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Use of recombinant gonadotropin-releasing hormone antigens for immunosterilization of beef heifers[1]

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ABSTRACT: The objectives of this study were to evaluate the effects of immunization against recombinant GnRH fusion proteins and growth promotants on onset of puberty, feedlot performance, and carcass characteristics of beef heifers. Heifers were immunized against an ovalbumin fusion protein containing 7 GnRH peptides (oGnRH, n = 12), a thioredoxin fusion protein containing 7 GnRH peptides (tGnRH, n = 12), a combination of oGnRH plus tGnRH (otGnRH, n = 12), or a combination of ovalbumin and thioredoxin (control, n = 11). Each heifer received a primary immunization containing 1 mg of protein in 1 mL of adjuvant injected into the mammary gland at wk 0 (mean age = 38 wk) and booster immunizations at wk 6 and 12. Six heifers within each treatment received Synovex H implants at wk –2. Weekly blood samples were collected from wk –2 to 26 for determination of serum progesterone concentrations and GnRH antibody titers. In GnRH-immunized heifers, GnRH antibody titers increased after the first booster injection, peaked after the second booster injection, and remained elevated through the end of the study ($P < 0.01$). Heifers immunized against oGnRH achieved greater ($P < 0.05$) GnRH antibody titers than tGnRH heifers but did not differ ($P = 0.20$) from otGnRH heifers. During the 26-wk study, ovulation was prevented ($P < 0.05$) in 10 out of 12, 12 out of 12, 11 out of 12, and 0 out of 11 tGnRH, oGnRH, otGnRH, and control heifers, respectively. At slaughter, uterine weights were lighter ($P < 0.01$) for GnRH-immunized heifers than control heifers. Synovex H-implanted heifers had greater ($P < 0.05$) ADG from wk –2 to 26, greater LM area, and lesser percentages of KPH, yield grade, and quality grade than nonimplanted heifers, regardless of the immunization treatment. Immunization against GnRH fusion proteins resulted in production of antibodies against GnRH that prevented ovulation in 92% of the heifers without affecting feedlot or carcass performance. Implanting heifers with Synovex H improved ADG, LM area, and yield grade. Improvements in delivery of the oGnRH vaccine may provide a feasible alternative to surgical spaying of heifers.

Key words: gonadotropin-releasing hormone, heifer, immunization, sterilization

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INTRODUCTION

Immunization against reproductive hormones has been used to control their function. Vaccines against GnRH have resulted in production of antibodies that inhibited function of endogenous GnRH, causing at least temporary sterilization in beef heifers (Johnson et al., 1988; Adams and Adams, 1990; Bell et al., 1997) and bulls (Adams et al., 1993; Jago et al., 1997; D'Occhio et al., 2001). Improved growth was observed among GnRH-immunized heifers, but not bulls, receiving growth promotants (Adams and Adams, 1990; Adams et al., 1990, 1993). Immunosterilization with a GnRH vaccine could benefit the beef industry by replacing surgical castration and preventing undesirable pregnancies in the feedlot. Previous research has been conducted with vaccines containing chemically conjugated antigens that, although efficacious, would not be acceptable in the United States because of guidelines established by the Food and Drug Administration (**FDA**). The FDA stipulates that the molecular structure of hormone antigens be known and homogenous between batches (M. Shoenemann, FDA, Rockville, MD, personal communication). One method of generating con-

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sistent and defined antigens is to use recombinant fusion proteins.

Recombinant GnRH fusion proteins that induced immune responses against endogenous GnRH in mice (Zhang et al., 1999; Quesnell et al., 2000) and cattle (Sosa et al., 2000; Aissat et al., 2002; Stevens et al., 2005) have been generated. Heifers immunized against a recombinant GnRH fusion protein produced antibody titers against GnRH that blocked estrous cycles for 60 to 238 d (Sosa et al., 2000). More recently, postpubertal heifers immunized against recombinant GnRH antigens had suppressed estrous activity within 7 wk of primary immunization (Stevens et al., 2005). The objectives of this study were to evaluate the effects of immunization against recombinant GnRH fusion proteins and growth promotants on the onset of puberty, feedlot performance, and carcass characteristics of beef heifers.

MATERIALS AND METHODS

Animals and Treatments

Crossbred beef heifers (n = 48) were stratified by age (263 ± 4.6 d) and BW (222 ± 5.8 kg) and within strata were randomly assigned to receive 1 of 3 immunosterilizing antigens or control treatment. Heifers within each treatment were again stratified by BW and assigned randomly to receive a single Synovex H (Fort Dodge Animal Health, Overland Park, KS) implant or no implant 2 wk before immunization. Synovex H implants contain 20 mg of estradiol benzoate and 200 mg of testosterone and are approved for use in feeder heifers. Immunosterilizing antigens were purified recombinant fusion proteins generated from plasmids encoding either thioredoxin or ovalbumin containing 7 GnRH peptides at predicted antigenic epitopes (Zhang et al., 1999; Quesnell et al., 2000). Treatments included immunization against ovalbumin-GnRH (**oGnRH**), thioredoxin-GnRH (**tGnRH**), a combination of ovalbumin-GnRH plus thioredoxin-GnRH (**otGnRH**) or a combination of ovalbumin plus thioredoxin (control). Each heifer received a primary immunization on wk 0 and booster immunizations on wk 6 and 12. Each immunization contained approximately equimolar concentrations of GnRH peptides in 0.50 mg of total protein (oGnRH), 0.25 mg of total protein (tGnRH), or 0.375 mg of total protein (otGnRH). Control heifers received 0.75 mg total protein (0.50 mg of ovalbumin and 0.25 mg of thioredoxin) at each immunization. Primary and booster immunizations were emulsified in 1 mL of modified Freund's complete and incomplete adjuvant (Calbiochem-Novabiochem Corp., La Jolla, CA), respectively and injected into 3 sites within the mammary gland (total volume = 2 mL). Heifers were group fed in 2 pens with an equal number of heifers from each treatment per pen. Heifers received corn-silage-based diets that were formulated to provide approximately 1.1 kg of ADG until wk 19 when rations were adjusted to provide approximately 1.2 kg of ADG among nonimplanted heifers. One control heifer died during wk 6 from pneumonia; thus samples and data from this heifer were removed from further analyses.

Blood Samples and Data Collection

Blood samples were collected from the coccygeal blood vessels of heifers weekly from wk –2 through 26 to determine serum progesterone concentration and antibody-binding activity against GnRH, ovalbumin, and/or thioredoxin. Blood was stored at 4°C for 24 h to allow blood samples to clot and then centrifuged for 20 min at 1,800 × *g* to separate serum. Serum was removed, and aliquots were stored at –20°C for determination of progesterone concentration and antibody activity.

Body weights of heifers were determined every 28 d from wk –2 through 26. Formation of granulomas in the mammary gland was assessed 2 wk after the first booster immunization and at wk 26 by a single technician to allow for subsequent evaluation of correlations between granuloma formation and immune response. Mammary glands received a subjective score of 0 to 5; 0 indicated the detection of no granulomas, and 5 indicated the presence of several granulomas or large granulomas. The mammary gland was also dissected at slaughter, and granuloma formation was recorded by the same technician.

Heifers were slaughtered at a local abattoir (up to 8 heifers per week) based on ultrasound backfat thickness (~1 cm) and BW (>450 kg) 0 to 16 wk after the end of the study (wk 26). Approximately equal numbers of heifers from each treatment were slaughtered on each date. Staggering of slaughter dates resulted in an average slaughter date of 38 wk (wk 0 = primary immunization). At slaughter, reproductive tracts were collected and the following measurements recorded: reproductive tract (including the cervix, uterus, oviducts, and ovaries) weight; uterine weight; ovary size (width × length × height); the number of small (<5 mm), medium (5 to 10 mm), and large (>10 mm) follicles; the presence of a corpus luteum; and uterine horn diameter at the external uterine bifurcation. Carcass traits including hot carcass BW, LM area (**LMA**), fat depth at the 12th rib, percentage of KPH, marbling score, USDA yield grade, and USDA quality grade were also collected after slaughter.

Progesterone Concentrations and Antibody Binding

Serum samples were evaluated in duplicate for progesterone concentration by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA) as described by Bellows et al. (1991). The intra- and interassay CV were 7.2 and 12.4%, respectively. The sensitivity of the progesterone assay was 0.04 ng/mL of serum. Heifers were classified as having ovulated 1 wk before the progesterone concentration of serum rose

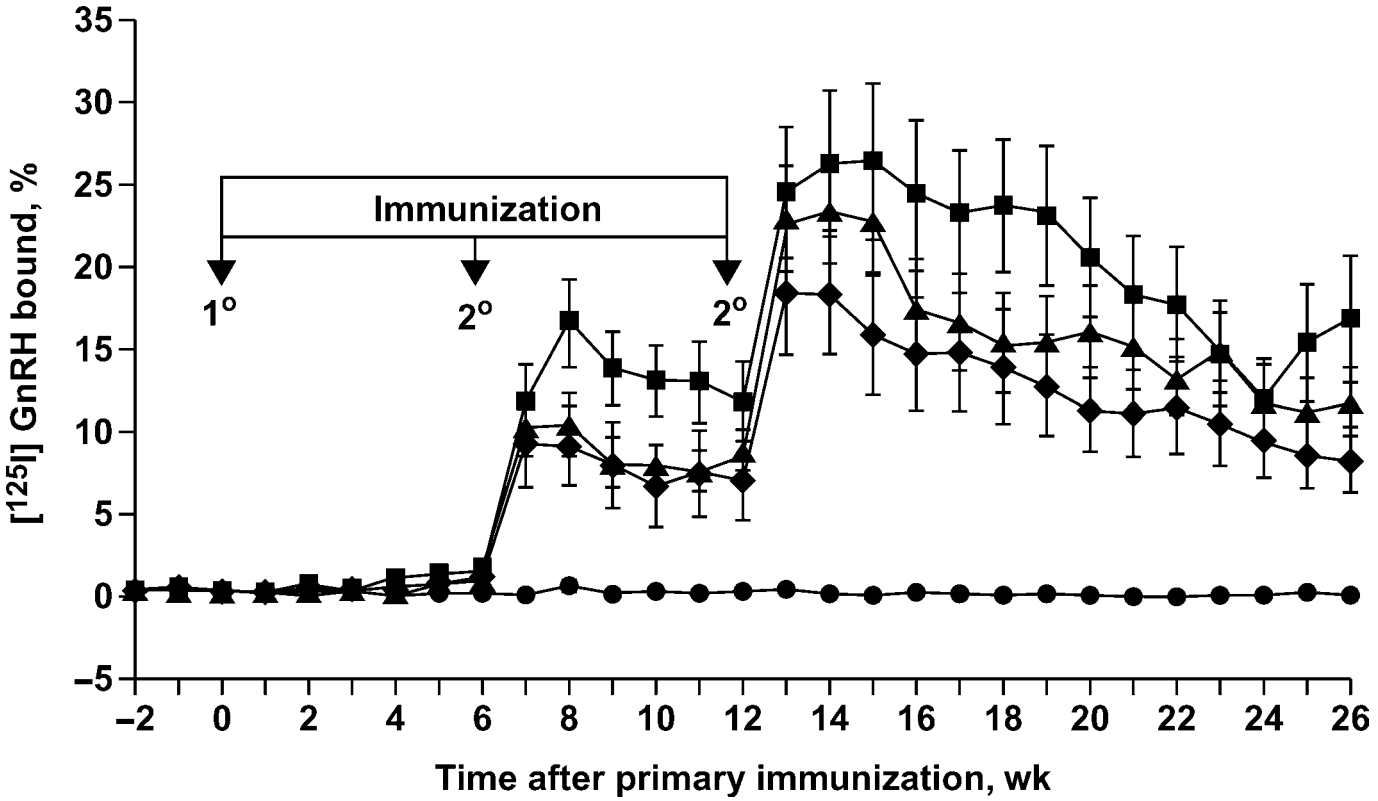

Figure 1. Mean percentage of [^{125}I]GnRH bound in a 1:1,000 dilution of serum from heifers immunized against thioredoxin-GnRH (black diamond) fusion protein, ovalbumin-GnRH (black square) fusion protein, a cocktail of thioredoxin-GnRH and ovalbumin-GnRH (black triangle) fusion proteins, or a cocktail containing thioredoxin and ovalbumin (control, black circle). Antibody activity differed between GnRH-immunized and control heifers from wk 7 through 26 ($P < 0.01$).

>1 ng/mL (Wheeler et al., 1982; and unpublished data of this assay in our laboratory). Likewise, heifers were classified as anovulatory if progesterone concentrations of serum samples were <1 ng/mL for 2 consecutive wk.

The percentage of [^{125}I]GnRH bound by antibodies in each serum sample (1:1,000 dilution) was quantified in duplicate using methods described by Johnson et al. (1988). Ovalbumin antibody activity was quantified in duplicate at a 1:10,000 dilution of serum as described by Zhang et al. (1999). Percentage of [^{125}I]thioredoxin bound for each serum sample (1:1,000 dilution) was quantified in duplicate using the RIA described by Stevens et al. (2005).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Vaccination treatment, Synovex implant treatment, sample day, and all 2- and 3-way interactions were independent variables in analyses of estrous cycle status and antibody activity. Immune responses were assessed as mean antibody binding (wk 0 to 26), peak antibody binding, and area under the curve (wk 0 to 26). Homogeneity of variances among GnRH × Synovex subclasses was tested using Bartlett's Test. For most response variables the within subclass variances were found to be significantly heterogeneous ($P < 0.01$). Thus, data were transformed to ranks, and normal scores were computed from the ranks according to Blom (1958). Transformed data were analyzed using the MIXED procedure in SAS. Vaccination treatment, Synovex treatment and their interaction, and slaughter date (carcass traits only) were independent variables in analyses of feedlot performance, carcass traits, and reproductive tract measurements. Upon observation of significant ($P < 0.05$) treatment effects, treatment means were separated using the PDIFF option of the LSMEANS statement. Relationships between granuloma formation and mean antibody-binding activity of GnRH-immunized heifers were quantified using Pearson correlation coefficients in SAS.

RESULTS

Antibody-binding activity against GnRH was greater ($P < 0.05$) in GnRH-immunized heifers than in control heifers from wk 7 through 26 (Figure 1). Each GnRH immunization treatment resulted in greater ($P < 0.01$) mean, peak, and area under the curve GnRH antibody-binding activity than was observed in the control group. Heifers receiving oGnRH produced greater ($P < 0.05$) GnRH antibody activity than heifers receiving tGnRH,

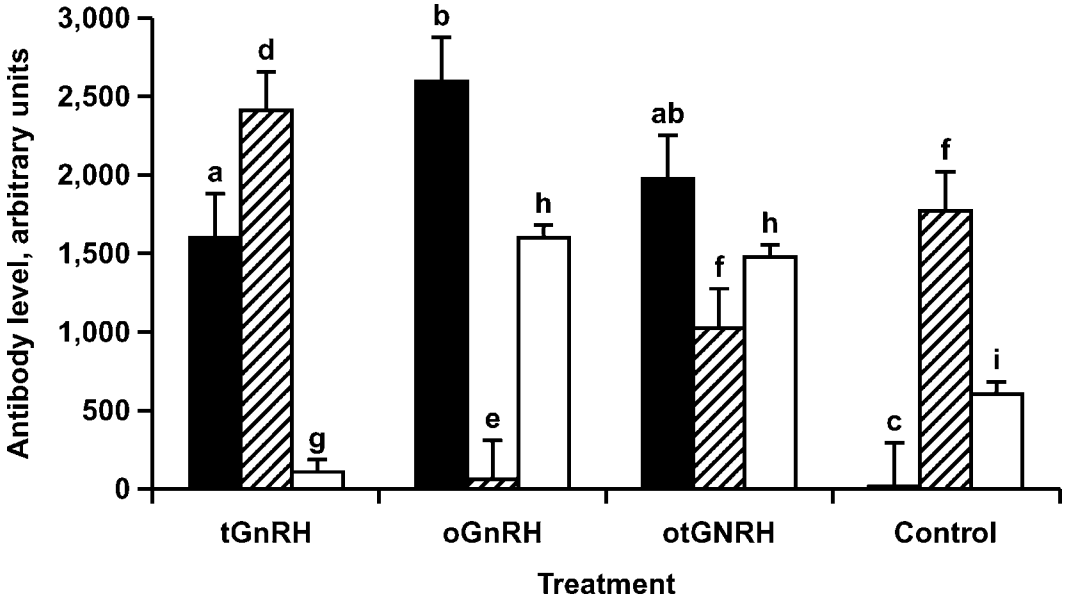

Figure 2. Mean areas under the curves for antibody responses against GnRH (solid bars), thioredoxin (hatched bars), and ovalbumin (open bars) for heifers immunized against thioredoxin-GnRH (tGnRH) fusion protein, ovalbumin-GnRH (oGnRH) fusion protein, a cocktail of tGnRH and oGnRH (otGnRH) fusion proteins, or a cocktail containing thioredoxin and ovalbumin (control). For like bars, antibody responses without a common letter are different ($P < 0.05$).

whereas otGnRH heifers had intermediate GnRH antibody responses (Figure 2).

Two oGnRH heifers and one otGnRH heifer were pubertal at the time of the primary immunization, and 5 oGnRH, 4 tGnRH, and 4 otGnRH heifers and 1 control heifer were pubertal at the time of the first booster immunization. Control heifers continued to reach puberty during the sampling period, and all were pubertal by wk 22 of the study. Within 3 wk of the first booster immunization, 92% of pubertal GnRH-immunized heifers became anovulatory, and only 3 GnRH-immunized heifers (2 tGnRH heifers and 1 otGnRH heifer) had serum progesterone concentrations >1 ng/mL at any time during the remaining 26 wk (Figure 3). From wk 9 until the end of the study, fewer GnRH-immunized heifers exhibited evidence of ovulation than control heifers ($P < 0.01$). At slaughter (38 ± 1.8 wk of study), 7 of 12 tGnRH, 9 of 12 oGnRH, 9 of 12 otGnRH, and 0 of 12 control heifers were anovulatory. Among GnRH-immunized heifers, those that were anovulatory at slaughter had greater ($P < 0.05$) mean, peak, and area under the curve GnRH antibody activities than heifers that had resumed cycling (Figure 4).

Granuloma scores at wk 8 (r = 0.47; $P < 0.01$) and at slaughter (r = 0.36; $P < 0.05$) were positively correlated to mean GnRH antibody activity, whereas the granuloma score at wk 26 (r = 0.15; $P = 0.37$) was not. Mean GnRH antibody-binding activity was only weakly correlated with heifer cyclicity status at wk 26 (r = –0.27; $P = 0.10$) and slaughter (r = –0.33; $P = 0.05$).

Differences were observed for antibody binding against ovalbumin and thioredoxin in heifers immunized against different antigens ($P < 0.05$). Antibody response profiles to ovalbumin and thioredoxin paralleled the GnRH antibody profiles in heifers immunized against these respective antigens. Heifers immunized against tGnRH produced greater ($P < 0.05$) thioredoxin antibody-binding activity than heifers immunized against oGnRH, otGnRH, or control heifers (Figure 2). Heifers immunized against oGnRH or otGnRH produced similar ovalbumin antibody binding that was greater ($P < 0.05$) than those of control heifers or heifers immunized against tGnRH (Figure 2).

A vaccine treatment × Synovex H implant interaction ($P < 0.01$) was observed for the GnRH antibody response. Synovex H-implanted heifers had less GnRH antibody activity when immunized with the tGnRH antigen and greater GnRH antibody activity when immunized with the oGnRH antigen compared with the non-implanted heifers (Table 1).

Reproductive tracts collected at slaughter revealed differences in percentage of heifers cycling between treatments. Fewer ($P < 0.01$) GnRH-immunized heifers were cycling (as evidenced by the presence of a corpus luteum or corpus albicans on at least one ovary) at slaughter (mean = 38 wk) than control heifers (42% tGnRH, 25% oGnRH, 25% otGnRH, and 100% control). The percentage of heifers cycling at slaughter did not differ ($P > 0.10$) between GnRH immunization treatments. Reproductive tract weight, uterine weight, ovarian size, and number of large follicles were less ($P < 0.05$) for GnRH-immunized compared with control heifers but did not differ among the different GnRH immunization treatments (Table 2). Uterine diameter tended ($P < 0.10$) to be smaller for GnRH-immunized compared with control heifers. A tendency ($P < 0.10$) for fewer

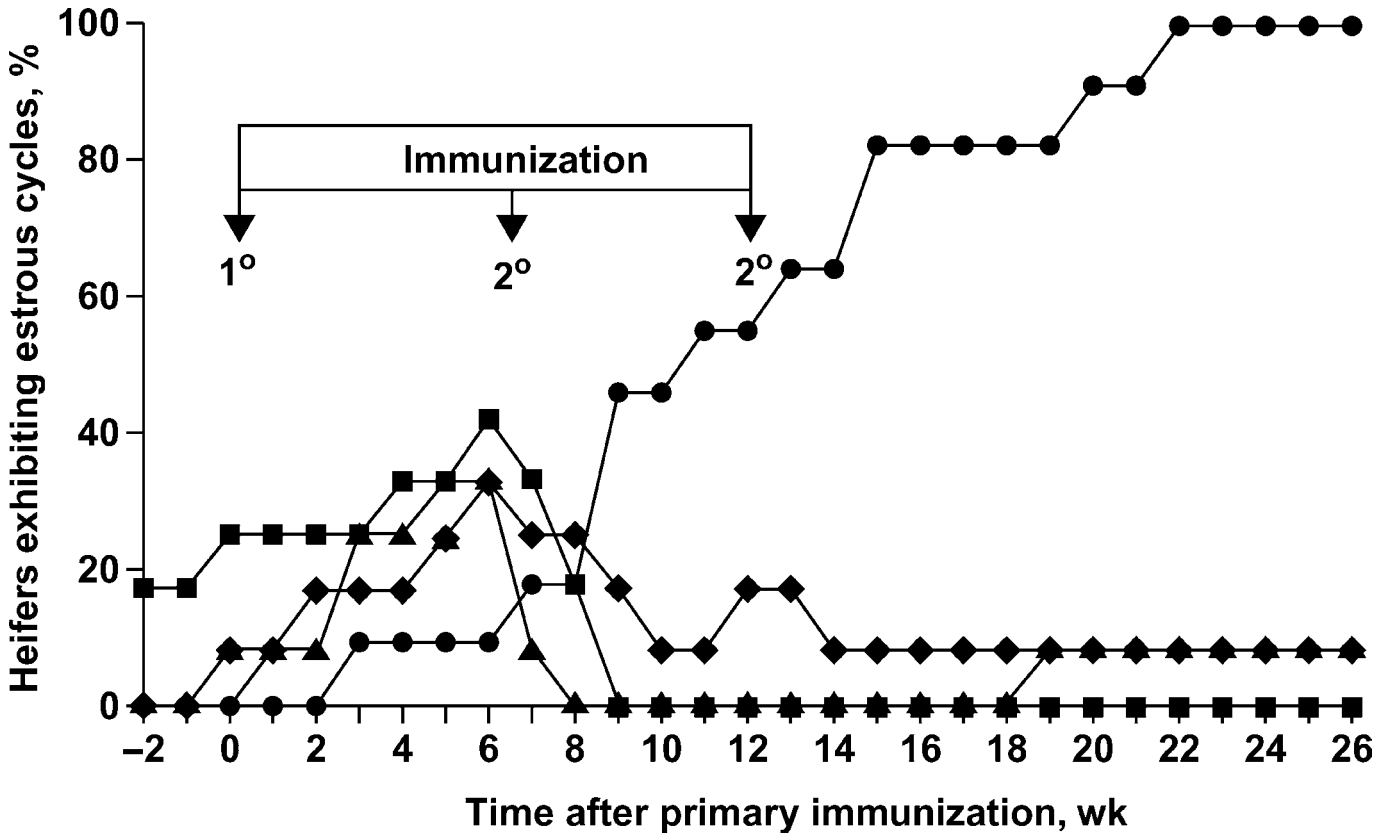

Figure 3. Percentage of heifers exhibiting estrous cycles, as determined by progesterone concentration >1 ng/mL, following immunization against thioredoxin-GnRH (tGnRH, black diamond) fusion protein, ovalbumin-GnRH (oGnRH, black square) fusion protein, a cocktail of tGnRH and oGnRH (otGnRH, black triangle) fusion proteins, or a cocktail containing thioredoxin and ovalbumin (control, black circle). A greater proportion of control heifers were cyclic from wk 9 to the end of the study ($P < 0.01$).

medium follicles was observed on ovaries of GnRH-immunized compared with control heifers, but the number of small follicles on ovaries of GnRH-immunized and control heifers did not differ ($P = 0.85$).

Weight gain of heifers did not differ ($P = 0.59$) among GnRH immunization treatments or between GnRH-immunized and control heifers. Average daily gains for heifers receiving tGnRH, oGnRH, otGnRH, and control treatments were 0.96 ± 0.04, 0.95 ± 0.05, 0.94 ± 0.03, and 0.98 ± 0.05 kg, respectively, during wk –2 to 26. However, heifers that received Synovex H implants had greater ($P < 0.05$) ADG (1.01 ± 0.03 kg) than heifers that did not receive implants (0.90 ± 0.03). The interval from the end of the study (wk 26) to slaughter (based on live BW and ultrasound backfat thickness) was 12 ± 1.8 wk and did not differ ($P = 0.86$) among immunization treatments nor between Synovex H-implanted and nonimplanted heifers ($P = 0.29$). Carcass characteristics (LMA, %KPH, USDA yield grade, and USDA quality grade) did not differ among heifers receiving different immunization treatments. Carcasses of Synovex H-implanted heifers had larger LMA and lower %KPH, yield grade, and quality grade ($P < 0.01$) than nonimplanted heifers.

DISCUSSION

The GnRH antibody response achieved in heifers immunized against the oGnRH antigen was completely effective at preventing estrus in these heifers between wk 9 and 26, and only 3 out of 12 were cycling at slaughter (~wk 38). The oGnRH antigen was evaluated previously in 4 pubertal heifers by Sosa et al. (2000), who reported that heifers became anestrus for 60 to 238 d. Each of the GnRH antigens used in the present study was evaluated previously in predominantly pubertal heifers (Stevens et al., 2005). The GnRH-immunized heifers in the present study produced GnRH and ovalbumin or thioredoxin antibody responses that were parallel to those reported by Stevens et al. (2005). Sosa et al. (2000) reported divergent GnRH and ovalbumin antibody responses among heifers immunized against the oGnRH antigen, which is typical of carrier-mediated immune suppression (Sad et al., 1991). Peak GnRH antibody-binding activity of heifers in this study occurred after the second booster immunization, similar to peak antibody response reported by Stevens et al. (2005). In contrast, peak GnRH antibody binding of heifers immunized against the oGnRH antigen by Sosa

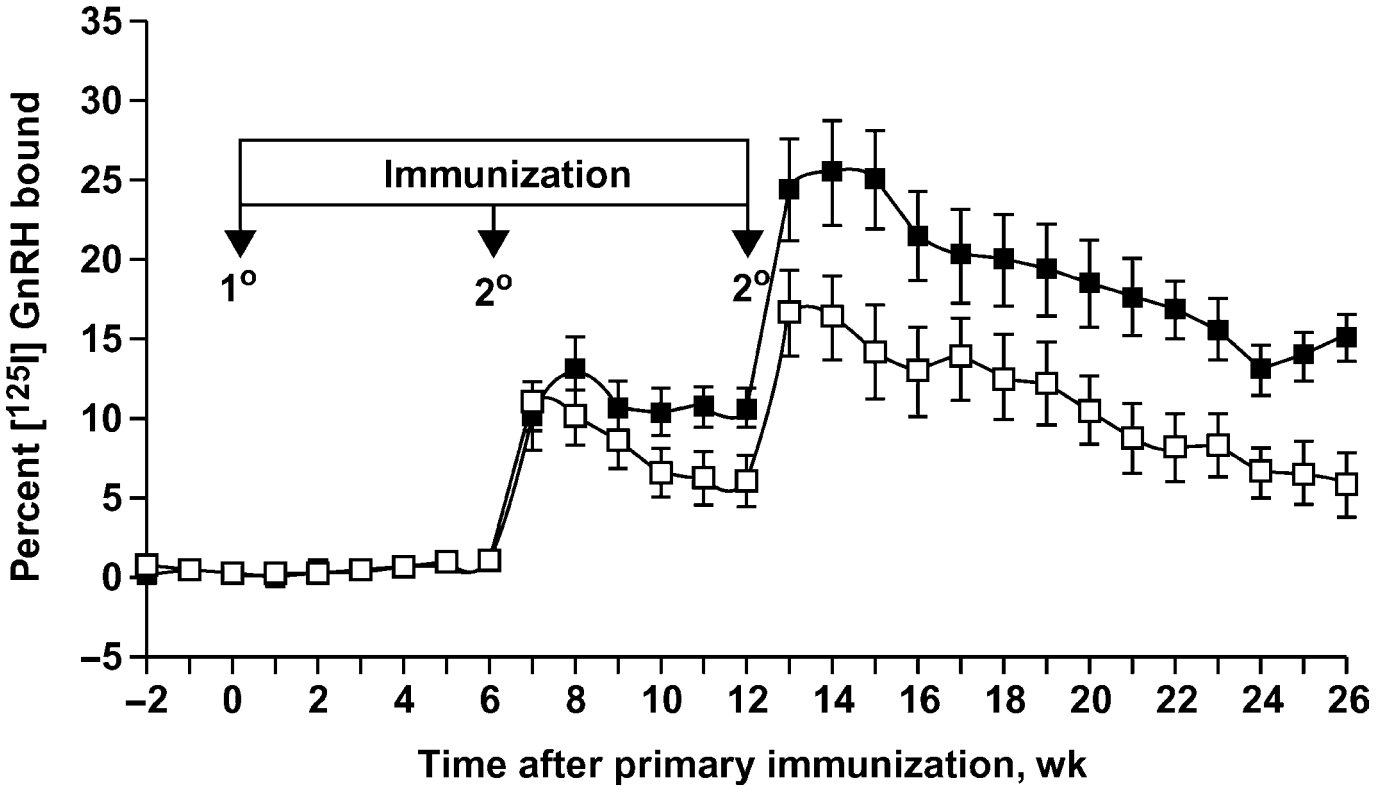

Figure 4. Mean percentage of [^{125}I]GnRH bound by a 1:1,000 dilution of serum from GnRH-immunized heifers that were anovulatory (black square; n = 25) or cyclic (white square; n = 11) at slaughter (approximately wk 38 of the study; $P < 0.05$).

et al. (2000) occurred before the second booster immunization. Because a portion of heifers immunized against the same oGnRH antigen in each of these 3 studies produced different antibody responses with respect to timing of the peak response, there may be genetic or physiological factors not yet identified that influence immune response of heifers.

Heifers immunized against tGnRH produced lower GnRH antibody responses than those immunized against oGnRH, whereas otGnRH-immunized heifers were intermediate in their response, similar to the report of Stevens et al. (2005) in predominantly pubertal heifers. Cook et al. (2001) reported improved GnRH antibody titers in heifers receiving implants containing trenbolone acetate and estradiol benzoate. However, in the present study, implanting heifers with Synovex H resulted in differential response between oGnRH- and tGnRH-immunized heifers.

The duration of immunosterilization among heifers immunized with the tGnRH antigen was not different from that of oGnRH- or otGnRH-immunized heifers. However, at slaughter (wk 38 of study), numerically more tGnRH-immunized heifers (5 out of 12) were cycling compared with oGnRH-immunized (3 out of 12) or otGnRH-immunized heifers (3 out of 12), indicating perhaps that the immunogenic properties of the tGnRH antigen were waning. The 17-wk duration of preventing ovulation observed in oGnRH-immunized heifers in the

Table 1. Least squares means for percentage of [^{125}I]GnRH bound by serum of heifers immunized against thioredoxin-GnRH (tGnRH) fusion protein, ovalbumin-GnRH (oGnRH) fusion protein, tGnRH/oGnRH (otGnRH) fusion protein cocktail, or a cocktail containing thioredoxin and ovalbumin (control), and also receiving Synovex H or no implant 2 wk before the initial immunization[1]

	tGnRH	oGnRH	otGnRH	Control
Synovex H-implanted	5.59 ± 2.27^{ab}	18.75 ± 2.25^{c}	9.88 ± 2.25^{bd}	0.83 ± 2.39^{b}
Nonimplanted	12.66 ± 2.25^{cd}	10.13 ± 2.24^{d}	12.79 ± 2.32^{cd}	0.90 ± 2.60^{b}

$^{a–d}$Means without a common superscript letter differ ($P < 0.05$).

[1]Means represent wk 1 through 26. Variance was heterogeneous among GnRH × Synovex H subclasses, so data were transformed to ranks and normal scores computed from the ranks (Blom, 1958).

Table 2. Mean values for uterine and ovarian characteristics of reproductive tracts collected at harvest in heifers immunized against thioredoxin-GnRH (tGnRH) fusion protein, ovalbumin-GnRH (oGnRH) fusion protein, tGnRH/oGnRH (otGnRH) fusion protein cocktail, or a cocktail containing thioredoxin and ovalbumin (control)

Trait	tGnRH	oGnRH	otGnRH	GnRH-immunized[1]	Control
Reproductive tract wt, kg	0.39 ± 0.05^{ab}	0.43 ± 0.04^{ab}	0.44 ± 0.06^{ab}	0.42 ± 0.05^{a}	0.56 ± 0.05^{b}
Uterine wt, kg	0.09 ± 0.02^{a}	0.10 ± 0.02^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.19 ± 0.01^{b}
Uterine diameter, cm	1.85 ± 0.15^{xy}	1.90 ± 0.11^{xy}	1.80 ± 0.10^{xy}	1.85 ± 0.07^{x}	2.11 ± 0.09^{y}
Ovarian size, cm^3	19.98 ± 3.06^{a}	17.18 ± 3.45^{a}	17.19 ± 2.45^{a}	18.12 ± 1.71^{a}	28.04 ± 3.07^{b}
Small follicles,[2] no.	54.17 ± 6.90	45.0 ± 7.02	63.92 ± 13.46	54.36 ± 5.55	51.55 ± 7.08
Medium follicles,[2] no.	1.08 ± 0.50^{xy}	2.08 ± 1.03^{xy}	1.67 ± 0.76^{xy}	1.61 ± 0.45^{x}	3.27 ± 0.82^{y}
Large follicles,[2] no.	0.42 ± 0.23^{ab}	0.50 ± 0.19^{ab}	0.83 ± 0.37^{ab}	0.58 ± 0.16^{a}	1.27 ± 0.19^{b}

^{a,b}Within a row, means with different superscripts are different ($P < 0.05$).
^{x,y}Within a row, means with different superscripts tend to differ ($P < 0.10$).
[1]GnRH-immunized heifers group includes all heifers receiving tGnRH, oGnRH, or otGnRH.
[2]Small, medium, and large follicles were defined as <5 mm, 5 to 10 mm, and >10 mm, respectively.

present study may be sufficient to prevent pregnancy of heifers on pasture, such as in stocker operations. A GnRH antigen plus adjuvant combination that would provide at least 6 mo of immunosterilization with fewer booster immunizations would be desirable by producers to prevent estrous cycles and pregnancy in heifers on pasture.

Researchers have reported differences in GnRH antibody-binding activity between heifers that remained anestrus and those that became cyclic (Adams and Adams, 1990) or pregnant (Bell et al., 1997). Although GnRH antibody-binding activity differed between heifers that were cyclic and heifers that were anestrus at slaughter, we are unable to identify a minimum threshold level of GnRH antibody-binding activity required to suppress estrus. Fourteen out of 25 heifers that were anestrus at slaughter had lesser mean GnRH antibody responses than the mean GnRH antibody response of cyclic heifers, and 2 out of 11 heifers that were cyclic at slaughter had greater mean GnRH antibody responses than the mean GnRH antibody response of anestrous heifers.

The GnRH antibody-binding activity of heifers in the present study and that of Stevens et al., (2005) appear to be lower than the GnRH antibody-binding activity of heifers immunized against chemically conjugated GnRH antigens (Adams and Adams, 1990; Johnson et al., 1988). However, another difference between the studies is that heifers in the recent studies were immunized with antigen in Freund's adjuvant that contained *Mycobacterium butyricum* (current study; Stevens et al., 2005) rather than *Mycobacterium paratuberculosis* (Johnson et al., 1988; Adams and Adams, 1990) as the immune stimulating agent. The differential response in peak GnRH antibody activity of heifers to the oGnRH antigen in this study and that of Sosa et al., (2000), who used the Z-Max adjuvant (Zonagen, Woodlands, TX), may also be related to adjuvant differences. The GnRH antibody binding from heifers in this study appears to be lower than the GnRH antibody binding obtained in bulls immunized with equimolar amounts of the otGnRH cocktail (Aissat et al., 2002) administered using the same Freund's adjuvant containing *Mycobacterium paratuberculosis*. The GnRH antibody-binding affinity was not determined for heifers in this study. Antibody binding affinity of serum has been measured in male mice immunized against the tGnRH and oGnRH fusion proteins and was reported to be quite variable between mice (Quesnell et al., 2000).

In the current study and in other studies, heifers immunized against GnRH had similar ADG as control heifers (Prendiville et al., 1995; Bell et al., 1997; Cook et al., 2001). However, Adams and Adams (1990) and Adams et al. (1990) reported reduced gains in GnRH-immunized heifers, and reduced gains were reversed with Synovex H implants. Heifers in the current study had increased ADG with Synovex H, but ADG was not affected by the interaction between GnRH immunization and implant. Heifers in the current study received a lower energy diet than those reported by Adams and Adams (1990) and Adams et al. (1990), which might have accounted for differences in BW gain responses. In agreement with findings of other studies, carcass traits were not affected by GnRH immunization (Prendiville et al., 1995; Cook et al., 2001). Increased LMA and carcass leanness among Synovex H-implanted heifers has been reported previously (Unruh, 1986; Garber et al., 1990).

Decreased ovarian size and number of medium and large follicles on ovaries of GnRH-immunized heifers is likely the result of reduced secretion of the gonadotropins, follicle stimulating hormone, and luteinizing hormone (Adams and Adams, 1986) and has been reported previously (Adams and Adams, 1990; Prendiville et al., 1995). Decreased uterine weight of GnRH-immunized heifers is likely due to decreased estrogen and progesterone synthesis from less active ovaries.

In summary, immunization against GnRH using oGnRH, tGnRH, or a cocktail of the oGnRH/tGnRH fusion proteins as antigens prevented estrous cycles in 92% of prepubertal heifers during the 26-wk experimental period. The oGnRH antigen resulted in the greatest

GnRH antibody response, and 100% of these heifers were anestrus 14 wk after the last booster immunization. Immunization against GnRH had no effect on ADG or carcass traits, but heifers receiving Synovex H implants had increased gain and leaner carcasses with larger ribeye area. Heifers in this study received 1 primary and 2 secondary immunizations. Although the oGnRH antigen has potential applications for preventing estrous activity and pregnancy among heifers destined for slaughter, further research is needed to facilitate the delivery system of this antigen to make it easier for producers to apply.

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