ANTISERUM TO AN INHIBIN ALPHA-CHAIN PEPTIDE NEUTRALIZES INHIBIN BIOACTIVITY AND INCREASES OVULATION RATE IN SHEEP

R. L. Meyer, K. M. Carlson, J. Rivier and J. E. Wheaton
University of Minnesota, St. Paul 55108

ABSTRACT

A synthetic fragment representing the N-terminal 25 amino acid residues of the α-subunit of ovine inhibin (α-IF) was coupled to human α-globulin (βα-G) and used as an antigen. In Exp. 1, ovine antiserum generated against α-IF-βα-G was shown in vitro to neutralize inhibin bioactivity contained in ovine follicular fluid. In Exp. 2, 18 lambs were immunized with 0.3, 0.6, and 1.2 mg α-IF-βα-G or equivalent doses of βα-G. Antibody titer to α-IF was detected only in serum from lambs immunized against α-IF-βα-G and was first detected 27 ± 2 d after primary immunization. Thereafter, antibody titers increased steadily. The degree of antibody responses was unrelated to antigen dose and differed among lambs. Plasma FSH concentrations were unchanged, whereas LH concentrations were lower (P < .001) in sheep immunized against α-IF-βα-G. Ovulation rate was increased (3.5 ± .5 vs 1.5 ± 1; P < .01) in lambs immunized against α-IF-βα-G. Ovulation rate was similar among animals receiving different antigen doses and increased with time after primary immunization (P < .01). At estrous periods occurring -34, 50, 74 and 107 d after primary immunization, respective ovulation rates were 157, 169, 207 and 450% of control values. Ovulation rate and antibody titer were correlated positively (r = .95; P < .01) within lambs. In Exp. 3, three lambs were immunized with 0.25 mg unconjugated α-IF; this was nonantigenic. In conclusion, the use of a synthetic fragment of the α-subunit of ovine inhibin as a hapten elicits an antibody capable of neutralizing inhibin bioactivity in vitro and increasing ovulation rate in vivo.

Key Words: Inhibin, Ovulation Rate, Ewes, Immunization


Introduction

Progress regarding the biochemistry and physiology of inhibin has been reviewed (Findlay, 1986; Burger et al., 1987; Ying, 1988; de Jong, 1988). Bovine, porcine and ovine inhibin have been purified from follicular fluid (FF); each consists of a 30,000 to 32,000 heterodimeric glycoprotein with disulfide-linked α- and β-subunits (Rivier et al., 1985; Robertson et al., 1985; Leversha et al., 1987).

Partially purified preparations of bovine FF and a recombinant DNA-derived protein corresponding mainly to the α-subunit of bovine inhibin have been used as antigens to increase ovulation rate of sheep (Henderson et al., 1984; O'Shea et al., 1984; Cummins et al., 1986; Forage et al., 1987). The hypothesis is that antibodies developed against these preparations neutralize endogenous inhibin and thereby increase FSH secretion and, consequently, ovulation rate.
Ability of antibody directed against fragments of the α-subunit of inhibin to neutralize inhibin in vitro was shown in 1985 (Rivier et al., 1985). Presence of epitopes eliciting inhibin-neutralizing antibody in short N-terminal sequences of the α-subunit suggest that synthetic fragments have potential as antigens to increase ovulation rate. In the present study, a peptide matching the N-terminal sequence of the α-subunit of ovine inhibin was evaluated in lambs for antigenicity, generation of inhibin neutralizing antisera and for effects on gonadotropin levels and ovulation rate.

**Materials and Methods**

**Antigens and Immunizations.** A 27 amino acid synthetic peptide fragment corresponding to the N-terminal 25 amino acid residues of the α-subunit of ovine inhibin, otx-(1-25)-Gly-Tyr-OH (α-IF), was conjugated to human α-globulin (ho-G) through its C-terminal Gly acid synthetic peptide fragment corresponding to the N-terminal sequence of the α-subunit of inhibin to neutralize antibody activity. Complete Freund’s adjuvant (complete initially, incomplete for boosters) was added and tubes were mixed immediately using a vortex mixer. Tubes were centrifuged at 1,520 × g for 20 min, supernatant fluid was aspirated and pellets were air-dried before counting. Binding percentages of the α-subunit of ovine inhibin was evaluated in lambs for antigenicity, generation of inhibin neutralizing antisera and for effects on gonadotropin levels and ovulation rate.

**Estimating Plasma Anti-α-IF Activity.** Relative capacity of antisera to bind [125I]-α-IF was determined by diluting plasma 1:10,000 in buffer (.05 M EDTA, .01 M phosphate buffered saline, pH 7.0) and incubating duplicate 100-μl aliquots with 200 μl 1% gelatin in .01 M phosphate buffered saline, pH 7.0 (gel-PBS) and [125I]-α-IF (25,000 cpm/100 μl gel-PBS) for 24 h at 4°C. Cold (−20°C) ethanol (1 ml) was added and tubes were mixed immediately using a vortex mixer. Tubes were centrifuged at 1,520 × g for 20 min; supernatant fluid was aspirated and pellets were air-dried before counting. Binding percentages of samples were calculated using the following formula: 100 × [(sample cpm − buffer cpm) + total cpm]. The CV averaged 9.9%. Binding was considered significant when it exceeded that of preimmunization serum samples by >2 SD. Plasma samples from Exp. 3 also were tested at dilutions of 1:2,000, 1:4,000 and 1:8,000. The α-inhibin fragment was radioiodinated using Iodogen5. Iodogen was dissolved in methylene chloride (.025 μg/μl) and 20 μl were evaporated in a 8-mm × 30-mm glass vial. Peptide (5 μg/50 μl 1 M PBS, pH 7.1) was transferred to the iodogen-coated reaction vial and incubated 2 min, after which 1 mCi sodium 125I was injected into the vial and the reaction was allowed to proceed for 1.5 min at room temperature with periodic tapping. The mixture was transferred to a .8-cm × 17-cm glass column of Sephadex G-25 equilibrated in .01 M PBS and eluted with gel-PBS. Radiolabeled α-IF eluted in 4 to 7 ml and had a specific activity of ~125 μCi/μg.

**Inhibin Bioassay.** Chronically ovarioctomized (ovx), mature blackfaced ewes were immobilized by cranial puncture using a captive bolt and killed by exsanguination. Pituitary glands were excised aseptically 10 min after death and placed in an ice-cold sterile-filtered Hank's balanced salt solution (Ca- and Mg-free, pH 7.4, HBSS-CMF), supplemented with 25 mM HEPES, 4 mM NaHCO3 and the antibiotics gentamicin (50 μg/ml), amphotericin B (2.5 μg/ml) and penicillin (50 U/ml)/streptomycin (50 μg/ml). The pars nervosa and adhering dura and vasculature were removed before dicing into ~1-mm3 pieces and centrifugation at 500 × g for 5 min. Tissue was resuspended in HBSS-CMF containing .5% collagenase (Type I-S, 211 U/mg), .2% protease dispaase (Type IX) and DNase (Type II) and incubated at 37°C for 50 min with intermittent trituration using a Pasteur pipette. Cells were separated by centrifugation (500 × g, 5 min), washed thrice in HBSS-CMF, then twice in .1% glucose Dulbecco’s modified Eagle media (DMEM), supplemented with L-glutamine (2 mM), 44 mM NaHCO3, the aforementioned antibiotics and 10% fetal calf serum. Cells were resuspended in the latter medium; unfiltered cells were >90% viable using the trypsin blue exclusion test. Pituitary yield was 3 to 4 × 107 cells. Cells were plated at 2 × 105 cells/well in a 24-well, uncoated Coming tissue culture plates and incubated at 37°C in a humidified 5% CO2, 95% air atmosphere for 3 d. Cells
were washed once with serum-free DMEM immediately before treatment media were imposed. After a 3-d incubation, media were aspirated, centrifuged (1,520 × g for 20 min) and stored at -20°C until assayed for FSH.

**Ovine Follicular Fluid.** At a commercial abattoir, ovaries were obtained from 120 mature ewes within 40 min after death. Ovaries were stored in crushed ice for 2 to 3 h, during which time a total of 35 ml of ovine follicular fluid (OFF) was aspirated from all surface antral follicles. Ovine follicular fluid was mixed with activated charcoal (35 mg, 250 to 350 mesh) and Dextran9 T70 (3.5 mg) for 24 h at 4°C and then centrifuged at 1,520 × g for 30 min at 4°C. The supernatant fluid was centrifuged at 18,000 × g for 1 h at 4°C; protein and 17β-estradiol (E2) concentrations of the supernatant fluid were determined. Protein was measured using a bicinchoninic-acid protein assay kit8. Inhibin bioactivity was assessed by adding .01 to 1.3 μl OFF in triplicate to cell culture wells.

**Experiment 1: Immunoneutralization of Inhibin Bioactivity.** Three 7-mo-old Finn × Dorset ewe lambs weighing 39 ± 1 kg were ovariectomized and 15 d later (d 0) immunized against 1.2 mg α-IF-hα-G. Booster immunizations were administered on d 14, 28 and 47 and blood samples were taken via jugular venipuncture on d 0, 21, 28, 33, 48, 55, 64 and 70. Serum was stored at -20°C before estimation of antibody titer against α-IF and testing of its ability to neutralize inhibin bioactivity of OFF.

Ability of serum to neutralize inhibin bioactivity was tested by adding 50-μl aliquots of serum drawn from lambs on d 0 (preimmunization) and d 64 to 1 ml of cell culture medium that contained 0, .01, .02, .04, .08, .16, .31, .62, 1.3, 2.5, 5.0 or 10.0 μl OFF. After a 24-h incubation at 4°C, test media were equilibrated to bioassay conditions and then transferred to cell culture wells and incubated with pituitary cells for 3 d.

**Experiment 2: Effects of Active Immunization Against α-IF-hα-G.** Eighteen 1/4 Finn × 3/4 Suffolk-Hampshire ewe lambs 6 to 7 mo of age and weighing 44 ± 1 kg were assigned randomly in equal numbers to six treatment groups. Three groups of lambs were immunized against α-IF-hα-G administered at doses of .3, .6 and 1.2 mg, and three with equivalent doses of hCG. Each antigen was given on d 0, 21, 61 and 94.

Estrus was synchronized twice during the fall with intravaginal progesterone-releasing devices, controlled internal drug release dispensers-type G (CIDR-G10). Devices were inserted on d 21 and 61 and withdrawn 12 d later, at which time 10 mg Lutalyse11 was i.m. injected. Vasectomized rams were penned with lambs from d 0, and beginning on d 21 lambs were observed daily for mating marks and standing estrus. Following CIDR-G removal, onset of estrus was checked at 6-h intervals for 60 h. Vasectomized rams were replaced with a fertile ram on d 101 and pregnancy diagnosis using ultrasound was performed 80 to 85 d after mating. Ovulation rate was determined 8 to 12 d after spontaneous and synchronized estrus by counting corpora lutea via endoscopy (Youngs, 1985).

Blood samples (3 ml) were taken at 0900 at 2- to 3-d intervals from d 0 to 77, and on the day of breeding to the fertile ram. Upon CIDR-G removal, blood samples were drawn at 6-h intervals for 60 h. Plasma (5 IU heparin/ml blood) was stored at -20°C until assayed for antibody titer to α-IF, FSH and LH.

**Experiment 3: Antigenicity of α-IF.** Three 1/4 Finn × 3/4 Suffolk-Hampshire ewe lambs 8 to 9 mo of age and weighing 52 ± 3 kg were immunized on d 0 and 18 with .25 mg unconjugated α-IF. Blood samples were taken at 2- to 3-d intervals from d -1 to 70. Plasma antibody titer was measured and compared to that developed in lambs immunized against the equivalent dosage of α-IF-hα-G in Exp. 2.

**Radioimmunoassay.** Ovine FSH levels were measured in duplicate 300-μl plasma and 50-μl media samples using a homologous double-antibody procedure. Antiserum (100 μl NIAMDD-anti-oFSH-1 diluted 1:6,000 in 1% normal rabbit serum-EDTA-PBS) was incubated 48 h with 200 μl gel-PBS with 5 mM sodium bicarbonate (gel-PBBS) and sample or standard (NIAMDD-oFSH-13). Radioiodinated NIAMDD-oFSH-1 (16,000 cpm/100 μl gel-PBBS) was added and incubated 48 h, after which precipitating antiserum (sheep anti-rabbit γ-globulin serum) was added. Assay binding averaged 30%; 3 ng FSH was mid-range on the standard curve. Sensitivity was .7 ng/tube and intra-assay and interassay CV.

9Pharmacia, Uppsala, Sweden.
11Upjohn, Kalamazoo, MI.
Figure 1. Patterns of antiserum development. Ovariectomized lambs were immunized (*) against α-IF-hCG (α-subunit fragment of ovine inhibin coupled to human α-globulin) and relative antibody titers to α-IF were estimated by capacity of serum to bind \[125^I\]α-IF.

were 7 and 8%, respectively. Radioiodination protocol followed the aforementioned iodogen procedure, incorporating 2.5 μg FSH/10 μl water, 40 μl .1 M PBS, 4 μg iodogen, 1 mCi \[125^I\] and a 15-min reaction period. Products were chromatographed on a Sepharose 4B-Concanavalin A column as described by Chappel (1981). Components of the LH RIA were NIH-LH-S19 for reference, LER-1374A for radioiodination and GDN #15 antiserum. Assay protocol followed that described by Niswender et al. (1969). Sensitivity was .05 ng/tube and intra-assay and interassay CV were 9 and 11%, respectively. Radioimmunoassay of E2 levels in oFF utilized a double-antibody E2 kit\(^{12}\). Ovine follicular fluid (1 to 10 μl) was diluted in gel-PBS and assayed directly. Sensitivity was 1 pg/tube.

Statistical Analyses. Inhibin bioassay responses were evaluated for effects of oFF dose using one-way analysis of variance. A two-way classification was used when the bioassay included oFF doses that had been preincubated with antiserum. Data analyzed consisted of FSH concentrations in test media expressed as a percentage of FSH concentrations in media from control (0 μl oFF) cell culture wells. Median effective dose (ED\(_{50}\)) corresponds to the volume of oFF that reduced FSH secretion by 50% (dose-response range = 100%). The maximum depression value represents the mean ± SE of the replicates of the oFF dose that produced the greatest inhibition of FSH secretion. Serial measurements made on the same animal (i.e., ovulation rate, FSH and LH values and antibody titer) were analyzed in a split-plot design for repeated measures (Gill and Hafs, 1971). Data from the four estrous periods monitored were used to calculate correlation coefficients between antibody titer and ovulation rate among and within animals.

Results

Experiment 1: Immunoneutralization of Inhibin Bioactivity. Development of plasma anti-α-IF activity differed among ovx lambs (Figure 1). Response was much greater in lamb #30 than in the other sheep.

Follicle-stimulating hormone secretion from cultured ovine gonadotropes was reduced (\(P < .001\)) by .01 to 1.3 μl oFF in a dose-response
manner (Figure 2). Maximum depression was 28.8 ± 1.8% of control and the ED₅₀ was .015 μl offF. Luteinizing hormone release was unchanged by offF, which contained 125 μg/μl protein and 5 pg/μl E₂. Ovine follicular fluid preincubated with 50 μl of preimmunization serum reduced FSH secretion similarly to offF alone (max depression = 21.0 ± .4%; ED₅₀ = .013 μl). Preincubation with 50 μl postimmunization serum from lamb #30 diminished (P < .001) inhibin bioactivity in .01 to 1.3 μl offF (ED₅₀ = .42 μl; Figure 2). Day-64 serum samples from lambs #35 and #45 were ineffective in neutralizing inhibin bioactivity in offF.

Experiment 2: Effects of Active Immunization Against α-IF-hα-G. Plasma from lambs immunized against α-IF-hα-G bound [¹²⁵I]-α-IF above (P < .001) that in control lambs, which was nondetectable. Binding increased over time (Figure 3). Individual means in treated lambs ranged from .5 to 18% and were unrelated to the dose of immunogen administered. Time from initial immunization to onset of significant antibody binding averaged 27 ± 2 d and varied independently of antigen dose.

Levels of FSH were unaffected by antigen type and dose (Figure 4). There was a tendency (P = .10) for means of α-IF-hα-G-immunized sheep (17.0 ± .4 ng/ml) to be lower than those of control animals (20.2 ± .4 ng/ml) throughout the trial, including estrous synchronization periods (Figure 5). This tendency existed before development of significant antibody titer. Plasma LH concentrations were decreased (P < .001) in animals immunized against α-IF-hα-G (Figure 4 and 5). This effect was evident 26 d after primary immunization. Intervals from withdrawal of CIDR-G to onset of estrus (32 ± 2 h) and to preovulatory LH surges (32 ± 2 h) were similar in lambs of both treatment groups during the first and second estrus synchronization periods. Highest preovulatory LH surge values were less (P < .01) in lambs immunized against α-IF-hα-G (Figure 5). Highest FSH values were concomitant and correlated (r = .78; P < .001) with LH.

In lambs immunized against α-IF-hα-G (Table 1), the ovulation rate increased (P < .01). Mean ovulation rate was 3.5 ± .5 in treated lambs and 1.5 ± .1 in lambs immunized against hα-G. Although the ovulation rate in treated sheep increased over time (P < .01) with a marked rise on d 101 to 111, it remained unchanged (P > .1) in control ewes. Ovulation rates of 8, 10 and 15 were recorded for individual lambs during the fourth endoscopy. Within lambs, antibody titers were
Figure 5. Plasma gonadotropin levels (mean ± SE) following controlled internal drug release dispensers-type G (CIDR-G) withdrawal on d 73. Data have been aligned to the highest LH level detected.

Correlated positively with ovulation rate (pooled r = .95; P < .01). Among lambs, correlation coefficients between antibody titer and ovulation rate for the four periods of estrus were .42, .88 (P < .01), .30 and -.02.

Lambs were penned with a fertile ram on d 101 and each of the lambs immunized against α-IF-hα-G was bred, as were five of eight control lambs. One control lamb was rebred 18 d later. Midgestation ultrasound scanning confirmed pregnancy in the nine α-IF-hα-G-immunized and five control lambs.

Experiment 3: Antigenicity of α-IF. Lambs immunized against unconjugated α-IF did not develop detectable antibody titer to α-IF. Plasma samples invariably had only background counts per minute when tested for binding of [125I]-α-IF.

Discussion
Inhibin bioactivity was neutralized by antisem developed against a synthetic peptide corresponding in sequence to the N-terminal 25 amino acids of the α-subunit of ovine inhibin. The analogous portion of native inhibin apparently has a similar configuration, and is available for antibody binding; presence of antibody at the site renders the molecule biologically inactive. In Exp. 1, antibodies to α-IF-hα-G were developed in ovx lambs in order to avoid confounding effects of endogenous inhibin and E2 in the inhibin bioassay. Ovarian follicles are the major source of inhibin in ewes (Tsonis et al., 1983). Preincubation of 50 µl antisem neutralized 67% of inhibin bioactivity in .01 to .3 µl OFF, after which the percentage dropped. Larger OFF volumes may have exceeded antisem binding capacity or provided enough E2 to depress FSH secretion. Larger OFF volumes contained sufficient E2 to produce concentrations capable of reducing FSH release (Miller et al., 1977; Tsonis et al., 1986). Antibodies generated to synthetic peptides patterned after the 1 to 6 and 1 to 30 residues of the α-subunit of porcine inhibin have been shown to recognize the parent molecule (Rivier et al., 1985; Cuevas et al., 1987).

In Exp. 2, animals were immunized four times and booster immunizations preceded estrus by about 2 wk. Onset of appreciable [125I]-α-IF binding followed the first booster immunization and antibody titers continued to increase. Ovulation rate increased coincidently with antibody titer. The mechanism by which immunoneutralization of inhibin affects ovulation rate is unclear. Immunoreactive levels of FSH were unchanged but LH levels were lower in α-IF-hα-G-immunized lambs. Speculation in this regard is that negative feedback effects of elevated E2 concentrations, arising from increased follicular mass, may have depressed LH levels and prevented FSH levels from increasing in response to inhibin neutralization. Higher E2 levels in the α-IF-hα-G-immunized lambs also would be consistent with their lower preovulatory LH surge values (Goodman and Karsch, 1980). Measurement of plasma E2 levels was attempted, but limited plasma volume and assay sensitivity prevented reliable quantification. Another possibility is a direct gonadal effect. Immunoneutralization of inhibin may have blocked a local inhibitory
IMMUNONEUTRALIZATION OF INHIBIN BIOACTIVITY

TABLE 1. OVULATION RATES OF LAMBS IMMUNIZED AGAINST α-IF-ha-G<sup>a</sup> OR ha-G<sup>b</sup>

<table>
<thead>
<tr>
<th>Trt</th>
<th>Dose, mg</th>
<th>Lamb #</th>
<th>Day of estrus</th>
<th>Day of estrus</th>
<th>Day of estrus</th>
<th>Day of estrus</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>34&lt;sup&gt;c&lt;/sup&gt;-35</td>
<td>45&lt;sup&gt;d&lt;/sup&gt;-55</td>
<td>74&lt;sup&gt;e&lt;/sup&gt;-76</td>
<td>101&lt;sup&gt;d&lt;/sup&gt;-111</td>
<td></td>
</tr>
<tr>
<td>α-IF-ha-G</td>
<td>.125</td>
<td>102</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>3.6 ± .7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>108</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>121</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>α-IF-ha-G</td>
<td>.25</td>
<td>109</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>3.3 ± .8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>129</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>α-IF-ha-G</td>
<td>.5</td>
<td>101</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>111</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>134</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td>2.2</td>
<td>2.7</td>
<td>2.9</td>
<td>6.3</td>
<td>3.5 ± .5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.5</td>
<td>.9</td>
<td>.4</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T3</th>
<th></th>
<th></th>
<th>34&lt;sup&gt;c&lt;/sup&gt;-35</th>
<th>45&lt;sup&gt;d&lt;/sup&gt;-55</th>
<th>74&lt;sup&gt;e&lt;/sup&gt;-76</th>
<th>101&lt;sup&gt;d&lt;/sup&gt;-111</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>hx-G</td>
<td>.125</td>
<td>114</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1.6 ± .2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>137</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>hx-G</td>
<td>.25</td>
<td>117</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1.3 ± .2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>128&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>hx-G</td>
<td>.5</td>
<td>119</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1.4 ± .3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>124</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>126</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td>1.4</td>
<td>1.6</td>
<td>1.4</td>
<td>1.4</td>
<td>1.5 ± .1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.2</td>
<td>.4</td>
<td>.2</td>
<td>.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Alpha-subunit fragment of ovine inhibin coupled to human α-globulin.

<sup>b</sup>Human α-globulin.

<sup>c</sup>Synchronized estrus.

<sup>d</sup>Spontaneous estrus.

<sup>e</sup>Lamb #128 died on d 57 from an unknown cause.

Action of inhibin on follicular development (Findlay et al., 1987).

Magnitude of immunological responses varied substantially among lambs. Variation was not attributable to immunogen dose, breed or age. Active immune responses typically differ among animals and are influenced by an individual's genetically determined ability to respond to determinants on an antigen (Schwacz, 1986). Such variation hinders development of inhibin immunoneutralization as a method to achieve a uniform and predictable increase in ovulation rate.

Lambs were penned with a fertile ram on d 101 of the experiment, and conception occurred at the first estrus thereafter in the nine lambs immunized against α-IF-ha-G and in four of eight control lambs. Perhaps immunoneutralization of inhibin exerted a maturational influence on folliculogenesesis. Such an effect has been reported (Al-Obaidi et al., 1987). Three of five lambs having ovulation rates of ≥6 had terminated pregnancy by midgestation. Incidence of embryo and fetal mortality and abortion are directly linked to ovulation rate (Robinson, 1951).

Unconjugated α-IF, tested under similar experimental conditions as conjugated α-IF, failed to elicit detectable antibody titer in lambs. Lack of antigenicity of α-IF may be due to its low molecular weight and 93% homology with the primary structure of the N-terminal region of the α-subunit of ovine inhibin.

Implications

A synthetic peptide duplicating the N-terminal 25 amino acids of the α-subunit of ovine inhibin was an effective antigen when conjugated to a carrier protein. The synthetic peptide and complete inhibin molecule appar-
ently contain similar epitopes to which antibody binding blocks inhibin's bioactivity. Active immunization of ewes against the conjugated peptide increases ovulation rate in relation to the degree of antibody titer developed. Results support a key role for inhibin in regulating ovulation rate.

**Literature Cited**


