TECHNICAL BULLETIN

Fertility response to commercially available GnRH products in lactating cows synchronized with the Double-Ovsynch protocol.

ABSTRACT

This study was designed to evaluate whether commonly used gonadorelin products that are commercially available in the United States have comparable ovulation and pregnancy per AI (P/AI) results in synchronized lactating dairy cows. A total of 1,411 lactating Holstein cows receiving the Double-Ovsynch (DOV) protocol with the addition of a second PGF2α (Day 25) treatment (Day 1: GnRH, Day 7: PGF2α, Day 10: GnRH, Day 17: GnRH, Day 24: PGF2α, Day 25: PGF2α, Day 26-pm: GnRH, Day 27: AI 16 hours after last GnRH) for first postpartum AI were randomized to receive one of the following GnRH products throughout the Double-Ovsynch protocol: Cystorelin® (CYS, gonadorelin diacetate, n = 484); Factrel® (FAC, gonadorelin hydrochloride, n = 482) or; Fertagyl® (FER, gonadorelin diacetate, n = 515). On Day 17 and Day 24 of DOV, a subgroup of cows (n = 487) were examined by ultrasound to identify whether ovulation had occurred following the third GnRH injection given at Day 17 in the DOV protocol. Circulating progesterone concentration was also measured on Day 17 of DOV (presumably Day 7 of the estrous cycle) in a subset of cows (n = 487) to evaluate the impact of circulating P4 on ovulatory responses. Proportion of cows ovulating at Day 17 tended (*P*=0.07) to differ among GnRH salts (gonadorelin hydrochloride=61.5% vs gonadorelin diacetate=72.7%) but was similar across GnRH products (FER=74.1% vs FAC=61.5% vs CYS=72.2%). In a further analysis, the logistic regression model indicated that predicted ovulation response to FAC was consistently lower than the other two GnRH products regardless of circulating P4 levels, although greater circulating P4 had a similar detrimental effect on ovulation across all GnRH products. Results on P/AI at 60 days differed across GnRH salts (*P* = 0.02) as well as commercial products (*P* = 0.04), in which P/AI was similar between FER (47.8%) and CYS (49.8%), but both were greater than FAC (42.0%). In conclusion, fertility following Double-Ovsynch was significantly lower for the hydrochloride-based GnRH product likely due to poorer ovulatory responses throughout the synchronization protocol.

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INTRODUCTION

Reproductive performance is a major driver of profitability in dairy farms. The use of GnRH and prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) in a coordinated fashion (i.e. Ovsynch) allows for cows to receive timed artificial insemination (TAI) without the need for detection of estrus. The use of Ovsynch protocol has been shown to increase the percentage of cows inseminated sooner after the end of the voluntary waiting period, thus increasing the proportion of cows that become pregnant earlier in lactation. The Double-Ovsynch is a later development of the original Ovsynch program, and is widely used in high-producing dairy herds in the United States.

In the United States, several GnRH products are commercially available and approved by the Food and Drug Administration to be used in association with breeding protocols for dairy and beef herds. These products may have different gonadotropin salts and diluent composition that may have an impact on ovulation. As a result, it is possible that lower ovulatory responses to GnRH during critical times in a synchronization program will likely decrease fertility in synchronized dairy cows and that needs further investigation. For example, in a study by Souza *et al*. 2009, both the LH surge and ovulatory response (Table 1) were less for cows treated with gonadorelin hydrochloride compared with cows receiving gonadorelin diacetate-based products; however, the effect of GnRH on P/AI was not tested in that study.

Therefore, the objective of the present study was to compare three commercially available GnRH products in terms of ovulatory response and P/AI in cows having their first postpartum AI synchronized using a modified Double-Ovsynch protocol with two $PGF_{2\alpha}$ treatments towards the end of the program to assure complete luteal regression.

Based on previous scientific information, our hypothesis was that ovulatory response and P/AI following Double-Ovsynch is less for cows treated with gonadorelin hydrochloride (Factrel®) than cows treated with gonadorelin diacetate-based products (Cystorelin® and Fertagyl®).

TABLE 1. Effect of 100 µg of various types of gonadorelin on ovulation after GnRH treatment on Day 7 in cattle.[5] Excerpt taken from Souza et al. (2009)

TREATMENT	NUMBER OF COWS	PERCENTAGE OVULATING TO GNRH (%)
CYSTORELIN®	146	76.7[a]
FACTREL®	132	55.3[b]
FERTAGYL®	140	73.6[a]
OVACYST®	140	85.0[a]

[a,b]Means within a column with different superscripts are different (P < 0.05).
**Ovulation assumed positive if at least one accessory CL was found on Day 14 in the same ovary as the dominant follicle observed on Day 7.*

MATERIALS AND METHODS

ANIMAL HANDLING, HOUSING AND FEEDING

Enrolled cows were housed in free-stall facilities on two commercial dairy herds (n total = 1,411; herd A = 614; herd B = 797) located in Wisconsin, from February 2016 through April 2017. Lactating Holstein cows (primiparous = 591 and multiparous = 910) were milked three times daily and fed twice daily a total mixed ration (TMR) that consisted of corn and alfalfa silage as forage with a corn and soybean meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cattle and cows had water *ad libitum*. All procedures, including injections, blood sampling, artificial insemination and ultrasonography, was conducted while cows were locked up at the feedline.

RANDOMIZATION AND HORMONAL PROTOCOL

Weekly cohorts of postpartum cows at 48 ± 3 days in milk (DIM) and producing 92.62 ± 7.92 lb/day were enrolled to receive the Double-Ovsynch protocol for their first postpartum AI as previously described, with the addition of a second $PGF_{2\alpha}$ treatment given 24 hours after the last $PGF_{2\alpha}$ during the Double-Ovsynch protocol to improve luteolysis. Thus, all cows received the modified Double-Ovsynch protocol, as follows: Day 1: GnRH, Day 7: $PGF_{2\alpha}$, Day 10: GnRH, Day 17: GnRH, Day 24: $PGF_{2\alpha}$, Day 25: $PGF_{2\alpha}$, Day 26-pm: GnRH, Day 27:AI 16 hours after last GnRH (Figure 3). All hormonal treatments for timed AI were given intramuscularly. The $PGF_{2\alpha}$ used was Estrumate (500 mcg of cloprostenol sodium). Then, within each herd, weekly cohorts of cows were assigned to receive one of the three types of GnRH throughout the entire hormonal program, as follows: 1) Cystorelin® (CYS, n = 484, 100 mcg of gonadorelin diacetate); 2) Factrel® (FAC, n = 482, 100 mcg of gonadorelin hydroclorate); 3) Fertagyl® (FER, n = 515, 100 mcg of gonadorelin diacetate). Breeding records from both herds were collected and stored.

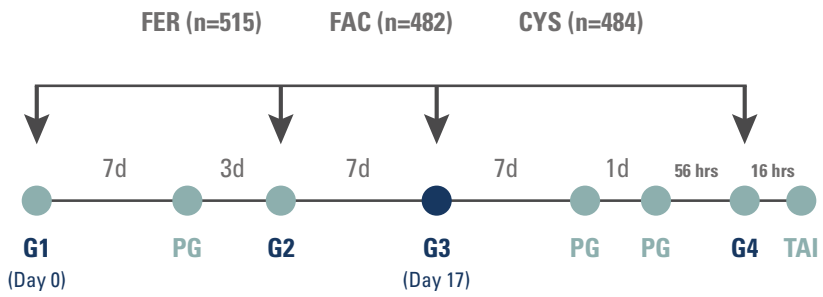

FIGURE 3. The Double-Ovsynch design that was used for the experiment.

OVARIAN ULTRASONOGRAPHY, OVULATORY RESPONSES AND PREGNANCY DIAGNOSIS

Ultrasonographic evaluations of the ovaries were performed in a subset of 487 cows (from herd B) on Day 17 and Day 24 of the Double-Ovsynch protocol to evaluate ovulation response to the three differing GnRH products. Data from ultrasound exams were used to determine presence of a dominant follicle greater than 10 mm and a mature CL on Day 17 and ovulation was assumed for cows with the appearance of a new CL structure on Day 24 at the same site of the ovulatory follicle detected the prior week. Pregnancy was diagnosed by a transrectal ultrasound examination 32 days after timed AI and reconfirmed 60 days after AI. Pregnancy loss was assumed for cows diagnosed pregnant on the first ultrasound exam on Day 32, but found open on Day 60 post AI.

BLOOD SAMPLING AND HORMONAL ASSAY

In order to measure circulating progesterone levels at the time of GnRH injection on Day 17, blood samples were collected just before the administration of GnRH on Day 17 in a subset of cows in herd B (n = 487).

STATISTICAL ANALYSES

Binomially distributed data (ovulation to GnRH on Day 17 and P/AI) were analyzed by logistic regression. Explanatory variables considered for inclusion in the models were treatment, farm, month of AI, parity (primiparous vs multiparous), circulating P4 at GnRH, ovulatory response to the GnRH on Day 17 and meaningful two-way interactions. The variables that were included in the final model for analysis of fertility were: treatment, parity and interaction between treatment and parity. A logistic procedure was utilized to produce continuous logistic regression curves for predicted ovulatory responses to the GnRH products across differing levels of circulating P4 on Day 17 of the Double-Ovsynch protocol. Tendency was assumed for *P*-values between 0.05 and 0.10, and significance when *P*-value was lower than 0.05.

RESULTS

Ovulatory response to GnRH on Day 17 of the Double-Ovsynch tended to differ between the type of GnRH salt. The regression model detected a trend (P=0.07) for lower ovulation rate for the hydrochloride-based GnRH compared to the diacetate salt. However, ovulation response to GnRH on Day 17 did not differ among the three commercial GnRH products (Figure 1). Furthermore, the predicted ovulatory response graph indicates lower ovulation results for cows treated with gonadorelin hydrochloride than cows treated with gonadorelin diacetate, as well as for cows treated with FAC compared to FER and CYS (Figure 2). In addition, as P4 concentration at the time of GnRH injection on Day 17 increased, ovulatory response decreased ($P < 0.01$) for all groups, and there was no interaction in terms of ovulation response between P4 concentration on D17 and type of salt or GnRH product (Figure 2).

Pregnancy per AI results at 32 days post TAI tended to differ among GnRH products and was greater for FER and CYS than FAC according to the analysis as shown in Table 2. In addition, P/AI was greater for cows treated with gonadorelin diacetate products than cows treated with gonadorelin hydrochloride (Table 2). Similarly, at 60 days after TAI, P/AI differed among groups and was greater for FER and CYS than FAC, and was also greater for cows treated with gonadorelin diacetate than cows that received gonadorelin hydrochloride (Table 2). Pregnancy loss between 32 and 60 days after TAI did not differ among GnRH products or GnRH salts (Table 2).

RESULTS CONT.

TABLE 2. Effect of GnRH product on pregnancies per AI (P/AI) and pregnancy loss in lactating Holstein cows treated with the Double-Ovsynch protocol at first postpartum AI.

	FACTREL	FERTAGYL	CYSTORELIN	GnRH PRODUCT	GnRH SALT[1]
	n = 482	n = 515	n = 484	*P-value*	*P-value*
P/AI 32d (%)	44.1[a]	49.3[b]	51.3[b]	0.07	0.03
P/AI 60d (%)	42.0[a]	47.8[b]	49.8[b]	0.04	0.02
PREG LOSS (%)	4.7	3.1	2.8	0.42	0.26

[a,b] Means with different letters within each line are different, P < 0.05;
[1]Contrast: Factrel (gonadorelin hydrochloride) vs Fertagyl + Cystorelin (gonadorelin diacetate) = 0.03.

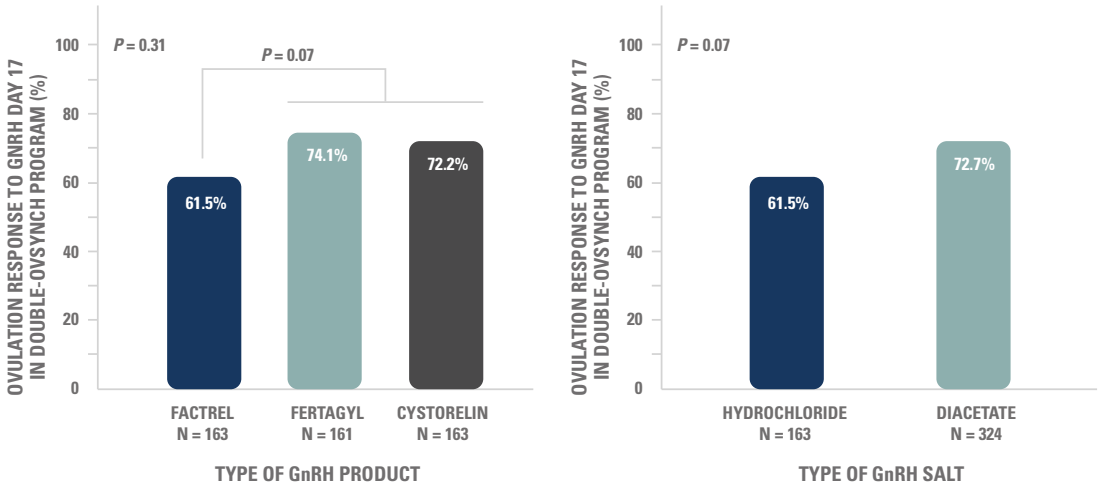

FIGURE 1. Ovulation response according to GnRH product (Cystorelin vs Factrel vs Fertagyl – left panel) or GnRH Salt (Hydrochloride vs Diacetate – right panel) on ovulation response to GnRH on Day 17 during the Double-Ovsynch protocol in lactating Holstein cows.

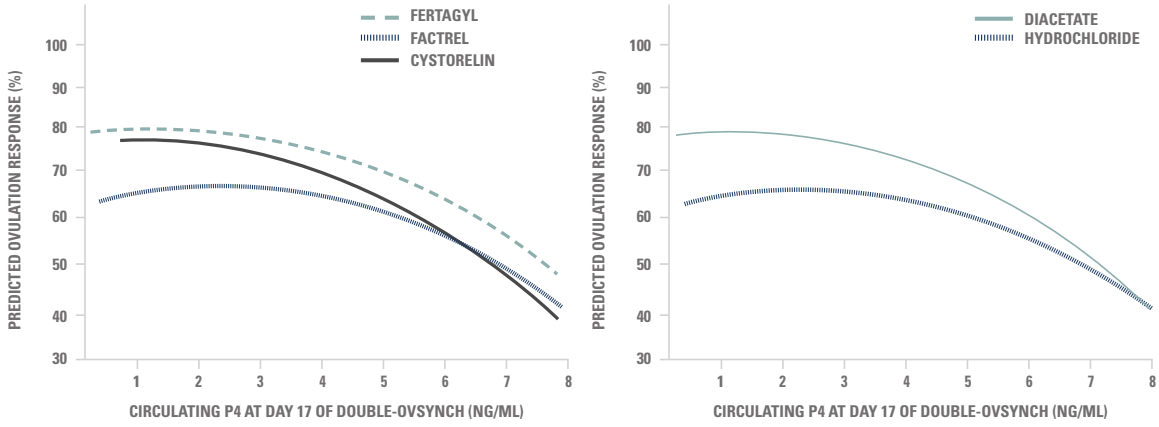

FIGURE 2. Estimated effect of GnRH product (Cystorelin vs Factrel vs Fertagyl – left panel) or GnRH Salt (Hydrochloride vs Diacetate – right panel) on ovulation response to GnRH on Day 17 during the Double-Ovsynch protocol in lactating Holstein cows.

DISCUSSION

A GnRH treatment induces a LH and FSH surge from the anterior pituitary and ovulation of a dominant follicle. Interestingly, a previous study has reported lower amplitude of the GnRH-induced LH surge and ovulatory response in cows treated with gonadorelin hydrochloride compared to cows treated with gonadorelin diacetate, which may explain the lower ovulatory responses as well as lower P/AI found in the current trial for gonadorelin hydrochloride. The exact mechanism by which gonadorelin diacetate induces a greater GnRH-induced LH surge and subsequent ovulatory response and possibly fertility is unknown, but our results suggest that the lower ovulation response to gonadorelin hydrochloride is independent from levels of circulating progesterone at the time of GnRH injection. Perhaps the hydrochloride salt has lower capability to cross the brain membranes to have access to GnRH receptors of the central nervous system. Alternatively, the two salts may differ in terms of stability and degradation over time, which may possibly lower its efficacy. Otherwise, these differing salts may have different perfusion patterns once applied in the animal. Further basic research exploring pharmacological features of these differing GnRH salts are critical to understand the reasons for their differing biological effects.

The logistic regression analysis indicate that greater circulating progesterone lowered ovulation results to all types of GnRH products and salts in the current study, and these findings are in alignment to previous reports. For example, Giordano, Fricke also evaluated the effect of circulating P4 concentrations at the time of the GnRH treatment. Cows with high (3.5 ng/mL) P4 concentrations had a lower peak of the GnRH-induced LH surge than cows with low (0.2 ng/mL) P4 concentrations (3.3 vs. 15.7 ng/mL). Altogether, when P4 concentrations exceed 1 ng/mL total LH secretion and LH peak were decreased. In a subsequent study, Giordano, Wiltbank evaluated the effect of increasing the dose of GnRH on ovulatory response. Interestingly, doubling the dose of GnRH increased ovulatory response only for cows with P4 concentrations exceeding 1 ng/mL, and no effect was observed for cows with P4 concentrations < 1 ng/mL. Thus, depending on the reproductive schedule used in a farm and the stage of the estrous cycle at beginning of the reproductive programs, increasing the GnRH dose might be something to be explored by the herd veterinarian to maximize synchronization in the breeding protocol.

In the current study, we only utilized cows at first postpartum AI synchronized with the Double-Ovsynch protocol to make sure most cows are at the same stage of the estrous cycle and with a responsive follicle when Ovsynch starts (Day 17 of Double-Ovsynch). Double-Ovsynch increases proportion of cows ovulating to the first GnRH of Ovsynch compared to the standard pre-synch with two $PGF2_{\alpha}$ treatments; and cows ovulating to the first GnRH of Ovsynch generally have greater P/AI than non-ovulating cows. However, when complete luteal regression at the end of the Double-Ovsynch protocol is not achieved, the expected increase in P/AI in cows ovulating to GnRH on Day 17 might be negated. This is the main reason why two $PGF2_{\alpha}$ treatments were used in the current experimental design. In fact, as reported by a number of research groups, incomplete luteal regression has a profound negative impact on P/AI. The bovine corpus luteum begins to acquire luteolytic capacity approximately 7 days after ovulation; thus, cows ovulating to the 1st GnRH of the Ovsynch protocol have greater chances of having incomplete luteal regression when a single $PGF2_{\alpha}$ treatment is used. The improved fertility following greater ovulation results to GnRH at beginning of Ovsynch (Day 17 in Double-Ovsynch) associated with successful luteolysis are likely related to enhanced embryo. For example, Cerri *et al*. 2009 evaluated the effect of ovulatory response to the first GnRH of Ovsynch on subsequent embryo development on day 7 after AI. Although fertilization rate did not differ, based on ovulatory responses of cows to the first GnRH (87.5% vs. 83.3%), the percentage of embryos classified as excellent and good quality was substantially decreased (38.9% vs. 77.5%) and the percentage of degenerate embryos was greater (22.2% vs. 5.0%) for cows that did not ovulate. The reduction in quality of embryos from cows failing to ovulate to the first GnRH of Ovsynch was attributed to ovulation of persistent/aged follicles. Thus, cows ovulating to the GnRH on Day 17 in our experimental design probably had greater P/AI due to improved responses to the remaining treatments during the Ovsynch protocol, and because they ovulate a more consistent size follicle at the end of the protocol thereby producing an embryo of better quality by day 7 after AI.

CONCLUSIONS

In conclusion, the gonadorelin hydrochloride-based GnRH salt (Factrel) tended to produce lower ovulation results on D17 of Double-Ovsynch compared to the diacetate salts (Fertagyl and Cystorelin). Interestingly, these differences in ovulatory response were unrelated to P4 concentrations at the beginning of Ovsynch. As a result, P/AI 60 days post AI was significantly lower for cows receiving Factrel during Double-Ovsynch compared to cows treated with either Fertagyl or Cystorelin, presumably, due to lower ovulation responses at critical times during the Double-Ovsynch program.

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