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# Immunization against gonadotropin-releasing hormone in dairy cattle: Antibody titers, ovarian function, hormonal levels, and reversibility

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## ABSTRACT

Suppression of cyclic activity in cattle is often desired in alpine farming and for feedlot cattle not intended for breeding. A cattle-specific anti-GnRH vaccination (Bopriva, Zoetis Australia Ltd., West Ryde, Australia) is approved for use in heifers and bulls in New Zealand, Australia, Mexico, Brazil, Argentina, Turkey, and Peru. Eleven healthy, cyclic Swiss Fleckvieh cows were included in the study and vaccinated twice with Bopriva 4 wk apart. Injection site, rectal body temperature, and heart and respiratory rates were recorded before and 3 d following each vaccination. Blood samples were taken weekly for progesterone and estrogen analysis and to determine GnRH antibody titer. Ovaries were examined weekly, using ultrasound to count the number of follicles and identify the presence of a corpus luteum. Thirty weeks after the first vaccination, the cows were subjected to a controlled internal drug-releasing device-based Select-Synch treatment. The GnRH antibody titers increased after the second vaccination and peaked 2 wk later. Estrogen levels were not influenced by vaccination, and progesterone level decreased in 7 of 11 cows up to 3 wk after the second vaccination and remained low for 10 to 15 wk following the second vaccination. The number of class I follicles (diameter $\leq$5 mm) was not influenced by vaccination, whereas the number of class II follicles (diameter 6–9 mm) decreased between 7 and 16 wk after the first vaccination. Class III follicles (diameter >9 mm) were totally absent during this period in most cows. The median period until recurrence of class III follicles was 78 d from the day of the second vaccination (95% confidence interval: 60–92 d). After vaccination, all cows showed swelling and pain at the injection site, and these reactions subsided within 2 wk. Body temperature and heart and respiratory rates increased after the first and second vaccinations and returned to normal values within 2 d of each vaccination. The cows in our study were not observed to display estrus behavior until 30 wk after the first vaccination. Therefore, a Select-Synch protocol was initiated at that time. Ten cows became pregnant after the first insemination (the remaining cow was reinseminated once until confirmed pregnancy). Bopriva induced a reliable and reversible suppression of reproductive cyclicity for more than 2 mo. The best practical predictor for the length of the anestrus period was the absence of class III follicles.

**Key words:** anti-gonadotropin releasing hormone (anti-GnRH), gonadotropin releasing hormone (GnRH), immunization, antibody titer, anestrus

## INTRODUCTION

Estrus inhibition is frequently requested by livestock owners. Preventing recurrent estrus is desired for alpine farming and for feedlot cattle not intended for breeding. These cows, when untreated, cause disturbance in the herd during estrus and, additionally, are often pregnant at slaughter. In contrast to surgical methods (ovariectomy; Hobson and Hansel, 1972; Bleul et al., 2005), mechanical contraception methods (foreign objects in the uterus; Turin et al., 1997), or permanent administration of progesterone derivatives (Burns et al., 1993; Jacobsen et al., 1995; Denicola et al., 1997; Petta et al., 1998), immunological techniques represent an animal-friendly method for controlling populations (Miller et al., 2000; Curtis et al., 2002). Immunization against GnRH has been used to suppress sexual behavior and to prevent the development of secondary sexual characteristics in males (Finnerty et al., 1998; Cook et al., 2000; Thompson, 2000; D'Occhio et al., 2001; Aïssat et al., 2002; Janett et al., 2003, 2009b, 2012a; Turkstra et al., 2005; Theubet et al., 2010) as

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well as in females (Dalin et al., 2002; Imboden et al., 2006; Elhay et al., 2007; Janett et al., 2009a). In heifers, administration of a human chorionic gonadotropin or LH vaccine (Johnson et al., 1988), as well as a GnRH vaccine (Johnson et al., 1988; Adams and Adams, 1990; Prendiville et al., 1995; Bell et al., 1997; Stevens et al., 2005), has been tested. It could be demonstrated that antibody titers, ovarian function, progesterone concentration, and uterus weight differed significantly after immunization with a recombinant GnRH antigen from nonimmunized heifers (Geary et al., 2006). Note that these studies all used different presentations of the GnRH antigen as well as different adjuvants. Immunization with GnRH prevented the growth of follicles >5 mm in diameter and induced anestrus. However, as in mature mares (Dalin et al., 2002; Imboden et al., 2006), the effect of the vaccine varied individually in terms of duration and behavior (Prendiville et al., 1995; Crowe et al., 2001). No information is available in the literature regarding reversibility to estrus in cows after vaccination against GnRH.

The aim of the present study was to evaluate the effect of an active immunization against GnRH (Bopriva, Zoetis Australia Limited, West Ryde, Australia) on GnRH antibody titer, plasma progesterone and estrogen concentrations, and ovarian activity. Of particular interest were the duration and reversibility of the vaccination-induced anestrus. We hypothesized that the vaccine would increase GnRH antibody titer, decrease the plasma concentrations of progesterone and estrogen, and reduce the number of follicles >5 mm in diameter and therefore induce a phase of anestrus of unknown length. Once pregnancy was confirmed, cows were terminated from the study.

## MATERIALS AND METHODS

### Animals and Inclusion and Exclusion Criteria

Eleven lactating Swiss Fleckvieh cows (cows A through M), from second to sixth lactation (median = third lactation) and 88 to 201 d postpartum (median = 137 d) were included in the study. Inclusion criteria were regular cyclicity before first treatment and uterine health. A uterine swab was taken in diestrus for microbiological and cytological analysis. Results of microbiological samples had to be negative on bacterial culture, and less than 5% neutrophils (as a proportion of total cells) had to be counted to demonstrate uterine health, according to Gilbert et al. (2005). Exclusion criteria were a history of preceding dystocia or caesarian section, acute or chronic endometritis, cystic ovarian disease, or general diseases (e.g., chronic mastitis, claw diseases).

### Animal Care and Housing

The cows were housed in tie-stalls on straw bedding. From May to October, the cows had daily access to pasture. During winter months, they had access to a courtyard 3 times a week. They were fed an individually calculated ratio of hay and concentrate determined on the basis of individual milk yield, had free access to water, and were milked twice daily.

### Treatment with GnRH Vaccine

The treatment consisted of 2 vaccinations (initial and booster) with the anti-GnRH vaccine Bopriva (Zoetis Australia Ltd.) 4 wk apart (d 0 and 30). Bopriva contains an analog of GnRH linked to a carrier protein combined with a synthetic aqueous adjuvant (400 μg of GnRH-protein-conjugate per mL) and is registered with claims to suppress testosterone blood levels in postpubertal bulls and estrus behavior in postpubertal heifers. The dosage used for both the initial and the booster vaccination was 400 μg of GnRH-protein-conjugate (1 mL of Bopriva). All injections were administered subcutaneously on the right side of the neck.

All animal experimentation was performed with permission and in accordance with Swiss law (Nr. BE 29/11).

### Clinical Evaluation and Side Effects

Clinical evaluation included the parameters of body temperature, heart and respiratory rates, and daily milk production. The injection site was meticulously inspected for signs of tissue reaction. Cows were examined directly before each vaccination (d 0) and daily on the following 3 d or until all parameters examined had returned to normal. Estrus behavior observation was conducted daily after feeding on pasture or in the courtyard.

### Blood Sampling, GnRH Antibody Titer, and Hormones

In all animals, blood was sampled weekly starting 3 wk before vaccination until pregnancy was confirmed. The blood samples were collected by venipuncture from the coccygeal vein into heparinized tubes and centrifuged (4,000 × *g*, 10 min), and plasma was then stored at −18°C for later analysis.

***GnRH Antibody Titers.*** Antibody titers of GnRH were determined by dissociation-enhanced lanthanide fluorescence immunoassay (**DELFIA**; PerkinElmer Pty Ltd., Glen Waverly, Australia) and results were expressed as relative light units (**RLU**). Briefly, 384-well,