J. Dairy Sci. 97:2816–2821
http://dx.doi.org/10.3168/jds.2013-7453

Evaluation of gonadotropin-releasing hormone hydrogen chloride at 3 doses with prostaglandin $F_{2\alpha}$ for fixed-time artificial insemination in dairy cows

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ABSTRACT

The objectives of the current study were to evaluate the efficacy and field safety of GnRH HCl administered at 3 doses in fixed-time artificial insemination (FTAI) programs (Ovsynch) in dairy cows. A common protocol was conducted at 6 commercial dairies. Between 188 and 195 cows were enrolled at each site (total enrolled = 1,142). Cows had body condition scores ≥2 and ≤4, were between 32 to 140 d in milk, and were clinically healthy. Within pen and enrollment day (enrollment cohort), cows were assigned randomly in blocks of 4 to each of 4 treatments: (1) 25 mg of $PGF_{2\alpha}$ on d 7 with FTAI 72 ± 2 h later (control); (2) 100 μg of GnRH on d 0, d 7 a dose of 25 mg of $PGF_{2\alpha}$, and the second administration of 100 μg of GnRH (T100) administered either at 48 ± 2 h (d 9) after $PGF_{2\alpha}$ with FTAI 24 ± 2 h later or 56 ± 2 h (d 9) after $PGF_{2\alpha}$ and FTAI 17 ± 2 h later; (3) same as T100 with both injections of 150 μg of GnRH (T150); and (4) same as T100 with both injections of 200 μg of GnRH (T200). Three sites selected the first option and 3 sites selected the second option for the timing of the second injection of all doses of GnRH. Cows were observed daily for signs of estrus and adverse clinical signs. Cows not returning to estrus had pregnancy diagnosis between 42 and 65 d following FTAI. Pregnancies per FTAI (P/FTAI) were analyzed as a binary variable (1 = pregnant, 0 = not pregnant) using a generalized linear mixed model with a binomial error distribution and a logit link function. The statistical model included fixed effects for treatment, random effects of site, site by treatment, enrollment cohort within site, and residual. Parity (first vs. second or greater) was included as a covariate. For demonstration of effectiveness, $\alpha = 0.05$ and a 2-tailed test were used. Fifty-two cows were removed from the study because of either deviation from the protocol, injury, illness, culling, or death. Among the remaining 1,090 cows, 33.9% were primiparous and 66.1% were multiparous. Back-transformed least squares means for P/FTAI were 17.1, 27.3, 29.1, and 32.2% for control, T100, T150 and T200, respectively. The P/FTAI for each GnRH dose differed from that of the control. No differences were detected in P/FTAI between GnRH doses. No treatment-related adverse events were observed. Mastitis was the most frequently observed adverse clinical sign, followed by lameness and pneumonia. This study documents the efficacy and safety of doses of 100 to 200 μg of GnRH as the HCl salt when used in Ovsynch programs.
Key words: gonadotropin-releasing hormone, fixed-time artificial insemination, dairy cow, Ovsynch

INTRODUCTION

The fixed-time artificial insemination (**FTAI**) program commonly called Ovsynch has been well described for use in dairy cattle (Pursley et al., 1995; Schmitt et al., 1996; Stevenson et al., 1996, 1999). This program consists of administration of GnRH, administration of $PGF_{2\alpha}$ 7 d later, and then, generally 48 or 56 h later, a second injection of GnRH is administered with FTAI 24 or 16 h later, respectively. These programs use 100 μg of GnRH for both injections because this was the labeled dose of GnRH in cattle for treatment of ovarian follicular cysts, the only label claim for GnRH products in the United States at the time these FTAI programs were developed. Limited evaluation of other doses of GnRH in these FTAI programs has taken place. Leslie et al. (2003) evaluated 100 and 200 μg of GnRH, with all possible dose combinations for first and second dose, in a 48-h Ovsych program and did not detect any overall difference in pregnancies to FTAI (**P/FTAI**) between treatments with an overall mean of 32%. Fricke et al. (1998) evaluated 50 or 100 μg of GnRH diacetate tetrahydrate for both administrations in an Ovsynch program in dairy cows and did not detect a difference in either the ovulation rate to the second dose of GnRH or P/FTAI. In general, a dose of 100 μg has been deemed acceptable for use in these FTAI programs.

Souza et al. (2009), using an ovulation model in dairy cows, reported a reduced ovulation rate in response to 100 μg of GnRH as the HCl salt, relative to ovulation rate in response to 100 μg of GnRH diacetate tetrahydrate. In contrast, no difference was detected in LH

Received September 3, 2013.
Accepted January 7, 2014.
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response following administration of the same dose of these 2 salts of GnRH. Only one product containing GnRH HCl is registered in the United States (Factrel injection, Zoetis). All other GnRH products contain either the diacetate tetrahydrate or acetate salt. The current study was conducted to evaluate the efficacy and field safety of the administration of GnRH HCl in FTAI programs when both administrations of GnRH in the treatment protocol were either 100, 150, or 200 μg of GnRH. Efficacy was measured as P/FTAI, determined at 42 to 65 d after FTAI.

MATERIALS AND METHODS

All aspects of this study were performed to meet FDA-CVM, Guidance for Industry #85, Good Clinical Practice, VICH GL9, Final Guidance, 9 May 2001, and was conducted under the authorization of FDA CVM Investigational New Animal Drug Authorization 011–912. The trial was conducted in a manner consistent with applicable local, state, and federal laws and regulations governing humane care of animals on commercial farms under veterinary supervision. The protocol was reviewed and approved by the Zoetis Veterinary Medicine Research and Development Ethical Review Board.

Locations and Animals

The study was conducted from October 2011 to April 2012 using a common protocol at each of 6 dairy farms producing milk for commerce located in New York, Michigan, Minnesota, Colorado, California, and Florida. These farms had facilities consistent with commercial operations within their geographical locations. At 5 sites, cows were housed in freestall barns with central feed alley with or without adjacent dry lots. Housing at the remaining site (CO) consisted of dry lots. All facilities had feed bunks with head lock-ups where observations were conducted and treatments were administered. Cows were Holstein at 5 sites and either Jersey or Jersey by Holstein cross at the Minnesota site. Cows were fed diets designed to meet or exceed NRC (2001) requirements for lactating dairy cows, and regional feed components were used. Cows were milked 3 times daily in herringbone, parallel, or rotary parlors.

All cows were subjected to a preenrollment physical exam and were clinically healthy at the time of enrollment. At enrollment cows were at least 32 and no more than 140 DIM with a BCS $\geq$2 and $\leq$4 using a 5-point scale. No reproduction products (GnRH, PGF, progesterone) had been administered within 20 d before enrollment.

Treatments and Experimental Design

The objective was to evaluate the effectiveness, as measured by P/FTAI, of each of 3 doses of GnRH HCl compared with the control. The study was powered (80%) to be able to detect an expected difference of 15 percentage points between each GnRH HCl dose and the control, assuming responses were 15% in control and 30% in each GnRH HCl dose, using a 2-sided test and $\alpha = 0.05$. Thus, based on the small difference in P/FTAI reported administering 50 or 100 μg of GnRH diacetate tetrahydrate in the Ovsynch program (Fricke et al. 1998), the current study was not designed to be able to detect potentially small differences that may occur between the selected doses of GnRH HCl.

Within each dairy, the study was conducted as a randomized complete block design with cows blocked by order-of-entry within a pen, without regard to parity. At most sites primiparous cows were housed in pens separated from multiparous cows. Once cows were declared eligible for enrollment they were entered onto the next line of an allotment form previously prepared by the sponsor (Zoetis VMRD). Using this allotment form, cows (n = 4) were entered in each block, with each of the 4 treatments randomized within block. Thus, blocks consisted of 4 cows enrolled from the same housing unit (pen) on the same enrollment day. An enrollment cohort consisted of all cows enrolled in the same pen or lot on the same day. Most sites enrolled more than one enrollment cohort on each day of enrollment. Incomplete blocks were allowed. With the expectation that not all cows would complete the study, a minimum of 188 cows were enrolled at each site, providing 2 blocks more than identified as needed by power calculations.

Treatments consisted of (1) an i.m. dose of 25 mg of $PGF_{2\alpha}$ (5 mL of Lutalyse Sterile Solution, Zoetis) on d 7 with FTAI 72 ± 2 h later (control); (2) an i.m. dose of 100 μg of GnRH (2 mL of Factrel injection) on d 0, on d 7 a dose of 25 mg of $PGF_{2\alpha}$, and each site selected to administer the second administration of 100 μg of GnRH (**T100**) on d 9 at either 48 ± 2 h after $PGF_{2\alpha}$ with FTAI 24 ± 2 h later (d 10) or 56 ± 2 h after $PGF_{2\alpha}$ with FTAI 17 ± 2 h later (d 10); (3) same timing of the treatment regimen as T100 with both administrations of 150 μg of GnRH (3 mL of Factrel injection; **T150**); and (4) same timing of the treatment regimen as T100 with both administrations of 200 μg of GnRH (4 mL of Factrel injection; **T200**). Three sites selected the earlier timing of the second administration of all GnRH treatments and 3 sites selected the later timing; both treatment options were considered representative of FTAI treatment regimens.

Following treatment initiation, cows were observed at least once daily for signs of estrus and for abnor-