Luteinizing hormone-releasing hormone fusion protein vaccines block estrous cycle activity in beef heifers


*Department of Animal Sciences, †Center for Reproductive Biology and ‡School of Molecular Biosciences, Washington State University, Pullman 99164

ABSTRACT: Two LHRH fusion proteins, thioredoxin and ovalalbumin, each containing seven LHRH inserts were tested for their ability to inhibit estrous cycle activity. The objective was to evaluate immune and biological responses from alternating the two fusion proteins in an immunization schedule. One hundred ten heifers were divided equally into 11 groups. Two control groups consisted of either spayed or intact, untreated heifers. Heifers in the other nine groups were immunized on wk 0, 4, and 9. Treatments were immunizations of the same protein throughout or alternating the proteins in different booster sequences. Blood was collected weekly for 22 wk, and serum was assayed for concentrations of progesterone and titers of anti-LHRH. At slaughter, reproductive tracts were removed from each heifer and weighed. Heifers with ≥1 ng/mL of progesterone were considered to have a functional corpus luteum and thus to have estrous cycle activity. All LHRH-immunized groups of heifers had a smaller (P < 0.05) proportion of heifers showing estrous cycle activity after 6 wk than the intact, untreated control group. There was no difference in number of heifers cycling between the immunized groups and the spayed heifers during wk 9 to 22. Anti-LHRH did not differ among immunized groups during wk 1 to 9. Starting at wk 10 and continuing through the conclusion of the study, there was an overall difference among treatment groups for anti-LHRH (P < 0.05). Uterine weights differed among treatments (P < 0.05), with intact control animals having heavier uteri than all other groups (P < 0.05). Uterine weights were negatively correlated with maximum LHRH antibody binding (r = −0.44). In summary, the LHRH fusion proteins were as effective as surgical spaying in suppression of estrous cycle activity, but alternating the two proteins in an immunization schedule did not enhance the immunological or biological effectiveness of the vaccine.

Key Words: Fusion Protein, Heifers, Immunization, Luteinizing Hormone-Releasing Hormone


Introduction

Approximately 12 million heifers are fed in feedlots each year; of these, 7.3% are pregnant when they enter the feedlot (USDA, 2000). Immunization against LHRH before entering the feedlot could sterilize heifers and prevent unwanted pregnancies that could decrease feedlot performance. To date, most LHRH sterilization vaccines used in female cattle have been produced using chemical conjugation techniques. Exceptions to this include techniques reported by Van der Zee et al. (1995), Cook et al. (2000), and Sosa et al. (2000), who used recombinant DNA technology to produce LHRH vaccines as a fusion protein for use in cattle. For a vaccine to be approved by the U.S. FDA, both within- and between-batch variability must be minimized, something that can be accomplished with a fusion protein much more easily than by using chemical conjugation (M. Schoenemann, FDA, Rockville, MD, personal communication).

Schutze et al. (1985) defined carrier-mediated immune suppression as preimmunization against a given carrier that will prevent the elicitation of an immune response to a new antigenic epitope when presented on the same carrier. Sad et al. (1991) reported carrier-mediated immune suppression that resulted in the inhibition of immune responses to a hapten linked to the same carrier used in the initial immunization. In the current study, two different fusion proteins were used...
Figure 1. Schematic structures of recombinant ovalbumin, ovalbumin-LHRH-7 (molecular weight [MW] = approximately 55 kDa), thioredoxin and thioredoxin-LHRH-7 (MW = approximately 22 kDa). The straight lines represent amino acids (18 to 381 for ovalbumin and entire sequence for thioredoxin). Open boxes represent LHRH insertion sites for ovalbumin (before AA 18, between AA 65 and 66, and between AA 97 and 98), and thioredoxin (before AA 1, between AA 34 and 35, and after AA 124). The closed box at the C-terminus represents the His-tag sequence used for His-bind affinity chromatography.

in an attempt to counteract possible carrier-mediated immune suppression. The hypothesis of this study is that by alternating the carriers in an LHRH immunization schedule, the number of unresponsive animals would decrease.

Materials and Methods

Preparation of Antigens and Immunization

Ovalbumin-LHRH-7 (OL) was prepared by recombinant DNA technology, as described by Zhang et al. (1999). An ovalbumin gene fragment (AA 18 to 381) was manipulated with the insertion of two LHRH sequences in tandem at the C-terminus (AA 381), monomers between AA 65 and 66 and between 97 and 98, and three sequences in tandem at the N-terminus (AA 18) (Figure 1). Purification of OL was performed as described by Zhang et al. (1999). Thioredoxin-LHRH-7 (TL) was prepared by recombinant DNA technology as described by Quesnell et al. (2000). A thioredoxin gene was manipulated with the insertion of two sequences in tandem at the C-terminus (AA 124), two sequences in tandem between AA 34 and 35 and three sequences in tandem at the N-terminus (AA 1; Figure 1). Thioredoxin-LHRH-7 was purified as described previously for OL.

All plasmids were expressed in Escherichia coli BL21 (DE3; ovalbumin) or E. coli AD494 (DE3; thioredoxin). Both OL and TL were found to be insoluble and were thus dissolved in 6 M urea, centrifuged, and filtered through a 0.45-μM membrane before purification. These recombinant proteins contain six histidines (His-Tag, Novagen, San Diego, CA) on the C-terminal end, which bind to heavy metals such as Ni$^{2+}$. To purify the protein, metal chelation affinity chromatography was conducted on a Ni$^{2+}$ column. The bound, purified protein was then eluted from the Ni$^{2+}$ column with buffer and SDS-PAGE (10% acrylamide for OL and 15% acrylamide for TL) was performed to determine whether a protein product of the correct size was present.

Primary immunizations were performed in a water-oil emulsion (containing Mycobacterium butyricum as the immunostimulant) with 0.4 nmol of the LHRH antigen. Both booster immunizations were administered in a water-oil adjuvant without M. butyricum with an equal molar amount of LHRH antigen.

Animals and Treatments

The Washington State University Institutional Animal Care and Use Committee approved all procedures performed on heifers in this study. One hundred ten heifers at an average BW of 355 ± 3 kg were purchased from a local sale yard. Before the beginning of the study, blood was collected two times, 14 d apart, to determine serum progesterone concentrations. Palpation per rectum and serum progesterone concentrations were used to determine estrous cycle activity and pregnancy in all heifers. Heifers were then divided into two groups, those that were exhibiting estrous cycle activity before the beginning of the study, and those that were not. If a heifer had less than 1 ng/mL of serum progesterone for three consecutive weeks at any time during the study, the heifer was considered to be anestrous at that time. Each group of heifers was then randomly and equally divided among the 11 treatment groups. Treatment groups were administered a primary immunization dose followed by two booster injections at 1-mo intervals. Immunizations were for either 0.4 nmol OL (OLOLOL), 0.4 nmol TL (TLTLTL), or 0.4 nmol of a combination of both proteins, as described in Table 1, with the combination immunization receiving 0.2 nmol of each fusion protein. Immunizations were given in one s.c. site on the neck of the animals. Ovariectomies were performed on 10 heifers using a transvaginal approach (Garber et al., 1990) before the start of the study to produce the spayed control heifers. This treatment group contained both cyclic and anestrous heifers before spaying.

Heifers were housed in 14 pens and fed a feedlot diet (barley and pea hay) throughout the course of the study. On average, heifers received 9.3 kg of feed/d of a diet consisting of 0.73 Mcal/kg of DE on a DM basis. Heifers were placed in pens based on BW so feed offered per heifer within pen was similar for all heifers.

Data Collection and Blood Samples

Blood samples were collected by coccygeal venipuncture to determine serum progesterone and LH concentrations, as well as antibodies against LHRH, oval-
bumin, and thioredoxin. Serum samples were collected weekly for each of 22 wk. Serum progesterone analysis was conducted for all 22 wk, whereas analysis for serum LH, anti-LHRH, anti-ovalbumin, and anti-thioredoxin antibodies was performed on samples collected weekly until wk 4 and then every other week for the remainder of the study. Heifers were weighed every 28 d, and reproductive tracts were removed and weighed at the time of slaughter. Uteri were trimmed of any visible fat and cut at the cervical/uterine junction before being weighed. Heifers were sent to slaughter when it was visually estimated that 70% would grade USDA Choice.

Statistical Analyses

Antibody titers for LHRH, thioredoxin, and ovalbumin were analyzed initially with repeated-measures ANOVA using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Due to interactions between treatments and time, these data were analyzed at each time period using a completely randomized design. The model contained 11 treatments, with cycling status of heifers before the beginning of the study and the interaction of the two, as well as heifer BW as a covariate. The interaction was not significant (P > 0.05) at any of the time periods and was therefore removed from the test. When overall F-ratios were significant, differences among treatments were tested using preplanned, nonorthogonal contrasts at the 5% probability level. A post hoc test was run to determine whether treatment groups containing two or more OL immunizations differed from treatment groups containing two or more TL immunizations. Average concentrations of LH, ADG, and uterine weight were analyzed using a one-way (11 treatments) ANOVA in a completely randomized design using the GLM procedure of SAS. A protected Waller-Duncan test statistic (SAS) at the 5% probability level was used to compare treatments. There were no differences among treatment groups for ADG or LH; therefore, treatment groups were pooled and compared to spayed and untreated heifers. Average daily gain and LH were also analyzed with a repeated-measures ANOVA. The model contained treatment, time, and the treatment × time interaction as sources of variation. Concentrations of progesterone measured each week were analyzed separately by one-way ANOVA using a completely randomized design with 11 treatments. When overall F-ratios were significant (P ≤ 0.05), differences were tested using preplanned, nonorthogonal contrasts at the 5% level.

Table 1. Experimental design to evaluate combinations of ovalbumin-LHRH (OL) and thioredoxin-LHRH (TL) immunization schedules in heifers

<table>
<thead>
<tr>
<th>Treatment groupa</th>
<th>Primary immunizationb</th>
<th>First booster (at wk 4)b</th>
<th>Second booster (at wk 9)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Spayed control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TL</td>
<td>TL</td>
<td>TL</td>
</tr>
<tr>
<td>4</td>
<td>TL</td>
<td>OL</td>
<td>TL</td>
</tr>
<tr>
<td>5</td>
<td>TL</td>
<td>OL</td>
<td>OL</td>
</tr>
<tr>
<td>6</td>
<td>TL</td>
<td>OL</td>
<td>OL</td>
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<tr>
<td>7</td>
<td>OL</td>
<td>OL</td>
<td>OL</td>
</tr>
<tr>
<td>8</td>
<td>OL</td>
<td>TL</td>
<td>OL</td>
</tr>
<tr>
<td>9</td>
<td>OL</td>
<td>OL</td>
<td>TL</td>
</tr>
<tr>
<td>10</td>
<td>OL</td>
<td>TL</td>
<td>TL</td>
</tr>
<tr>
<td>11</td>
<td>OL+TL</td>
<td>OL+TL</td>
<td>OL+TL</td>
</tr>
</tbody>
</table>

a Each treatment group contained 10 heifers.
b Each immunization provide a total of 0.4 nmol of fusion protein, with the OL+TL immunizations providing 0.2 nmol of each fusion protein.

Iodination of Thioredoxin

One Iodo-bead (Pierce, Rockford, IL) was washed with 1.0 mL of 0.1 M NaPO4 buffer (pH 6.5), dried on filter paper, and placed into a 1.5-mL microcentrifuge tube. One hundred microliters of 0.1 M NaPO4 (pH 6.5) was added to the microcentrifuge tube along with 10 μL of [Na125I] (1 mCi) and allowed to react for 5 min. Five micrograms of thioredoxin (Sigma Chemical Co., St. Louis, MO) in 35 μL of 0.1 M NaPO4 (pH 6.5) was then added to the tube and allowed to react for 8 min. The reaction was stopped by transferring the solution to a 3-mL column of anion exchange resin (1-X8 resin; Bio-Rad Industries, Hercules, CA) and eluted with 0.05 M PO4 buffer until approximately 4 mL of elute was collected.

Hormone and Antibody Concentrations

Progesterone concentrations were quantified by a single-antibody RIA using a [125I]-progesterone Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA). Luteinizing hormone concentrations were quantified by double-antibody RIA (Adams et al., 1975). The percentage of [125I]-LHRH bound for each sample was quantified at a 1:1,000 dilution with the [125I]-LHRH binding assay described by Johnson et al. (1988). The percentage of [125I]-thioredoxin bound for each sample was quantified by a radioligand binding assay. The sera were diluted 1:1,000 in EDTA-PBS, pH 7.0. Two hundred microliters of each diluted serum was added to assay tubes containing 400 μL of PBS gel, pH 7.0 (0.1% gelatin) and 200 μL of normal bovine serum diluted 1:400 in EDTA-PBS. Sera were incubated for 24 h at 4°C with 30,000 cpm of iodinated thioredoxin in 100 μL of PBS-gelatin. Following the 24-h incubation, 200 μL of sheep anti-bovine γ-globulin diluted 1:15 in PBS, pH 7.0, was added as a second antibody to precipitate the antibody-bound labeled hormone. Assay tubes were again incubated at 4°C for 24 h, followed by addition of 1 mL of PBS (pH 7.0). Tubes were centrifuged at 1,500 × g for 20 min, the supernatant fluid was poured off, and the pellet was counted in a gamma counter for bound radioactivity. The ELISA described by Zhang et al. (1999) was used to determine ovalbumin antibody concentration at a 1:10,000 dilution.
The LHRH fusion proteins developed measurable antibody titers against the spayed control, and treated groups (Figure 3). As expected, measurable antibody titers were not detected in either the spayed control or untreated control heifers during any of the 22 wk of the study (Figure 3). Pretreatment estrous cycle activity did not influence anti-LHRH antibody titers at any time during the study. Beginning on wk 10 and continuing through the conclusion of the study, anti-LHRH antibody titers differed among the treatment groups. During wk 10, the OLOLOL group had higher \( P = 0.03 \) anti-LHRH antibody titers than the TLTLTL group. Neither OLOLOL nor TLTLTL differed from the cocktail (combination) group. During wk 14, the OLOLOL and cocktail groups were not different from one another \( P = 0.74 \), whereas both groups had greater \( P < 0.05 \) anti-LHRH titers than the TLTLTL groups. A similar trend was noted for wk 18 and 22. Antibody titers to LHRH were greatest \( P < 0.05 \) in the OLOLOL and combination-treated heifers and least in the TLTLTL heifers.

Antibody titers for thioredoxin, as indicated by radioligand binding assay, did not differ during the first 3 wk of the study. Beginning at wk 4 and until the conclusion of the study, the percentage of binding differed \( P < 0.05 \) among treatment groups (Figure 3). Antibody titers for ovalbumin, as indicated by ELISA, did not differ during the first week of the study \( P = 0.84 \); however, from wk 2 through the conclusion of the study, ovalbumin antibody titers as expressed by optical density differed \( P < 0.05 \) among treatment groups (Figure 3).

Average circulating concentrations of LH during the study differed by treatment group \( P < 0.01 \), with spayed control heifers having greater concentrations of LH than all other treatment groups (Table 2). By pooling all immunized groups and comparing them with the intact control and spayed control groups, it was determined that the spayed control heifers had greater \( P < 0.01 \) circulating concentrations of LH, whereas there was a trend for the immunized heifers to have a lower circulating concentration \( P = 0.06 \) of LH than the intact control heifers (Table 2). Repeated-measures analysis indicated a difference \( P < 0.05 \) among treatment groups for concentration of LH, but there were no time or treatment \( \times \) time interactions between treatment groups.

Average Daily Gain

Heifer BW did not differ significantly among treatment groups at the beginning of the study. Average daily gain did not differ \( P = 0.20 \) among treatment groups from wk 4 through the conclusion of the study; however, pooling all immunized groups and comparing them to the intact control and the spayed control groups indicated that the immunized heifers had a lower \( P < 0.01 \) ADG than the intact control heifers but were not different from the spayed control heifers (Table 2).
Figure 3. Percentage of heifers exhibiting estrous cycle activity (bars), percentage of $^{125}$I-LHRH bound (◆), percentage of $^{125}$I-thioredoxin bound (■), and ovalbumin antibody titers (▲) after LHRH immunization using different carriers. Thioredoxin (TL) given at 0.4 nmol per immunization, ovalbumin (OL) given at 0.4 nmol per immunization, and a combination given at 0.2 nmol each of TL and OL. Arrows indicate times of immunization. Anti-LHRH antibody titers were elevated ($P < 0.05$) in all immunized groups of heifers by wk 6, and there were fewer ($P < 0.05$) heifers exhibiting estrous cycle activity at this time compared with intact control heifers.
Table 2. Mean (±SEM) serum concentrations of LH, uterine weight, and ADG during the 22-wk study for intact control, spayed control, and pooled immunized treatment heifers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>LH, ng/mL</th>
<th>Uterine weight, g</th>
<th>ADG, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>10</td>
<td>0.3 ± 0.1a</td>
<td>210 ± 6a</td>
<td>1.2 ± 0.1a</td>
</tr>
<tr>
<td>Spayed</td>
<td>10</td>
<td>0.9 ± 0.1b</td>
<td>80 ± 13b</td>
<td>1.0 ± 0.1b</td>
</tr>
<tr>
<td>Immunized</td>
<td>89</td>
<td>0.1 ± 0.1a</td>
<td>90 ± 4b</td>
<td>1.0 ± 0.1b</td>
</tr>
</tbody>
</table>

a,bValues within the same column with different superscripts differ, P < 0.05.

Uterine Weight

Uterine weight among treatment groups differed (P < 0.01), with the intact control heifers having heavier uteri than all other treatment groups (Table 2). The spayed and immunized heifers had similar uterine weights with the exception of the TL/TLTL and OLO-LOL groups. The TL/TLTL groups had a heavier (P = 0.05) uterine weight than the OLOLOL group (106 ± 15 vs. 70 ± 5 g, respectively).

Correlation Coefficients Between Traits

There was a positive (r = 0.95) and high correlation (P < 0.001) between mean LHRH antibody titer and high LHRH antibody titer. There was a negative correlation (P < 0.05) between high LHRH antibody titer and uterine weight and a positive correlation (P < 0.05) between starting BW and final BW, uterine weight and final BW, and ADG and final BW. There was no correlation between ADG and starting BW, antibody titer (mean or maximum) and either starting BW, final BW, or ADG (Table 3).

Carrier-Mediated Immune Suppression

Carrier-mediated immune suppression may be defined as an inhibition to the hapten portion of a vaccine after repeated immunizations with the same carrier protein (Sad et al., 1991). As can be seen in Figure 3, after each immunization, antibody titers increased (P < 0.05) for both the carrier protein and LHRH (the hapten portion of the vaccine). Thus, no carrier-mediated immune suppression was observed in this study.

Discussion

Immunoneutralization of LHRH disrupts the endocrine cascade that allows for follicular growth and ovulation, ovarian steroidogenesis and displays of behavioral estrus (Adams and Adams, 1986). Thus, serum LH concentrations are lowered when heifers are immunized against LHRH and the uterus of immunized heifers reverts back to a more regressed state. Adams and Adams (1990) showed that pituitary concentrations of both LH and LHRH receptors are decreased by approximately 50% in immunized animals vs. their unimmunized contemporaries. In the current study, this disruption of the endocrine cascade is readily apparent after the seventh week of the study, when there was a decrease in the number of heifers showing estrous cycles. This trend of decreased numbers of heifers showing estrous cycles continued until wk 13, when the number of heifers exhibiting estrous cycle activity in all treated groups was 3 of 89 animals. Thus, at this time, only 3% of the treated heifers were showing estrous cycles compared with 90% of the intact control heifers.

Evidence of a carrier-mediated immune suppression, as suggested by Sad et al. (1991), was not evident in this study. Treatment groups receiving each immunization with the same carrier had an increase in anti-LHRH antibody titers, similar to what was seen when alternating carrier proteins. This is also evident by the lack of differences in the number of heifers exhibiting estrous cycle activity in each treatment group throughout the study. Luteinizing hormone-releasing hormone antibody titers were significantly elevated in all treatment groups compared with the intact control and spayed control heifers by the 10th wk of the study.

Serum concentrations of LH were as expected for the different treatment groups. Spayed control heifers had the greatest serum concentration of LH when taken as an average throughout the study, which is consistent with previous observations after castration in cattle (Kiser et al., 1981).

Average daily gain did not differ among LHRH treatment groups, which is similar to that reported by Pre-

Table 3. Correlation coefficients between different traits of all immunized beef heifers

<table>
<thead>
<tr>
<th>Variable</th>
<th>ADG</th>
<th>Final BW</th>
<th>Starting BW</th>
<th>Uterine weight</th>
<th>Maximum antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean antibody titer</td>
<td>−0.10</td>
<td>−0.08</td>
<td>−0.01</td>
<td>−0.42a</td>
<td>0.95a</td>
</tr>
<tr>
<td>Maximum antibody titer</td>
<td>−0.06</td>
<td>−0.02</td>
<td>0.03</td>
<td>−0.44a</td>
<td></td>
</tr>
<tr>
<td>Uterine weight</td>
<td>0.29a</td>
<td>0.22a</td>
<td>0.03</td>
<td>−0.44a</td>
<td></td>
</tr>
<tr>
<td>Starting BW</td>
<td>0.15</td>
<td>0.73a</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final BW</td>
<td>0.78a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aP < 0.05.
... from that of Adams and Adams (1990) when heifers did not receive an anabolic steroid implant. A more relevant comparison can be made when all immunized animals are pooled and compared with the intact control and spayed control heifers. This approach revealed that the intact control group had a greater ADG than both the immunized and spayed control groups as would be expected for animals with an endogenous source of estrogens (Lammers et al., 1999). The spayed control groups did not differ from the immunized groups as would be expected due to large decreases in circulating estrogens. Adams et al. (1990) showed that Synovex H implants allow for BW gains in spayed and LHRH-immunized heifers that are similar to those of intact heifers. From a production standpoint, this lack of endogenous steroid production while animals are being treated with an LHRH vaccine will negatively affect growth. However, it is important to note that the objectives for this vaccine are to block estrous cycle activity and pregnancy, much the same as spaying is used on range heifers before entering the feedlot in the western United States. Immunization is not being proposed as a replacement for use of melengestrol acetate in the feedlot. Once LHRH-immunized heifers enter the feedlot, it will be necessary for producers to use growth promotants, similar to those used with spayed heifers, to overcome the lack of ovarian steroid production. There are other options to a LHRH vaccine that may not have the same effect on growth. For instance, a conjugated porcine zona pellucida vaccine has been developed for horses and deer (Kirkpatrick et al., 1997) that still allows for normal estrous cycle activity, while blocking sperm binding to ovulated ova.

One interesting observation was made when assessing antibody titers for a given carrier protein. When boosting with one carrier, we continue to observe an increase in antibody titer to the opposite carrier. This would indicate an increase in the antibody production of the carrier not being boosted for. For this to occur, both fusion proteins must have an epitope in common. The C-terminal His-tag, which is common to both fusion proteins, may cross-react when producing antibodies. This trend in boosting antibody production of the carrier was seen for both carrier proteins and may also be a reason that no carrier-mediated immune suppression was seen in this study (Figure 3).

Figure 3 indicates that two heifers in the spayed control group resumed estrous cycle activity before the end of the study. This is most likely due to a small amount of ovarian tissue having been left following ovariectomy, which could have allowed a small amount of luteal tissue to develop. Similar results have been reported by Garber et al. (1990) when using a similar procedure for spaying heifers. Although heifers in this study did not have discernable ovaries at the time of slaughter, enough tissue apparently remained, allowing for progesterone concentrations to be elevated relative to those observed in heifers not exhibiting estrous cycle activity. It is possible that other sources of progesterone also contributed to the elevated concentrations of progesterone (adrenal progesterone), but serum progesterone in immunized heifers, which should have exhibited similar patterns, remained depressed. The fact that some heifers resumed estrous cycle activity before the conclusion of the study lends credence to the idea that the effects of the vaccine are reversible. Although these heifers were not subjected to a bull for breeding and their ability to become pregnant was not tested, resumption of estrous cycle activity is a strong indication that the effects of the vaccine may be reversible in some heifers. Bishop et al. (1996) found that LHRH-immunized heifers could be induced to ovulate with gonadotropins, also supporting the idea that heifers are able to undergo folliculogenesis and subsequent ovulation with proper stimuli. Because no animals immunized with two or more OL immunizations resumed cycling, and because the study was concluded after 22 wk, it was impossible to determine whether reversibility of the vaccine in these treatment groups would occur. By the conclusion of the study, 17.5% (7 of 40 animals) of the animals treated with two or more TL immunizations were exhibiting estrous cycle activity as determined by serum concentrations of progesterone. Of these seven heifers, three were in the TLTLTL treatment group. A difference in the effectiveness of the two fusion protein LHRH vaccines was obvious in this study. It is probable that protein folding may allow more of the LHRH inserts in the fusion protein to be exposed, thereby making this protein more effective in producing antibodies against LHRH than the TL fusion protein.

**Literature Cited**


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