi $^{125}$I) revealed only one protein peak, but the immunoreactivity of all fractions from Sephadex G-100 was low (less than 50% with R5-4 at a final dilution of 1:1,200). In addition, none of these fractions was suitable for use as an assay tracer because the response curves for FSH standards and serial dilution of sheep plasma were not parallel.

Alternate radioiodination methods were used in an effort to produce a more useful assay tracer. These were the lactoperoxidase method (Miyachi et al., 1972, 1973), the Bolton and Hunter (1973) method and the modification of the chloramine T method described by Butt (1972). Although these methods were used on only a limited scale and always in combination with gel filtration chromatography, none proved satisfactory. The $^{125}$I-FSH had either a low specific activity (less than 10 $\mu$Ci/ $\mu$g) or a low immunoreactivity (less than 50% binding with 1:1,200 R5-4). Possibly, the $^{125}$I-FSH prepared by these methods could have been used as an assay tracer if additional chromatography on HTP had been performed, but such chromatography was not attempted.

An important step in the preparation of immunoreactive assay tracer was adsorption chromatography of the $^{125}$I-FSH on HTP. HTP has been used previously to purify FSH from ovine pituitary extracts (Sherwood et al., 1970). All assay tracers used in the four animal studies discussed here were prepared from the .05 M effluents from HTP. The mean immunoreactivity of these assay tracer was 77 ± 4% (n = 4 iodinations). Tests of immunoreactivity on the pre-HTP, .005 M and .5 M effluents from HTP from these same iodinations averaged 52 ± 10, 64 ± 3 and 34 ± 8%, respectively. The elution of $^{125}$I-FSH from HTP is more completely characterized in figures 1 and 2. A portion of the combined fractions 33 to 38 (figure 1) was added to ovine pituitary extract and rechromatographed on HTP as shown in figure 2. The major part of the $^{125}$I-FSH and immunoassayable FSH eluted with .05 M buffer, while most of the immunoassayable LH and very little immunoassayable FSH eluted with .5 M buffer. These large relative differences between immunoassayable FSH and immunoassayable LH demonstrate the low cross-reactivity of LH in the FSH assay.

Antiserum. Each collection of antiserum from the six immunized rabbits was screened for identification of those with both adequate titer to be used in a RIA at a final dilution of at least 1:16,000 and sufficient specificity to permit accurate measurement of FSH in samples that also contained LH. Each of the six
rabbits produced at least one antiserum that bound more than 15% of the 125I-FSH, but none of the antisera was specific. One microgram of LH nearly eliminated binding of 125I-FSH by each antiserum from five of the six rabbits.

Antiserum R5-4 was an exception. The reduction in binding by LH was only 25%, which suggested the antiserum could be used to assay FSH if the cross-reacting LH antibodies could be neutralized by adsorption. Initially, the adsorption procedure was to preincubate antiserum R5-4 with LH. Figure 3 compares the preadsorbed antiserum R5-4 PA and unadsorbed R5-4 for their capacity to bind 125I-FSH in the presence of various amounts of LH. LH inhibited binding by about 10% with R5-4PA and by about 25% with R5-4. Based on these data, LH cross-reactivity was less than 1.8% for each antiserum. However, the small cross-reactivity value should obscure neither the serious lack of specificity nor the inaccuracy that would result if the unadsorbed R5-4 were used to assay FSH in plasma samples that contained LH. Note in figure 3 that LH and FSH had similar potencies between 90 and 80% bound (10 and 20% inhibition), which is the binding range for many plasma samples. Calculations of LH cross-reactivity based on 10% inhibition of binding rather than the more traditional 50% inhibition yielded values for R5-4PA of 12 and 136%, respectively.

The specificity of R5-4PA may have been acceptable, but the antiserum was not a suitable reagent for measuring FSH in plasma because serial dilutions of plasma and FSH standards were not parallel (data not shown). The reason for the lack of parallelism was suspected to involve an interaction between plasma and soluble LH-antibody complexes in the preabsorbed antiserum. This suggested an alternate adsorption procedure. Immunoabsorption was employed because all antibodies in antiserum R5-4 that bound to the beads of LH-Sepharose would be removed by centrifugation. Because the immunoabsorbed antiserum R5-4IA was acceptably specific and usually resulted in a parallel response between plasma and FSH, no attempt was made to either confirm or explain differences between R5-4PA and R5-4IA.

The specificity of the antiserum R5-4IA is shown in figure 4. Cross-reactivity with LH, TSH, growth hormone and prolactin relative to FSH was less than .5%. The inhibition of binding by five ovine plasma samples is also shown in figure 4.

The accuracy of the assay based on recovery of FSH (NIH-S10) from three plasma samples is shown in table 2. The regression equation for the mean recovery values was $y = -5.8 + 1.1 (x)$, $r^2 = .999$. Accuracy, based on the ratio of biological to immunological potency of various FSH preparations, is presented in table 3. The ratios ranged from .4 to 1.7, with a mean of $1 \pm .2$, for seven FSH preparations from three species. Although the assay appears to be capable of measuring bovine and porcine FSH, no attempt was made to confirm this supposition. Sensitivity of the assay in relation to NIH-FSH-S10 was 5.5 ng and the intra- and interassay coefficients of variation were both less than 15% (Midgley et al., 1969).

TABLE 2. RECOVERY OF OVINE FOLLICLE STIMULATING HORMONE (FSH) FROM OVINE PLASMA

<table>
<thead>
<tr>
<th>NIH-FSH-S10 added, ng</th>
<th>FSH recovered from 100 μl of plasma, ng&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EP-3</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>40</td>
<td>33</td>
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<tr>
<td>80</td>
<td>82</td>
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<tr>
<td>160</td>
<td>123</td>
</tr>
<tr>
<td>320</td>
<td>356</td>
</tr>
<tr>
<td>640</td>
<td>682</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each mean recovery value ($n = 2$) has been adjusted for immunoassayable FSH in the respective plasma sample. Immunoassayable FSH concentrations in plasma samples EP-3, EP-4 and 153 were 251 ± 11, 144 ± 3 and 44 ± 8 ng/ml, respectively.
### TABLE 3. COMPARISON OF THE BIOLOGICAL AND IMMUNOLOGICAL POTENCY OF SEVERAL FOLLICLE STIMULATING HORMONE (FSH) PREPARATIONS WITH R5-4IA ANTISERUM

<table>
<thead>
<tr>
<th>FSH preparation</th>
<th>Biological potency (in relation to NIH-FSH-S1)</th>
<th>Immunological potency(^c)</th>
<th>Biological: immunological potency (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value at 50% inhibition, ng</td>
<td>Value in relation to NIH-FSH-S1</td>
<td></td>
</tr>
<tr>
<td>Ovine</td>
<td>Official Potency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH-S1</td>
<td>1.0</td>
<td>324</td>
<td>1.0</td>
</tr>
<tr>
<td>NIH-S5</td>
<td>1.4</td>
<td>123</td>
<td>2.6</td>
</tr>
<tr>
<td>NIH-S10</td>
<td>1.1</td>
<td>103</td>
<td>3.1</td>
</tr>
<tr>
<td>HP-G4-150</td>
<td>50.0</td>
<td>7</td>
<td>47.6</td>
</tr>
<tr>
<td>Porcine NIH-P1</td>
<td>.8</td>
<td>687</td>
<td>.5</td>
</tr>
<tr>
<td>Bovine NIH-B1</td>
<td>.5</td>
<td>361</td>
<td>.9</td>
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<td>USDA-BP1</td>
<td>71.9</td>
<td>8</td>
<td>42.1</td>
</tr>
<tr>
<td>USDA-BP1 β-sub</td>
<td>NT(^b)</td>
<td>3.7</td>
<td>87.6</td>
</tr>
<tr>
<td>USDA-BP1 α-sub</td>
<td>NT(^b)</td>
<td>1,084</td>
<td>.3</td>
</tr>
<tr>
<td>Mean</td>
<td>1.0 ± .2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)See "Antisera" section in text for sources of biological potency estimates.
\(^b\)NT = not tested.
\(^c\)Immunological potency was determined from the amount of test hormone in relation to NIH-FSH-S1 required to inhibit binding by 50%.

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**Animal Studies**

**Study 1. Ovariectomy.** The concurrent increases in concentrations of FSH and LH after ovariectomy are shown in figure 5. Data have been pooled because differences between anestrous and luteal ewes were not significant. At surgery, plasma FSH was 75 ± 5 ng/ml and LH was .7 ± .1 ng/ml. One day later, FSH had increased two fold and LH had increased fourfold. By 15 days, the increases were four- and eightfold, respectively. L'Hermite et al. (1972) reported two- to fourfold differences in FSH concentrations between intact and ovariectomized ewes.

**Study 2. Estrus.** The profiles for FSH and LH around estrus have been normalized to the highest LH value and are shown in figure 6a. Mean peak LH (average of the highest LH value for each ewe) was 64.1 ± 9.3 ng/ml and occurred 10 ± 1 hr after the onset of estrus. The highest plasma FSH concentration observed in each ewe in study 2 (201 ± 22) occurred within 2 hr of the highest plasma LH concentration. The magnitude of the increase in concentration was considerably greater for LH. Plasma LH increased 34-fold over values observed 8 hr before the peak, while plasma FSH increased only 1.9-fold. Figure 6a also shows that a second increase in plasma FSH occurred 22 ± 1 hr after the first, but it was in the absence of a concurrent increase in plasma LH.

The present findings on the changes in FSH around estrus are in general agreement with results obtained in heterologous assays by L'Hermite et al. (1972) and McNeilly et al. (1976), but both FSH peaks in our study were less pronounced than those reported by Salamonsen et al. (1973b) and Pant et al. (1977).

**Study 3. Estradiol Treatment.** Figure 6b shows changes in FSH and LH in ovariectomized ewes injected with estradiol. Estradiol caused the expected peak in plasma LH. At the time of the estradiol injection, LH averaged 4.5 ± 6 ng/ml. About 16 hr later, LH peaked at 76 ± 19 ng/ml. The highest plasma FSH concentration also occurred between 14 and 18 hr post-injection, but this mean was not different from that observed before the injection (334 ± 36 vs. 270 ± 49 ng/ml). For the first 6 hr after treatment, estradiol tended to suppress the concentrations of both FSH and LH. Plasma FSH also tended to be low during the
In general, the profile of FSH and LH after estradiol injection appeared similar to the pattern observed around estrus (compare figure 6b with figure 6a). Howland et al. (1978) reported that estradiol induced small FSH peaks in two of five ovariectomized ewes.

Study 4. Releasing Hormone. Figure 7 shows changes in plasma FSH and LH in ovariectomized ewes treated with either a single dose of 30 or 90 μg of LH-RH/FSH-RH or with three doses of 30 μg spaced 1 hr apart. Each of the three LH-RH/FSH-RH treatments caused significant increases in mean peak concentrations of both FSH and LH (Table 4). The interval from injection of LH-RH/FSH-RH to the peak concentrations in ewes that received only a single dose ranged from 82 ± 10 min for LH after administration of the 30-μg dose to 105 ± 11 min for FSH after administration of the 90-μg dose. Among ewes receiving three doses, the mean interval from initial dose to peak value was 125 ± 10 min for LH and 145 ± 5 min for FSH. The interval from dose to peak value was similar to that observed in intact rams and anestrous ewes but longer than that reported for castrated male sheep (Pelletier, 1976).

The magnitude of the response to each of the three LH-RH/FSH-RH treatments was considerably greater for LH than for FSH. The increase was seven- to 15-fold for LH vs about twofold for FSH (figure 7). Kinder et al. (1976) observed an LH response to LH-RH/FSH-RH of similar magnitude in anestrous ewes.

Figure 7 shows that the administration of TRH had no appreciable effect on either plasma FSH or LH concentration as determined by either the FSH RIA or the LH RIA. In contrast, the concentration of prolactin in the same plasma samples increased from 125 ± 34 ng/ml before TRH to 1,670 ± 92 ng/ml 15 min afterward, and was 871 ± 221 at 3 hr after TRH (data not shown). Presumably, TSH also increased in response to TRH. Davis (1975) reported significant increases in both prolactin and TSH in ewe lambs treated with 100 μg of TRH.

The present studies provided biological confirmation that the FSH RIA measured changes in plasma FSH independent of changes in plasma LH and, presumably, plasma TSH. Clear evidence was obtained both from estrous ewes, in which a second FSH peak occurred in

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**TABLE 4. PLASMA FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) IN OVARIECTOMIZED EWES TREATED WITH LH-RH/FSH-RH**

<table>
<thead>
<tr>
<th>LH/RH/FSH-RH treatment, μg</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml ± SE</td>
<td>ng/ml ± SE</td>
</tr>
<tr>
<td>Before treatment&lt;sup&gt;a&lt;/sup&gt;</td>
<td>376 ± 49</td>
<td>548 ± 61</td>
</tr>
<tr>
<td>30</td>
<td>414 ± 43</td>
<td>753 ± 79*</td>
</tr>
<tr>
<td>90</td>
<td>546 ± 51</td>
<td>732 ± 56*</td>
</tr>
<tr>
<td>30 × 3</td>
<td>504 ± 85</td>
<td>964 ± 143**</td>
</tr>
</tbody>
</table>

<sup>a</sup>Within columns, comparisons were not different.

<sup>b</sup>Peak value for each ewe was determined from samples taken at 15-min intervals for 4 hr after initial LH-RH/FSH-RH injections.

<sup>c</sup>Different (P < 0.05) from value for control (0) group according to Dunnett's "t" test adjusted for unequal variances.

<sup>d</sup>Different (P < 0.01) from value for control (0) group according to Dunnett's "t" test adjusted for unequal variances.
the absence of an increase in LH, and from TRH-treated ovariectomized ewes, in which plasma FSH remained unchanged while plasma prolactin, and presumably plasma TSH, was greatly increased. In addition, different relative changes in plasma FSH and plasma LH were observed during certain treatments. The relative increases in FSH and LH were of similar magnitude after removal of the ovaries, and the relative decreases were similar during the first few hours after treatment with estradiol. In contrast, relative changes in plasma LH were greater than those in plasma FSH during the LH surge at estrus, after treatment with LH-RH/FSH-RH and during the estrogen-induced LH surge.

Results indicate that a homologous ovine FSH RIA has been developed with adequate specificity and sensitivity to reliably quantify FSH in ovine plasma. Success was attributed to two key procedures: (1) chromatography of the radioiodinated FSH on HTP, which improved the immunoreactivity of the assay tracer, and (2) immunoabsorption of the anti-ovine FSH serum, which greatly improved the specificity of the antisera and allowed parallel responses of ovine FSH standard and ovine plasma. In general, the plasma FSH profiles obtained with this homologous ovine FSH assay agreed with profiles obtained previously with the specific heterologous FSH assays that have employed one or more nonovine FSH reagents.

Literature Cited


Pant, H. C., C.R.N. Hopkinson and R. J. Ritzpatrick. 1977. Concentration of oestradiol, progesterone, luteinizing hormone and follicle-stimulating hor-
mone in the jugular venous plasma of ewes during the oestrus cycle. J. Endocrinol. 73:247.


