# IMMUNIZATION OF BEEF HEIFERS AGAINST GONADOTROPIN RELEASING HORMONE: EFFECTIVENESS OF THE PROTEIN THAT IS CONJUGATED TO GnRH

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### Story in Brief

Thirty-six Angus x Hereford heifers were used to determine if the protein that is conjugated to gonadotropin releasing hormone (GnRH) influences the antigenicity and the development of antibody titers. Heifers (6 per treatment) received a primary immunization against GnRH conjugated to either human serum albumin (HSA-GnRH), ovalbumin (OA-GnRH) or keyhole limpet (KL-GnRH), or heifers were immunized against the proteins (HSA, OA or KL). were emulsified in Freund's incomplete adiuvant Antigens diethylaminoethyl-dextran (DEAE). Booster immunizations were given at wk 4 and 12 of treatment. Luteal activity was suppressed in heifers immunized against GnRH for 23, 16 and 12 wk (P<.01; OA-GnRH, KL-GnRH and HSA-GnRH, respectively) when compared with heifers immunized against the carrier proteins (control). Antibodies titers against GnRH were greater for 19, 5 and 9 wk (P<.01: OA-GnRH, KL-GnRH and HSA-GnRH, respectively) when compared with control heifers. Body weights and body condition scores were not influenced by treatment. Immunization against GnRH conjugated to OA emulsified in Freund's incomplete adjuvant and DEAE dextran caused production of antibodies against GnRH and suppressed luteal activity for 23 wk when compared with control heifers. This procedure may be useful to prevent pregnancy in stocker heifers.

(Key Words: Heifers, Immunization, GnRH.)

#### Introduction

Gonadotropin releasing hormone (GnRH) is a decapeptide secreted by the hypothalamus in the brain. GnRH specifically binds to its receptors in the pituitary and stimulates synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH and LH are synthesized by the same gonadotrops, and stimulate follicular growth and ovulation.

Active immunization against GnRH results in delayed puberty and causes cessation of estrous cycles (Wettemann and Castree, 1994). Emulsification of the antigen in Freund's incomplete adjuvant and DEAE dextran is an effective

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method to produce a sufficient antibody response with minimal granuloma production at the site of injection (Duggan et al., 1992).

Since GnRH is a small peptide it must be conjugated to a larger protein to enhance its antigenicity (Fraser, 1980). Therefore, the objective of the present experiment was to evaluate the effect of conjugation of GnRH to different proteins on the production of antibodies against GnRH.

## **Materials and Methods**

Thirty-six yearling Angus x Hereford heifers weighing 279±7 kg were used. Heifers were blocked by body weight (BW) and body condition score (BCS; 1=emaciated, 9=obese) and assigned to 6 treatments in a 2 x 3 factorial design. Heifers (6 per treatment) received a primary immunization against GnRH conjugated to either human serum albumin (HSA-GnRH), ovalbumin (OA-GnRH) or keyhole limpet hemocyanin (KL-GnRH), or heifers were immunized against the carrier protein (HSA, OA or KL). Antigens were emulsified in Freund's incomplete adjuvant and a 33% solution of DEAE dextran. Primary immunization injections (4 ml) were given subcutaneously and intramurally at six sites in the mammary gland. Booster immunizations were given at wk 4 and 12 of treatment. Blood samples were obtained weekly for 40 wk to determine titers against GnRH by measuring binding of 125I-Concentrations of progesterone in weekly blood GnRH to diluted serum. samples were used to assess luteal ovarian function. Body weight, BCS and mammary gland score (MGS; 1=no granuloma production, 6=open lesions) were recorded every 28 days. The effect of treatments on percentage of heifers cycling, antibody titers, body weight and BCS were analyzed by split plot analyses of variance (main plot was treatment, subplot was week) and orthogonal constrasts used to determine differences between treatment means.

## Results and Discussion

Luteal activity was suppressed in heifers immunized against GnRH. At 20 wk none of the heifers immunized against GnRH conjugated to any protein had luteal activity, whereas, all control heifers had luteal activity (Table 1). Luteal function was reduced for 23, 16 and 12 wk (P<.01) for heifers treated with OA-GnRH, KL-GnRH, HSA-GnRH, respectively, when compared with heifers immunized against the carrier proteins.

Antibody titers against GnRH (1:10,000 dilution) were greater for 19, 5 and 9 wk (P<.01) for heifers immunized against OA-GnRH, KL-GnRH, HSA-GnRH, respectively, when compared with control heifers (Figure 1). Within a week after the first booster, titers against GnRH were greater (P<.01) for the OA-GnRH group compared with control heifers, and the difference was maintained longer for this treatment (23 wk) than for the other treatments.

Body weight and BCS were not influenced by treatment (Figure 2). Mammary gland score (MGS) was increased after the primary immunization,

Table 1. Percentage of heifers cycling after immunization against GnRH.

Treatment	Week of treatment				
	0	4	12	20	30
HSA	60	40	`80	100	60
HSA-GnRH	33	50	33	0	83
OA	50	100	100	100	67
OA-GnRH	. 33	<b>5</b> 0	17	0	33
KL	50	67	100	100	83
KL-GnRH	17	33	67	0	67
CONTROL	53	71	94	100	71
IMM. GnRH	27	44	39	0	61

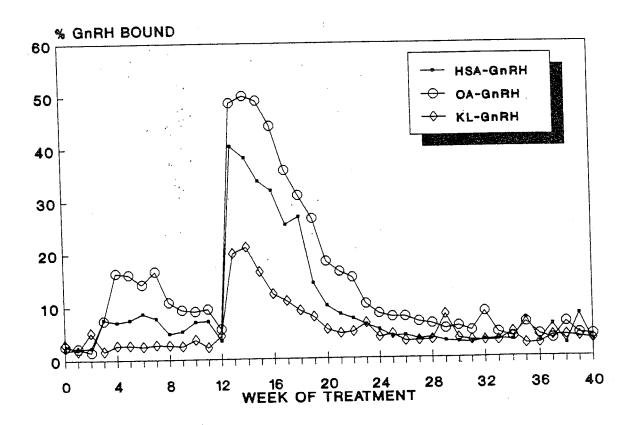


Figure 1. Effect of proteins on antisera titers against GnRH.

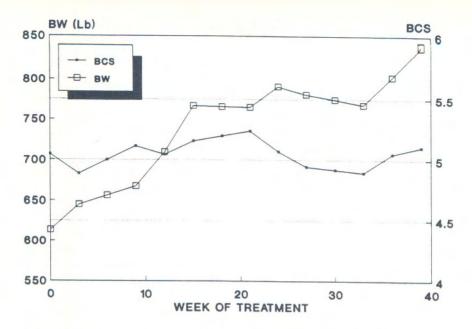


Figure 2. Body weight (BW) and body condition score (BCS) of heifers.

indicating the presence of granulomas at the site of injection. At week 40, the MGS was  $1.8 \pm 0.1$ , but no differences between treatments were observed.

In summary immunization against GnRH conjugated to ovalbumin emulsified in Freund's incomplete adjuvant and DEAE dextran caused significant production of antibodies against GnRH and suppressed luteal activity for 23 weeks when compared with control heifers. This procedure may be effective in preventing pregnancies in stocker heifers.

#### **Literature Cited**

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