Evaluation of gonadotropin-releasing hormone hydrogen chloride at 3 doses with prostaglandin F_{2α} 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_{2α} on d 7 with FTAI 72 $\pm$ 2 h later (control); (2) 100 μ g of GnRH on d 0, d 7 a dose of 25 mg of PGF_{2α}, and the second administration of 100 μ g of GnRH (T100) administered either at 48 $\pm$ 2 h (d 9) after PGF_{2α} with FTAI 24 $\pm$ 2 h later or 56 $\pm$ 2 h (d 9) after PGF_{2α} and FTAI 17 $\pm$ 2 h later; (3) same as T100 with both injections of 150 μ g of GnRH (T150); and (4) same as T100 with both injections of 200 μ g 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 μ g 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_{2α} 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 μ g 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 μ g of GnRH, with all possible dose combinations for first and second dose, in a 48-h Ovsynch 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 μ g 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 μ g 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 μ g of GnRH as the HCl salt, relative to ovulation rate in response to 100 μ g 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 μ g 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 ≥ 2 and ≤ 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 μ g 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 α} (5 mL of Lutalyse Sterile Solution, Zoetis) on d 7 with FTAI 72 ± 2 h later (control); (2) an i.m. dose of 100 μ g of GnRH (2 mL of Factrel injection) on d 0, on d 7 a dose of 25 mg of PGF_{2 α} , and each site selected to administer the second administration of 100 μ g of GnRH (**T100**) on d 9 at either 48 ± 2 h after PGF_{2 α} with FTAI 24 ± 2 h later (d 10) or 56 ± 2 h after PGF_{2 α} with FTAI 17 ± 2 h later (d 10); (3) same timing of the treatment regimen as T100 with both administrations of 150 μ g of GnRH (3 mL of Factrel injection; **T150**); and (4) same timing of the treatment regimen as T100 with both administrations of 200 μ g 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-

mal clinical signs. Cows observed in estrus before d 13 were not inseminated, except for FTAI on d 10. Cows observed in estrus on d 13 or later were inseminated, completed the study that day, and were classified as not pregnant for statistical analyses. Cows with abnormal clinical signs were treated following site-specific treatment prescriptions.

Cows were AI by personnel normally conducting AI following site-specific procedures. Early pregnancy diagnosis was conducted only if this was a site-specific procedure and no sooner than 27 d after insemination. Cows found not pregnant completed the study that day and were classified as not pregnant to FTAI. All cows found to be pregnant to early pregnancy diagnosis and those cows not returning to estrus had a final pregnancy diagnosis conducted during the interval of 42 and 65 d post-FTAI. However, within a site, all final pregnancy diagnoses were conducted within a 7-d interval, such as during 45 ± 3 d after FTAI.

Statistical Analyses

P/FTAI. The response for effectiveness was P/FTAI calculated as [(number of cows pregnant at final pregnancy diagnosis)/(number of cows inseminated at FTAI – number of cows removed from analysis)] $\times$ 100. Cow was the experimental unit. Pregnancy per FTAI was 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 and random effects of site, site by treatment, enrollment cohort within site, and residual. The model included parity (first parity vs. second and greater) as a covariate. The term used for testing treatment differences was the site by treatment interaction. Timing for the second administration of GnRH HCl, either 48 or 56 h after PGF_{2 α} , was not included in the model because this was confounded with site. No comparisons were made between these treatment regimen options. Least squares means, standard errors, and 95% confidence intervals were constructed. Back-transformed least squares means and back-transformed 95% confidence intervals are reported unless otherwise stated. For demonstration of effectiveness, an $\alpha = 0.05$ and a 2-tailed test were used.

Field Safety. Safety data were summarized using the VeDDRA system for organ classifications but not analyzed.

RESULTS AND DISCUSSION

Between 188 and 195 cows were enrolled at the 6 sites with a total enrollment of 1,142 cows. Twenty-three

cows were removed from the study because of significant noncompliance with protocol (deviations), including incorrect treatment administration, administration of hormonal products or AI by farm personnel outside the protocol, incorrect allotment, and so on. Another 29 cows were removed from the study because of injury, illness, culling, death, or other reasons. Data from these 52 cows were included in summaries and analyses up to the day of the deviation or removal; extraneous data collected beyond this day were removed from summaries and analyses data set.

P/FTAI

Included in the statistical analyses were 1,090 cows for P/FTAI, with 370 first parity cows (33.9%) and 720 cows in second or greater parity (66.1%). The P/FTAI and total cows included in statistical analyses, summarized by site and treatment, can be found in Table 1. Back-transformed treatment group least squares means for P/FTAI were 17.1, 27.3, 29.1, and 32.2% for control, T100, T150, and T200, respectively (Table 2). The P/FTAI was greater ($P \leq 0.0123$) in each GnRH HCl treatment dose than for the control. Summed across sites, the P/FTAI in first parity cows was 25.4% (94/370) and in second or greater parity cows was 28.75% (207/720); parity did not have a significant effect ($P = 0.36$) on P/FTAI. These P/FTAI to the Ovsynch program are within the range reported by others; Stevenson et al. (1999) reported a 22.1% (experiment 1) and 35.6% (experiment 2) P/FTAI, Jobst et al. (2000) reported 30.1% P/FTAI, and Pursley et al. (1997) reported 37% P/FTAI.

This improvement in P/FTAI in the GnRH HCl treatments relative to control was expected. The control, consisting of a single administration of PGF_{2 α} with FTAI 72 h later, would not be expected to synchronize ovulation in cows in the first 5 d of the estrous cycle, and the estrus in some cows responding to PGF_{2 α} treatment would be before or after the FTAI at 72 h, resulting in reduced fertility. In the Ovsynch treatment regimen, the LH released in response to the first injection of GnRH results in ovulation of any large follicles present on the ovary. This is followed by the recruitment and development of a new follicle that is expected to ovulate in response to the LH released following the second injection of GnRH. The PGF_{2 α} is administered to initiate the luteolytic process, resulting in regression of corpora lutea present on ovaries. The FTAI is scheduled, relative to the second administration of GnRH, to optimize conception. Furthermore, P/FTAI can be increased by inclusion of hormonal treatment programs administered before the Ovsynch protocol. Such programs are designed to have cows at the ideal time of

Table 1. Percentage of cows pregnant to fixed-time AI (FTAI) and total cows by site and treatment

Site	Experimental group								Total pregnant	
	Control ¹ pregnant		T100 ² pregnant		T150 ³ pregnant		T200 ⁴ pregnant			
	%	n	%	n	%	n	%	n	%	n
A	21.7	46	33.3	45	50.0	44	41.7	48	36.6	183
B	9.1	44	26.0	50	31.3	48	32.6	46	25.0	188
C	17.8	45	40.9	44	18.2	44	31.8	44	27.1	177
D	18.2	44	11.1	45	25.6	43	28.3	46	20.8	178
E	21.7	46	30.4	46	34.0	47	34.1	44	30.1	183
F	19.5	41	29.8	47	22.9	48	31.1	45	26.0	181
Total	18.0	266	28.5	277	30.3	274	33.3	273	27.6	1,090

¹Control was a single dose of 25 mg of PGF_{2α} with FTAI 72 ± 2 h later.

²T100 was a dose of 100 µg of GnRH on d 0, a dose of 25 mg of PGF_{2α} on d 7, a second dose of GnRH either 48 ± 2 h after PGF_{2α} administration with FTAI 24 ± 2 h after GnRH or 56 ± 2 h after PGF_{2α} with FTAI 17 ± 2 h after second dose of GnRH.

³T150 was the same as T100 with 150 µg of GnRH for both doses.

⁴T200 was the same as T100 with 200 µg of GnRH for both doses.

the estrous cycle to maximize ovulation response to the first GnRH administered in the Ovsynch protocol. One program, commonly called Presynch, uses 2 injections of PGF_{2α} at a 14-d interval, with the second injection administered 10 to 14 d before initiation of Ovsynch protocol (Moreira et al., 2001; El-Zarkouny et al., 2004). An alternate program is the double-Ovsynch protocol. This program consists of a pre-Ovsynch program with no FTAI and the second administration of GnRH 7 d before initiation of a regular 56-h breeding-Ovsynch program (Souza et al., 2008).

Differences in P/FTAI between GnRH HCl doses were small, 1.8 to 4.9 percentage points, and no differences were detected (T100 vs. T150, $P = 0.67$; T100 vs. T200, $P = 0.26$; T150 vs. T200, $P = 0.47$). Thus, no significant effect on P/FTAI was detected by increasing the dose of GnRH from 100 to 200 µg. However, the current study was not designed with sufficient power to detect potentially small differences in P/FTAI between GnRH doses as has been documented in other studies. Fricke et al. (1998) reported that cows administered 50 µg of GnRH diacetate tetrahydrate for both administrations of GnRH had synchronized ovulation

response to the second dose of an Ovsynch treatment regimen not different from that of cows administered 100 µg (84.9 vs. 83.1%), respectively. In addition, P/FTAI at 56 d after FTAI were not different, at 39.6 vs. 40.7%, respectively. Giordano et al. (2012) reported LH response to doses of 100 and 200 µg of GnRH diacetate tetrahydrate to dairy cows in either a high- or low-progesterone status. The LH response was greater in cows administered 200 µg in either progesterone status, though lower concentrations of LH were observed in cows in the high-progesterone status regardless of dose. In addition, Giordano et al. (2013) evaluated the effect of increasing the dose of GnRH diacetate tetrahydrate from 100 to 200 µg for the first administration of GnRH in the breeding-Ovsynch part of a double-Ovsynch FTAI protocol. Ovulation response was greater ($P = 0.01$) in cows administered 200 µg compared with cows administered 100 µg (66.6 vs. 57.5%, respectively); however, no difference was detected in P/FTAI (40.8 vs. 34.6%) at 74 d after FTAI.

These data suggest that, although LH release from the pituitary and incidence of ovulation both increase in response to doses of GnRH greater than 100 µg, P/

Table 2. Percentage of cows pregnant to fixed-time AI (FTAI) presented as back-transformed LSM with 95% CI and probability of difference between each GnRH dose and control

Treatment ¹	Animals (n)	LSM	SE	Lower 95% CI	Upper 95% CI	P -value ²
Control	266	17.1	2.6	12.4	23.1	
T100	277	27.3	3.2	21.0	34.6	0.0123
T150	274	29.1	3.3	22.5	36.6	0.0051
T200	273	32.2	3.4	25.3	39.9	0.0011

¹Control was a single dose of 25 mg of PGF_{2α} with FTAI 72 ± 2 h later; T100 was a dose of 100 µg of GnRH on d 0, a dose of 25 mg of PGF_{2α} on d 7, a second dose of GnRH either 48 ± 2 h after PGF_{2α} administration with FTAI 24 ± 2 h after GnRH or 56 ± 2 h after PGF_{2α} with FTAI 17 ± 2 h after second dose of GnRH; T150 was the same as T100 with 150 µg of GnRH for both doses; T200 was the same as T100 with 200 µg of GnRH for both doses.

² P -value for comparison of treatment within row with control.

FTAI is not increased to doses up to 200 µg. In addition, response to 50 µg of GnRH diacetate tetrahydrate was similar to that of 100 µg, as measured by ovulation response or P/FTAI. In toto, these data support that under a variety of Ovsynch programs 100 µg of GnRH is on a plateau of the dose response curve as measured by P/FTAI.

Field Safety

No abnormal clinical signs were attributed to treatment administration. Of the abnormal clinical signs documented over all sites, mastitis ($n = 88$), lameness ($n = 21$), and pneumonia ($n = 9$) had the greatest incidence of occurrence, representing 62.0, 14.8, and 6.3% of all abnormal clinical signs documented, respectively. These are common clinical conditions for dairy cows at this stage of lactation and are not considered to be treatment related.

An adverse health event was documented in 118 cows, with 26, 30, 27, and 35 cows in control, T100, T150, and T200, respectively. Therefore, no apparent trends were observed in the incidence of abnormal clinical signs detected as the dose of GnRH HCl increased. Of these 118 cows, only 88 had a determination of pregnancy status, the remaining being removed from the study. For these 88 cows, the mean P/FTAI were 29.4, 16.7, 23.8, and 46.2% for control ($n = 17$), T100 ($n = 24$), T150 ($n = 21$), and T200 ($n = 26$), respectively, and 29.5% overall. A total of 15, 23, 17, and 20 cows in control, T100, T150, and T200, respectively, were documented with mastitis, with only 12, 19, 14, and 17 of these cows with a determination of pregnancy status, the remaining being removed from the study. These cows had mean P/FTAI of 41.7, 10.5, 21.4, and 52.9%, respectively, and 30.6% overall. These data should be interpreted with caution because cows removed from the study were not included in these mean P/FTAI calculations and the reason cows were removed may have been as a consequence of an adverse health event. Therefore, these P/FTAI may be inflated. In contrast, 1,002 cows did not have any adverse health event documented. These cows had a mean P/FTAI of 17.3, 29.6, 30.8, and 32.0% for control, T100, T150, and T200, respectively, and 27.4% overall.

CONCLUSIONS

Dairy cows administered 100, 150, or 200 µg of GnRH as the HCl salt in Ovsynch FTAI programs had P/FTAI greater than that of cows administered PGF_{2α} with FTAI 72 h later. Under the conditions of the current study, increasing the dose of GnRH from 100 to 200 µg had no detected effect on P/FTAI. No abnormal

clinical signs were observed that were attributed to treatment with GnRH HCl at doses ≤ 200 µg of GnRH. These data support the effectiveness and field safety of the use of 100 to 200 µg of GnRH, as the HCl salt in the Ovsynch FTAI protocol in lactating dairy cows as measured by P/FTAI and incidence of abnormal health events.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support provided by personnel at the six dairies where this study was conducted and the participation of the following investigators in the conduct of the study: Paul Busman (Sparta, MI); Michael Capel (Geneseo, NY); Ricardo Chebel (Saint Paul, MN); Greg Goodell (Greeley, CO); Kenneth Mitchell (Tulare, CA); and Jose Santos (Gainesville, FL).

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