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Comparison of gonadorelin products in lactating dairy cows: Efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles

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Abstract

This study evaluated whether the four gonadorelin products that are commercially available in the United States produce comparable ovulation responses in lactating cows. Dairy cows at 7 d after last gonadotropin-releasing hormone (GnRH) treatment of Ovsynch (Day 7), with a corpus luteum (CL) ≥15 mm and at least one follicle ≥10 mm, were evaluated for response to GnRH treatment. Selected cows were randomized to receive (100 μg; im): (1) Cystorelin (n = 146); (2) Factrel (n = 132); (3) Fertagyl (n = 140); or (4) Ovacyst (n = 140). On Day 14, cows were examined for ovulation by detection of an accessory CL. Circulating luteinizing hormone (LH) concentrations were also evaluated in some cows after treatment with 100 μg (n = 10 per group) or 50 μg (n = 5 per group) GnRH. Statistical analyses were performed with the procedures MIXED and GLIMMIX of the SAS program. Percentage of cows ovulating differed ($P < 0.01$) among groups, with that for Factrel being lower (55.3%) than that for Cystorelin (76.7%), Fertagyl (73.6%), or Ovacyst (85.0%). There was no effect of batch, parity, or follicle size on ovulation response, but increasing body condition score decreased ovulation response. There was a much greater LH release in cows treated with 100 μg than in those treated with 50 μg, but there were no detectable differences among products in time to LH peak, peak LH concentration, or area under the LH curve and no treatment effects nor treatment by time interactions on circulating LH profile. Thus, ovulation response to Factrel on Day 7 of the cycle was lower than that for other commercial GnRH products, although a definitive mechanism for this difference between products was not demonstrated.

Keywords: GnRH; Gonadorelin; LH profile; Ovary; Ovulation rate

1. Introduction

The preovulatory dominant follicle produces increasing amounts of estradiol-17β, eventually stimulating a surge in gonadotropin-releasing hormone (GnRH) from the hypothalamus and behavioral estrus [1]. The GnRH molecule is a decapeptide that binds with high affinity to a specific GnRH receptor, triggering an increase in free intracellular calcium and other intracellular effector pathways [2,3]. Normally, GnRH is produced by hypothalamic neurons, transported to the pituitary gland by the hypophyseal-portal vascular system, with subsequent activation of GnRH receptors on the gonadotrope cells in the anterior

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hypophysis stimulating release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the circulation [3,4]. In cattle, this gonadotropin surge can cause ovulation of dominant follicles, normally ~24 to 32 h after the GnRH surge [5,6].

The structural determination of GnRH was soon followed by commercial production, U.S. Food and Drug Administration (FDA) approval, and marketing of GnRH products for the livestock industry. Today, GnRH has become one of the most utilized reproductive hormones, with four GnRH products currently marketed in the United States, each a salt of the native GnRH molecule. The primary indications for exogenous GnRH treatment have been treatment of follicular cysts [7], repeat-breeder cattle near the time of artificial insemination (AI) [8], ovulation of follicles after AI [9], or in association with timed artificial insemination (TAI) protocols (e.g., Ovsynch) [6,10]. The most extensive use of GnRH in the dairy industry currently appears to be in TAI protocols, with most dairy herds using a TAI protocol during routine reproductive management [11]. For any of these clinical or management uses of GnRH, an adequate LH surge is essential for a successful outcome.

Some studies have compared various dosages and/or types of GnRH analogues [12–17]. However, to our knowledge, only two studies have directly compared gonadorelin products in cattle. In one study, published in a master's thesis [15], a comparison was made between Cystorelin and Factrel in beef cattle. No significant differences were detected in percentage of cows pregnant when the two gonadorelin products were used in various synchronization protocols (Ovsynch, Cosynch, or Select-synch). This study also compared the LH profiles after GnRH treatment and reported no differences between the products in maximal LH concentration or area under the curve although there were some differences in timing of LH responses [15]. Another study [16] compared three gonadorelin products (Cystorelin, Fertagyl, and Factrel) in non-lactating Holstein cows or beef heifers. In two of the experiments, there was a tendency ($P < 0.10$ and $P = 0.11$) for differences between GnRH products in mean LH concentrations, and in the third experiment, this difference was significant ($P < 0.01$). In all cases, the Cystorelin product had the highest LH response, either numerically or significantly. Thus, these experiments provided an indication that there were differences among gonadorelin products in biological response in cattle. There were also reported differences in percentage of cows that ovulated to different GnRH products [16]; however, the numbers of cows per treatment group in that study (n = 19, 19, and 7) were not sufficient to provide a valid comparison of ovulation to the GnRH products.

Evaluation of ovulation in response to GnRH treatment represents a key biological response that would indicate efficacy of GnRH products. Nevertheless, size and functional state of the dominant follicle at the time of GnRH and phase of estrous cycle may be critical determinants of whether a cow will ovulate after GnRH treatment. For instance, Sartori et al. [18] found that only cows with growing follicles that were at least 10 mm would ovulate in response to LH treatment in lactating dairy cows. In addition, the magnitude of the GnRH-induced LH response was lower when cows were treated in the luteal phase (peak LH of ~4 ng/mL) compared with that when cows were treated in the follicular phase (peak LH ~14 ng/mL) [15]. A recent study also reported that cows with lower circulating progesterone have greater LH release after GnRH [19]. To reduce these confounding factors, this trial evaluated GnRH products only on 1 d of the cycle (Day 7) when medium circulating progesterone concentrations and the dominant follicle of the first follicular wave should be present in nearly all cows.

Thus, this study was designed to test the biological activity in lactating dairy cows of the four gonadorelin products that are currently available in the United States. Two biological end points were chosen in these studies. The major end point was percentage of cows that ovulated to treatment with a single 100 μg dose of each gonadorelin product on Day 7 of the estrous cycle. In addition, a subset of cows was evaluated for LH profile after treatment with 50 or 100 μg of each of the GnRH products. Our research hypothesis was that there would be differences in one or more of the GnRH products in ability to cause ovulation or LH release. This study was done on a commercial dairy in high-producing lactating dairy cows, as this would represent a major environment for use of commercial GnRH products. Differences between cows in parity, body condition score (BCS), and follicle size were expected in this experiment and were evaluated for effects on ovulation responses, as well as for potential interactions between these variables and GnRH treatment effects.

2. Materials and methods

2.1. Experimental pharmaceuticals

All GnRH analogues were purchased by a private-practice veterinarian in the traditional pharmaceutical market. Two batches of each of the four GnRH products

were used: Cystorelin was from Merial Limited (Iselin, NJ, USA; batch 097003-A and 090553-A); Factrel was from Fort Dodge Animal Health Inc. (Fort Dodge, IA, USA; batch 431339 and 431343); Fertagyl was from Intervet Inc. (Millsboro, DE, USA; batch 22048-R and 23165); Ovacyst was from IVX Animal Health, Inc. (St. Joseph, MO, USA; batch 4060729 and 3060636). All treatments were given as intramuscular injections.

2.2. Animals, management, and experimental design

Cows used in this experiment were housed during January 2005 to April 2005 in a free-stall facility in Juneau, Wisconsin. Cows were milked 3 times per day and fed a total mixed ration (TMR) that consisted of corn silage 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 [20]. All cows in the study started to receive bovine somatotropin (500 mg/dose; Posilac, Monsanto Co., St. Louis, MO, USA) at ~60 d postpartum, with treatment repeated every 14 d throughout the experiment.

A total of 488 lactating dairy cows (170 primiparous and 318 multiparous) were used in the study. All cows received the Ovsynch protocol (100 μg GnRH [Ovacyst]; 7 d later, 25 mg prostaglandin F2α [Prostamate; IVX Animal Health, Inc.]; 56 h later, GnRH; 16 h later, TAI) prior to experimental treatments on Day 7 after the final GnRH treatment. Some cows that were detected nonpregnant after the first Ovsynch procedure received a subsequent Ovsynch protocol and received another experimental treatment. Therefore, a total of 588 experimental (7 d after AI) GnRH treatments were performed. Cows averaged 153.1 ± 3.8 d in milk (DIM; mean ± SEM) at the time of experimental GnRH treatments.

Seven days after the last GnRH treatment of the Ovsynch protocol (Day 7), all cows were examined by transrectal ultrasonography (4.5- to 8.5-MHz broadband transducer; Easi-Scan; BCF Technology Ltd., Livingston, UK). At this time, cows were selected for subsequent continuation in the project. Only cows that had a corpus luteum (CL) ≥15 mm in diameter and at least one follicle ≥10 mm in diameter continued in the trial. All selected cows were blocked by parity and then randomly assigned to one of four treatments. A separate person was in charge of randomizing the previously selected cows into treatment groups and giving the appropriate GnRH treatment. Seven days later (Day 14), all experimental cows were examined again by ultrasonography for detection of accessory corpora lutea. Ovulation was assumed to have occurred if at least one accessory CL ≥15 mm was detected on Day 14 on the same ovary that previously contained the dominant follicle(s) observed on Day 7. The person doing the ultrasound examination did not know the treatment group of the cows being examined. Body condition score was recorded on Day 7, using a scale from 1 to 5 [21]. A subset of cows (n = 60) was evaluated for LH profiles after experimental GnRH treatments. This subset of cows received either the recommended dose (100 μg; n = 10 cows per treatment group [4 primiparous and 6 multiparous/group]; DIM = 150.2 ± 8.1) or half of the recommended dose (50 μg; n = 5 cows per treatment [n = 2 primiparous and 3 multiparous/group]; DIM = 147.5 ± 9.7) of one of the four GnRH products. Blood samples were collected at 0 h, 30 min, 1 h, 2 h, 3 h, and 4 h after GnRH treatments. All animal procedures were approved by the Animal Care Committee of the College of Agriculture and Life Sciences at the University of Wisconsin-Madison.

2.3. Hormone assay

Blood samples were collected by puncture of coccygeal vessels, immediately refrigerated, centrifuged at 1600 × *g* for 20 min, and serum was collected and subsequently stored at −20 °C until assayed for LH. The circulating concentrations of LH were determined by radioimmunoassay, as previously described [22]. The intra-assay and interassay CVs were 2.3% and 3.6%, respectively. The sensitivity (calculated as the average from all of the assays using two standard deviations above the total binding) was 0.16 ng/mL.

2.4. Statistical analyses

All values are expressed as mean ± SEM. The analyses of circulating LH concentrations were performed using the procedure MIXED of the SAS program [23]. The model included the effects of treatment, time, the interaction between treatment and time, and cow, which was treated as a random effect and was the subject for the repeated measures. Data transformation (log base 10) was used in the comparisons of area under the curve of LH profiles in order to attain normality. Ovulation was a binary response variable and was analyzed with the GLIMMIX procedure of SAS Version 9.1 for Windows [24], with cow treated as a random effect. Some variables such as month (January, February, March, and April), BCS

(≤2.5, 2.75, 3.0, and ≥3.25), GnRH batch within treatment, parity (primiparous and multiparous), and size of the largest dominant follicle (≤13 mm, 14 to 15 mm, and ≥16 mm) on Day 7 were inserted in the model as class variables. The continuous variable DIM was used as a covariate in the model. All variables and interactions were initially used in the statistical models. Only variables with $P < 0.15$ were kept in the final model, which accounted for effects of treatment, BCS, and DIM. P values < 0.05 were considered significant, and P values between 0.05 and 0.10 were discussed as tendencies. The terminology cow or breeding was used interchangeably to define our experimental unit (breeding). Only cows receiving the full dose of GnRH (100 μg) were used in the analyses of ovulation response.

3. Results

3.1. Circulating LH profile

There were no effects ($P > 0.10$) of batch within treatment in the LH profile for either the 50 or 100 μg dose of GnRH. Thus, the LH profiles from the two batches for all GnRH products were combined in the analyses. The average circulating LH profiles throughout the 4 h of blood sampling for both 50 μg or 100 μg GnRH doses are shown in Fig. 1. The time to peak, LH peak concentration, and area under the LH curve for the two doses of all LH products are summarized in Tables 1 and 2. All GnRH products caused a dramatic increase in circulating LH and therefore there was an effect of time ($P < 0.01$) for both the 50 or 100 μg dose of GnRH. There was a much greater response to the 100 μg dose than to the 50 μg dose, with roughly a doubling in maximal concentration of LH with the higher dose (50 μg = 11.55 ng/mL vs. 100 μg = 23.05 ng/mL) and ∼ 80% increase in area under the LH curve (50 μg = 26.18 vs. 100 μg = 46.70).

There was no detectable effect of treatment with either 50 μg ($P = 0.15$) or 100 μg ($P = 0.83$) of GnRH products. There also was no interaction of treatment by time in the circulating LH concentrations for either GnRH dose (Fig. 1). In the cows treated with the 50 μg dose of GnRH, there were some differences between products at 1 h and 2 h after treatment (Fig. 1A). However, there were no detectable differences at any time in LH concentrations in cows treated with 100 μg of GnRH (Fig. 1B).

There were no differences between GnRH products for time to LH peak or LH peak concentration in cows treated with either 50 μg (Table 1) or 100 μg GnRH (Table 2). In cows treated with 50 μg GnRH, the area under the curve for LH tended to be greater ($P = 0.10$) for Ovacyst than for Factrel (Table 1). In contrast, there were no detectable differences between GnRH products in area under the curve for LH when 100 μg GnRH was used (Table 2).

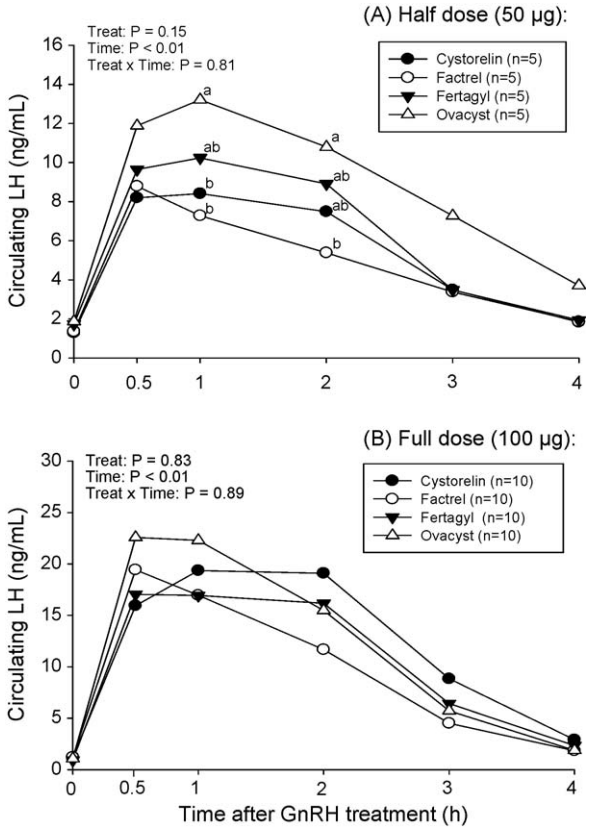

Fig. 1. Effect of treatment with various GnRH products on circulating LH concentrations in dairy cows: (A) half dose (50 μg/dose; n = 5 per group); (B) full dose (100 μg/dose; n = 10 per group). Treatments with the various GnRH products were performed at 0 h. [a,b]Means within each time are different ($P < 0.05$).

3.2. Ovulation response

The total percentage of cows that ovulated to each GnRH product is shown in Table 3. There was an effect ($P < 0.01$) of treatment, BCS, and DIM on percentage of cows ovulating to GnRH treatment. Logistic regression analysis indicated that Factrel had a lower percentage of cows that ovulated compared with the other three GnRH products. There was no significant difference between the percentage of cows that ovulated to Cystorelin, Fertagyl, or Ovacyst (Table 3).

There was no effect of month ($P = 0.68$) and no interaction of month by treatment ($P = 0.67$) on

Table 1
Effect of 50 μg of various types of gonadorelin on LH secretion in cattle

Treatment	Number of cows	Time to LH peak (h)	LH peak (ng/mL)	Area* under the curve of LH profile (ng^2)
Cystorelin	5	0.9 ± 0.3	10.4 ± 2.3	22.6 ± 6.6
Factrel	5	0.8 ± 0.3	9.6 ± 1.5	19.9 ± 4.2^B
Fertagyl	5	0.9 ± 0.3	11.9 ± 2.3	24.9 ± 6.9
Ovacyst	5	0.9 ± 0.3	14.3 ± 2.1	37.3 ± 7.2^A

^{A,B}Means within a column with different superscripts tended to be different (P = 0.10).
* Calculated by the trapezoidal method.

Table 2
Effect of 100 μg of various types of gonadorelin on LH secretion in cattle

Treatment	Number of cows	Time to LH peak (h)	LH peak (ng/mL)	Area* under the curve of LH profile (ng^2)
Cystorelin	10	1.1 ± 0.2	23.1 ± 7.8	52.5 ± 7.3
Factrel	10	1.0 ± 0.2	21.6 ± 3.6	39.9 ± 4.5
Fertagyl	10	1.2 ± 0.3	22.2 ± 3.9	45.2 ± 6.4
Ovacyst	10	1.1 ± 0.2	25.3 ± 6.9	49.2 ± 10.4

* Calculated by the trapezoidal method.

Table 3
Effect of 100 μg of various types of gonadorelin on ovulation after GnRH treatment on Day 7 in cattle

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.

percentage of cows that ovulated to GnRH. There was also no effect of batch on percentage of cows that ovulated to each GnRH product. Cows treated with either batch of Factrel were found to have the numerically lowest ovulation response that was observed in the experiment. In addition, there was no effect of batch within treatment (P = 0.68) in ovulation response.

Body condition score had an effect (P < 0.01) on ovulation response (Fig. 2). This effect of BCS was independent of GnRH product as indicated by the significant effect of BCS but lack of significant interaction between BCS and treatment (P = 0.58). Unexpectedly, cows with higher BCS (≥3.25) had the lowest ovulation response (60% ovulated), whereas cows with the lowest BCS had the numerically highest ovulation response (83%). This was also observed, at least numerically (Fig. 2), in ovulation data for each individual GnRH product at higher BCS (always the lowest ovulation response for each GnRH) and lower BCS (always the highest ovulation response for each GnRH).

There was no effect (P = 0.84) of follicle size on Day 7 on the percentage of cows that ovulated to GnRH (Fig. 3). In all follicle size categories, cows treated with Factrel had the lowest ovulation response to GnRH treatment (see Fig. 3). However, there was no overall treatment by follicle size interaction (P = 0.51).

There was no effect of parity (P = 0.25) on ovulation response to GnRH (Fig. 4). Nevertheless, there was a numerically lower ovulation response to each GnRH product in primiparous cows than in multiparous cows, with overall ovulation in 69% of primiparous cows and 75% of multiparous cows. There was an effect of GnRH product within each parity class, but no overall interaction of treatment by parity (P = 0.93) on ovulation response was found.

4. Discussion

This study tested whether the four gonadorelin products that are available in the United States differ in efficacy. The most important result related to gonadorelin efficacy is shown in Table 3. It is clear that Factrel had a much lower efficacy for induction of ovulation than that of the other three gonadorelin products. This reduced efficacy from Factrel was consistent, whether data were analyzed by batch, month of treatment, BCS, size of dominant follicle at the time of GnRH treatment, or parity of cows (Figs. 2–4). This reduction in ovulation efficacy by Factrel was consistent with the results of a previous study [16], although number of

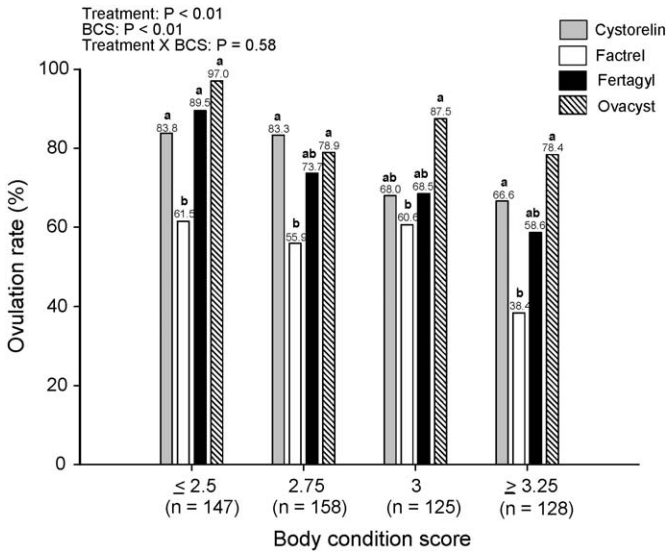

Fig. 2. Percentage of dairy cows that ovulated to each GnRH product after separation by BCS at the time of GnRH treatment. [a,b]Comparisons made within each BCS class; different letters between columns are different ($P < 0.05$).

cows evaluated for ovulation in that study was insufficient to provide reliable data. In this study, a relatively large number of cows were evaluated (>100 cows per treatment group) allowing valid assessment of induction of ovulation by GnRH products. Further, the person doing the ultrasound evaluations (used to determine eligibility for the study and to determine ovulation outcome) did not have information on treatment group of cow at the time of the examinations. Thus, it was striking that the ovulation efficiency of Factrel was found to be more than 20 percentile points lower than the average ovulation results from the other products (78.4% vs. 55.3%).

In spite of these dramatic differences in ovulation efficiency, there were no extraordinary differences in circulating LH profiles detected after treatment with the various gonadorelin products. There were some differences in area under the curve and circulating LH concentrations at 1 and 2 h after treatment when the lower dose of GnRH was used, but not with the prescribed dose (100 μg) used to evaluate ovulation efficacy. In contrast with our results, a previous study [16], working with dry cows (Experiment 2), reported differences in peak LH and mean LH concentrations after treatment with Cystorelin versus Factrel or Fertagyl. Also, Martínez et al. [16], working with beef

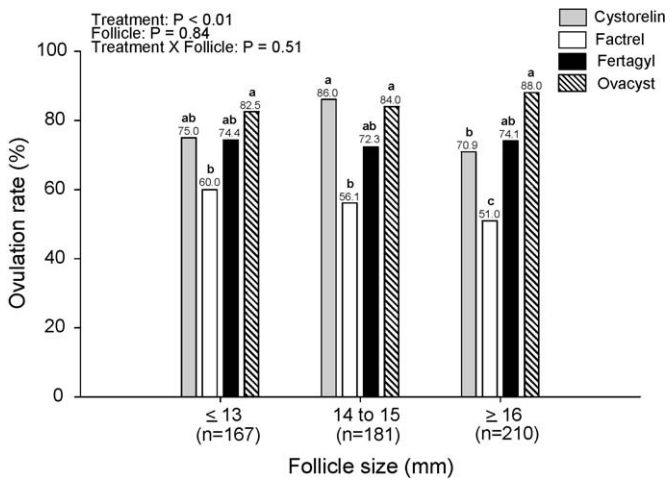

Fig. 3. Percentage of dairy cows that ovulated to each GnRH product after separation of cows by size of the largest ovarian follicle at time of GnRH treatment (Day 7). [a–c]Comparisons made within each follicle size class; different letters between columns are different ($P < 0.05$).

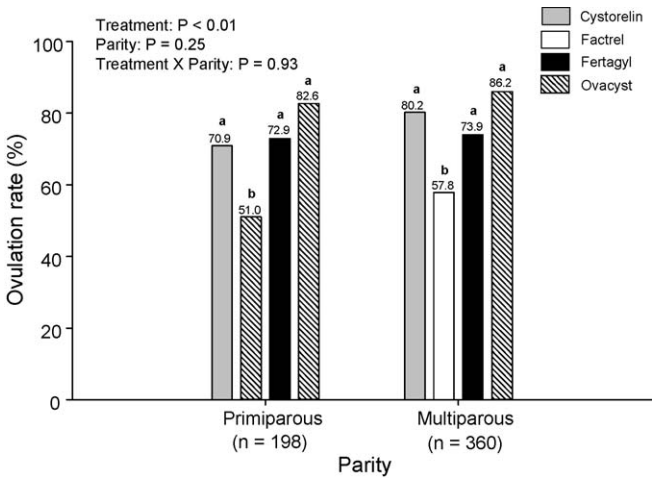

Fig. 4. Percentage of cows that ovulated to each GnRH product arranged by parity. [a,b]Comparisons made within each parity class; different letters between columns are different ($P < 0.05$).

heifers (Experiment 3), reported greater mean LH concentrations 1.5 h after GnRH treatments in heifers receiving Cystorelin compared with that in heifers receiving Fertagyl and Factrel. A study in sheep also found that Factrel induced lower secretion of LH than did either Cystorelin or Fertagyl [15]. In our study, Factrel was numerically lower than all other products for area under the LH curve at a dose of either 50 or 100 μg, but these differences did not reach statistical significance. Thus, although our study did not detect dramatically lower LH release from Factrel than from the other gonadorelin products, it seems likely that decreased LH secretion was the underlying biological basis for the lower ovulation efficiency of Factrel. Perhaps there were certain subsets of cows with less GnRH responsiveness that may have been present in the larger ovulation study but not in the smaller LH profile study.

There are some differences in chemistry and diluents among the GnRH products. Although Cystorelin, Fertagyl, and Ovacyst contain gonadorelin diacetate tetrahydrate, Factrel uses a different GnRH salt, gonadorelin hydrochloride. There are also some differences in the amount of benzyl alcohol (Cystorelin = 9 mg; Factrel = 2 mg; Fertagyl = 9 mg; Ovacyst = 9 mg) and sodium chloride (Cystorelin = 7.47 mg; Factrel = 6 mg; Fertagyl = 7.47 mg; Ovacyst = 7.48 mg) used per milliliter of the final solution in these GnRH products. These differences may be related to differences in ovulation efficacy or circulating LH profiles observed in this study and in previous studies. However, batch differences cannot be ruled out, as only two batches of each GnRH product were used in this study.

Combining all GnRH treatment groups, there were only 73% of cows that ovulated after GnRH treatment, with none of the treatment groups approaching 100%. This is surprising, because all cows used in the trial should have been at Day 7 of the estrous cycle with a dominant follicle (>10 mm) present on the ovary. Previous studies suggest that 100 μg GnRH treatment of beef cows produced much lower peak LH concentrations during the luteal phase (<4 ng/mL) than during the follicular phase (>12 ng/mL), suggesting a role for progesterone and/or estrogen in GnRH-induced LH secretion [15]. Similarly, a recent report has also found a large effect of circulating progesterone concentrations on LH release in response to GnRH in crossbred heifers [19]. Thus, GnRH treatment on Day 7 of the estrous cycle may, at times, require greater doses of GnRH to produce ovulatory concentrations of LH, possibly due to increased circulating progesterone. This is important, as 100 μg Factrel may produce sufficient LH concentrations during the follicular phase, even though it may not be sufficient during the luteal phase. In addition, increasing the dose of GnRH from 50 μg to 100 μg, regardless of GnRH treatment, dramatically increased LH peak (200%; from 11.55 to 21.05 ng/mL) and area under the LH curve (178%), indicating that a maximally effective dose of GnRH had not been reached by 50 μg. There is also evidence that doses > 100 μg GnRH continue to increase circulating LH (Souza AH and Wiltbank MC, unpublished observations), indicating that the prescribed dose was still not a maximally effective dose on Day 7 of the cycle. A previous study suggested that use of 50 μg

rather than 100 μg GnRH in the Ovsynch protocol did not decrease fertility [25]. However, a lower ovulation rate was observed when cows were treated on Day 7 with 50 μg rather than 100 μg GnRH (Sartori R and Wiltbank MC, unpublished results using Cystorelin). Ovulation to the first GnRH treatment of Ovsynch is associated with increased fertility [26]. Thus, to improve fertility during the Ovsynch protocol, it seems important to maximize ovulation rate to the first GnRH treatment of Ovsynch. Consideration of all these results seems to encourage use of a higher dose of GnRH, rather than a lower dose, at the first GnRH treatment of Ovsynch. Nevertheless, the findings that there was no decrease in fertility after implementation of Ovsynch with a half-dose of Cystorelin [25] or with Factrel rather than Cystorelin [15] potentially indicate that reduced efficacy of the first GnRH treatment of Ovsynch may not be critical for fertility with this protocol. In summary, it is not possible at this time to extrapolate these results on ovulation efficacy with different GnRH products or doses to differences in fertility.

Interestingly, regardless of treatment group, cows with greater BCS had a lower ovulation response. As might be expected, there was a good correlation between BCS and DIM ($r = 0.26$; $P < 0.01$), with BCS increases corresponding with increases in DIM (BCS $\leq$ 2.5, DIM = 132.7 $\pm$ 5.3; BCS = 2.75, DIM = 138.1 $\pm$ 5.5; BCS = 3.0, DIM = 143.7 $\pm$ 6.4; BCS $\geq$ 3.25, DIM = 198.3 $\pm$ 9.6). Thus, later DIM and not BCS alone may be responsible for the decreased ovulation efficiency. Cows later in lactation will (on average) have lower milk production, and this may increase circulating progesterone concentrations [27,28]. Greater circulating progesterone may decrease GnRH-induced LH secretion [15,19], producing the decreasing ovulation efficiency. This speculation may again be consistent with greater doses of GnRH being needed to maximize ovulation when cows are treated with GnRH during the luteal phase.

In conclusion, lactating cows treated during the luteal phase had decreased ovulation response to Factrel compared with that to Cystorelin, Fertagyl, or Ovacyst. These treatment differences did not appear to be related to batch, month, BCS, size of dominant follicle, or parity.

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