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Effect of gonadorelin, lecirelin, and buserelin on LH surge, ovulation, and progesterone in cattle

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ABSTRACT

Analogs of gonadoliberin (GnRH) are widely used in cattle to synchronize estrus and to induce ovulation, as well as for the treatment of ovarian cysts. The aim of this study was to compare the plasma profiles of LH and progesterone and the follicular dynamics in response to the administration of gonadorelin, lecirelin, or buserelin at the dose recommended to induce ovulation. In addition, the biological response to a half dose of lecirelin was assessed. Twelve healthy Holstein female cows were divided into four sequence groups, according to a Latin square design and received the four treatments during the four periods of the study. Before each period, the estrous cycle was synchronized, and on Day 6 or 7 of the ensuing cycle, the time at which it was most likely to have a dominant follicle, 100 μg of gonadorelin, 25 μg of lecirelin, 50 μg of lecirelin, or 10 μg of buserelin was administered to the cows. Blood samples were regularly collected for up to 4 days after the GnRH administrations. The plasma LH response was evaluated for up to 6 hours after administration, and the plasma progesterone response and ovarian follicular dynamics were evaluated for up to 4 days. There was a significantly lower LH release after gonadorelin treatment compared to lecirelin at the doses of 25 or 50 μg and the buserelin treatment. The mean maximal LH concentration after gonadorelin treatment was 2.5 lower than after lecirelin or buserelin treatment and was reached 1 hour earlier. Four days after the GnRH administration (i.e., at Days 10–11 of the estrous cycle), the overall mean increase in plasma progesterone concentration was 70% and did not differ between the treatment groups. The percentage of disappearance of the dominant follicle (84.8% of ovulation and 4.3% of luteinization) after GnRH treatment was high (73%, 82%, 100%, and 100%, for gonadorelin, lecirelin at the doses of 25 and 50 μg, and buserelin, respectively) and did not differ between the GnRH treatments. The follicle disappearance was followed by the emergence of a synchronous follicle wave within 2 days in almost all the heifers. Altogether, our data show that the three GnRH analogs, at the doses indicated for the induction of ovulation or at a half dose for lecirelin, are almost equally effective to induce the disappearance of the dominant follicle at Day 6 to 7 of the estrous cycle.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide produced by GnRH neurons in the basal hypothalamus. It stimulates the synthesis and secretion of FSH and of LH from the anterior pituitary.

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In veterinary medicine, natural GnRH and different GnRH agonists (The term GnRH used hereafter in this article includes gonadorelin and the commercially available analogs lecirelin and buserelin.) are widely used in the therapy of bovine cystic ovarian disease or to induce ovulation at the time of insemination [1,2]. In addition, GnRH analogs are currently extensively used in dairy cattle to control follicular dynamics and ovulation in fixed timed insemination protocols (e.g., Ovsynch) with most dairy herds in North America, Australia, and New Zealand.

The design of GnRH agonists has been directed toward stabilization of the molecule and increasing the affinity of the agonist for the GnRH receptor [3]. In particular, the replacement of the carboxy-terminal glycinamide terminus with alkyl amines has produced nonapeptides with a prolonged duration of action combined with increased potency [3].

Some of the commercially available GnRH analogs and agonists currently approved and marketed for use in cattle in Europe and North America are as follows: native GnRH (gonadorelin diacetate or diacetate tetrahydrate) and two nonapeptides used as the acetate, lecirelin and buserelin. Lecirelin differs by substitution of D-tertiary leucine for glycine at position 6 and the replacement of glycine by ethylamide at position 10. Buserelin has a D-serine at position 6 and ethylamide at position 10.

Numerous studies have compared the effect of various doses and/or types of GnRH analogs in cattle [4–10]. However to our knowledge, no *in vivo* study has evaluated the effects of lecirelin and compared them to those of gonadorelin and buserelin.

For any of the clinical or management uses of GnRH analogs, the key biological responses to evaluate efficacy of GnRH products are an adequate LH surge, the disappearance of the dominant follicle, and the formation of an accessory CL. However, the size and the functional state of the dominant follicle at the time of GnRH administration and the phase of the estrous cycle may be critical determinants of the response to GnRH treatment [11,12]. In addition, the steroid environment is known to influence the magnitude and duration of the GnRH-induced LH release [13]. Furthermore, in ovulation synchronization program in cattle, it was reported that response to Ovsynch differed according to the stage of the estrous cycle at which the cow received the first GnRH treatment [14]. Gonadotropin-releasing hormone treatment initiated on Days 5 to 9 of the estrous cycle resulted in ovulation in almost all cows and in high circulating progesterone (P4) levels at the time of the PGF2α treatment, 7 days later. These data provided the physiological basis for presynchronization strategy that attempted to maximize the number of cows at a more optimal stage of the estrous cycle (Days 5–9) at the first GnRH administration.

To reduce the variability associated with these physiological factors, our clinical trial evaluated the biological response to the GnRH products on Day 6 or 7 of the cycle when medium circulating P4 concentrations and the dominant follicle of the first follicular wave should be present in nearly all heifers [15].

The aim of this study was to compare the biological activity, in terms of LH and P4 responses and follicular dynamics, of three different GnRH analogs, gonadorelin, lecirelin, and buserelin, that are commercially available in Europe. These GnRH products were administered intramuscularly at the doses recommended for the induction of ovulation. In addition, for lecirelin, the biological response to a half dose of 25 μg was assessed. The secretion of LH in response to GnRH and the dynamics of the ovarian dominant follicle, i.e., its disappearance or its luteinization, were evaluated during the luteal phase of the estrous cycle. Plasma P4 levels were also assessed to evaluate if the formation of the accessory CL induced by the GnRH analogs was associated with increased levels of P4.

2. Materials and methods

2.1. Animals

All animal procedures were carried out in accordance with accepted standards of humane animal care under agreement number 31-1155545 from the French Ministry of Agriculture and were approved by the national ethics committee. Twelve Holstein heifers aged between 17 and 24 months, weighing 326 ± 28 kg at the beginning of the study and showing normal estrous cyclicity, were included in the trial. Animals were individually identified with ear tags and were fed with about 3.5 kg/head/day of an 18% protein commercial concentrate, 5 kg/animal/day of hay, and straw *ad libitum*. In addition, during the period between the treatments, the heifers were grazed in a meadow.

2.2. Experimental design

Each heifer was assigned randomly to one of the four treatment sequences (three heifers by treatment sequence) according to an equilibrated orthogonal Latin square design for four periods. The four groups of three heifers received all four GnRH treatments during the four periods but in a different order. Before the first experimental period, estrus was synchronized in the heifers by a P4 device (PRID Delta; CEVA Santé Animale, Libourne, France) associated with 150-μg d-cloprostenol (Reprostenol; Vetoquinol France) and 400-UI eCG (Synchro-Part PMSG, CEVA Santé Animale). The starting date of the animal phase (Day 0) was the day of the behavioral estrus as detected by frequent dedicated observations. Six or 7 days after the detection of estrus, the heifers received a GnRH administration. Seven days after the GnRH administration, i.e., on Day 13 to 14 of the previous estrous cycle, the heifers were given intramuscular 150-μg d-cloprostenol (Reprostenol; Vetoquinol France—MAH and Manufacturing Company, Fatro, Italy) to induce luteolysis. The subsequent period of the orthogonal Latin square began at Day 0 corresponding to a new estrus.

For each period, the cows were submitted to a follow-up including ovarian examinations and the determination of the time course of plasma P4 and LH concentrations.

2.3. Drug administration

The GnRH products to be tested were administered as a single intramuscular injection in the neck area, during the

luteal phase, at Day 6 (seven heifers) or 7 (38 heifers). Gonadorelin diacetate tetrahydrate (Cystoreline; CEVA Santé Animale) was administered at a dose of 100 μg per animal, corresponding to 2 mL of solution. Lecirelin acetate (Reproreline; Vetoquinol France—MAH and Manufacturing Company, Fatro) was administered at the doses of 25 and 50 μg per animal, corresponding to 1 and 2 mL of solution, respectively. The dose of buserelin acetate (Receptal; MSD, France) was 10 μg per animal, corresponding to 2.5 mL of solution.

2.4. Blood sampling

Blood samples were collected from the coccygeal vessel or the jugular vein into heparinized vacutainer tubes. Blood samples for LH determination were obtained just before the GnRH administration and at 15 minutes, 30 minutes, 1, 2, 3, 4, and 6 hours after the GnRH administration. Blood samples for P4 determination were obtained just before the GnRH administration and at Days 1, 2, 3, and 4 after the GnRH administration.

Blood samples were immediately chilled in ice and centrifuged (3000 × *g*, 10 minutes) within 2 hours of collection. Plasma samples were stored at −20 °C until assayed.

2.5. Clinical follow-up of cows

A transrectal ultrasound examination of the ovaries was carried out on a daily basis starting 24 hours before the administration of GnRH and every day until 4 days after the GnRH administration. Ovarian ultrasonography was performed using a real-time B-mode scanner (MyLab One, Hospimedi) equipped with a 10-MHz linear-array rectal transducer. For each examination, the location of the largest ovarian structures (CL and follicle) and the diameter of the largest follicle were recorded. A large follicle 10 mm or greater in diameter observed the day before GnRH administration was considered morphologically to be a dominant follicle. The day of the emergence of a new follicular wave was defined by the small follicles growing above 4 to 5 mm [16].

One heifer in the sequence 4 was not treated with gonadorelin during period 2 because it did not show estrus after cloprostenol treatment. One heifer, for which the diameter of the dominant follicle was 7.8 mm at the time of the lecirelin treatment at the dose of 25 μg, was excluded from the analysis of the data for this fourth period.

2.6. Analytical assays

The plasma LH concentrations were measured by a commercial immunoassay (LH Detect; ReproPharm). The mean intra-assay and interassay coefficients of variation for three quality control pools were respectively lower than 14.4% and 16.4%. The limit of quantification was validated at 0.6 ng/mL.

The plasma P4 concentrations were determined by a commercial immunoassay (Ovucheck Plasma; AES Laboratoire). The mean intra-assay and interassay coefficients of variation of three quality control pools were lower than 17.1%, 11.5%, and 9.9%, and the limit of quantification was validated at 1 ng/mL.

2.7. Data analysis

Descriptive statistics were used, and data are presented as the mean (±standard deviation [SD]).

The LH concentrations below the limit of quantification of 0.6 ng/mL were arbitrarily fixed at 0.3 ng/mL. The LH response to GnRH administration was assessed by calculating the area under the LH concentration curve ($AUC_{LH0\text{-}6h}$) by the trapezoidal method from time 0 (time of GnRH injection) to 6 hours post-GnRH administration. No correction was made to account for the basal LH concentration. C_{maxLH} and T_{maxLH} for LH were determined directly from the raw data.

To remove some within- and between-animal variability in P4 secretion, the P4 plasma concentrations obtained after the administration of GnRH were corrected by removing the plasma concentration at time 0 (before GnRH administration). The P4 response to GnRH administrations was assessed by calculating the area under the corrected plasma P4 curve ($AUC_{P4D0\text{-}D4}$) by the trapezoidal method from time 0 (time of GnRH injection) to Day-4 post-GnRH administration. C_{maxP4} and T_{maxP4} for P4 were determined directly from the raw data.

2.8. Statistical analysis

The statistical analyses were performed on Systat 12.0 (SPSS, Inc.). To check the comparability of the treatment groups at time 0, just before the GnRH administration, the diameter of the largest follicle and the plasma P4 and LH concentrations were analyzed by a two-way ANOVA, with sequence, treatment and their interaction as fixed-effect factors and the cows nested within sequence as a random-effect factor. Data transformation ($\log_{10}$) was used for the analysis of LH and P4 concentrations.

The $AUC_{LH0\text{-}6h}$, C_{maxLH}, and T_{maxLH} in response to the different GnRH analogs were analyzed by a two-way ANOVA, with sequence, treatment and their interaction as fixed-effect factors and the cows nested within sequence as a random-effect factor, followed if necessary by a *post hoc* test of Tukey. Data transformation ($\log_{10}$) was used for the analysis of $AUC_{LH0\text{-}6h}$, C_{maxLH}, and T_{maxLH} to obtain variance homogeneity and normality.

The parameters of the P4 response to GnRH ($AUC_{P4D0\text{-}D4}$, C_{maxP4}, and T_{maxP4}) were analyzed by a two-way ANOVA, with sequence, treatment, and their interaction as fixed-effect factors and the cows nested within sequence as a random-effect factor. Because of heterogeneity of variance among groups, $AUC_{P4D0\text{-}D4}$, C_{maxP4}, and T_{maxP4} were log transformed before analysis.

The day of the emergence of the follicular wave was compared using a two-way ANOVA, with sequence, treatment, and their interaction as fixed-effect factors. The percentages of cows that ovulated or for which the dominant follicle disappeared (ovulation or luteinization) were compared by a logistic regression with sequence, treatment, and their interaction as fixed-effect factors.

3. Results

3.1. Plasma LH concentrations

The mean basal plasma LH concentration observed before each GnRH treatment was low (overall mean ± SD, 0.92 ± 1.00 ng/mL) and did not differ as a function of the GnRH analogs or of sequence (ANOVA, not significant [NS]). The plasma LH concentrations versus time after an intramuscular administration of gonadorelin, lecirelin at doses of 25 and 50 μg, and buserelin in one representative heifer are shown in Figure 1.

A peak plasma LH concentration was clearly identified by 1 to 2 hours post-GnRH and, after a delay of 6 hours, the plasma concentrations returned to control values for all four GnRH treatments. The pharmacodynamic parameters describing the LH response to GnRH are given in Table 1. For $AUC_{LH0\text{-}6h}$ and C_{maxLH}, there was a significant effect of the interaction between sequence and treatment (ANOVA, $P = 0.006$ and 0.037, respectively). The mean $AUC_{LH0\text{-}6h}$ after a gonadorelin treatment (18.2 ± 8.20 ng. h/mL) was lower than the values obtained after lecirelin administration at the doses of 25 and 50 μg (49.7 ± 19.3 and 50.5 ± 16.3 ng.h/mL, respectively) or after buserelin treatment (47.7 ± 11.1 ng.h/mL), but this effect was significant only for three sequences (ANOVA, *post hoc* Tukey test, $P < 0.02$) probably because of the reduced number of heifers in sequence group 4.

The mean C_{maxLH} was 2.5 lower after a gonadorelin treatment (6.92 ± 2.72 ng/mL) than after lecirelin at the doses of 25 and 50 μg or buserelin treatments (16.9 ± 7.63, 17.9 ± 5.86, and 16.4 ± 5.70 ng.h/mL, respectively). However, this effect was significant or tended to be significant only for two or three sequence groups (for lecirelin at the dose of 25 μg: $P = 0.097$, 0.001, and 0.031; for buserelin: $P = 0.06$, 0.007, and 0.002 for the first three sequences, respectively and for lecirelin at the dose of 50 μg: $P = 0.012$ and $P < 0.001$ for the first and third sequence groups, ANOVA, *post hoc* Tukey test). The maximal LH concentration was attained 1 hour earlier for the gonadorelin treatment compared to lecirelin and buserelin treatments (ANOVA, *post hoc* Tukey test, $P < 0.001$).

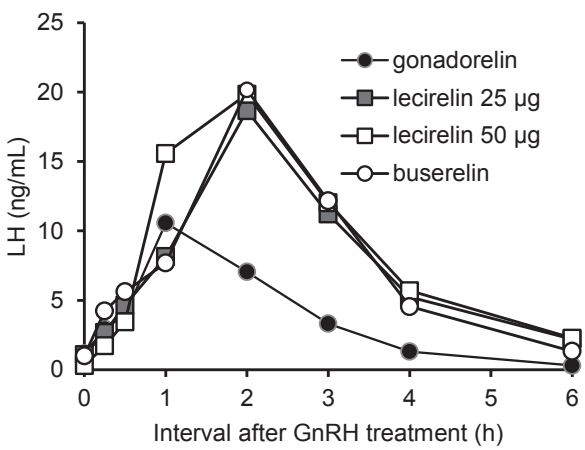

Fig. 1. Time course of plasma LH concentrations (ng/mL) after an intramuscular administration of gonadorelin (100 μg), lecirelin at a dose of 25 and 50 μg, and buserelin (10 μg) in a representative heifer on Days 6 or 7 after estrus.

3.2. Plasma progesterone concentrations

Figure 2 shows the mean (±SD) plasma P4 concentration (ng/mL) versus time after an intramuscular administration of gonadorelin, lecirelin at the dose of 25 and 50 μg, and of buserelin in 12 cows. The overall mean plasma P4 concentration before the GnRH administration was 5.21 ± 1.50 ng/mL, and the mean plasma P4 concentration observed before the gonadorelin administration was slightly higher (ANOVA, *post hoc* Tukey test, $P = 0.05$) than that before the lecirelin administration at the dose of 50 μg (5.95 ± 1.50 vs. 4.53 ± 1.18 ng/mL). This difference was corrected by calculating P4 plasma concentrations obtained after the administration of GnRH minus the value of plasma P4 level obtained before the administration of the different GnRH analogs.

Four days after the GnRH administration (i.e. at Day 10–11 of the cycle), the circulating P4 concentrations were high (8.34 ± 1.94 ng/mL, 4.81–14.5 ng/mL). The overall mean magnitude of the increase of plasma P4 concentration was 3.13 ± 1.71 ng/mL and was rather variable between the periods or the cows (range, 0.54–8.81 ng/mL). The three basic pharmacodynamic parameters describing the P4 response to GnRH, i.e., $AUC_{P4D0\text{-}D4}$, C_{maxP4}, and T_{maxP4} were not affected by the sequence, the GnRH treatment, or the interaction between sequence and treatment (ANOVA, NS, Table 2).

There was no relationship between the P4 response ($AUC_{P4D0\text{-}D4}$) and LH exposure ($AUC_{LH0\text{-}6h}$) after the GnRH administration.

3.3. Follicular dynamics

On Day 0 of each period, corresponding to Day 6 to 7 of the estrous cycle, all the heifers had a CL and a dominant follicle with a diameter of 10 mm or greater (mean ± SD, 12.90 ± 1.33 mm) and the size of the dominant follicle was not different in function of the GnRH treatments or of sequence (ANOVA, NS).

The overall percentage of disappearance or luteinization of the dominant follicle within 48 to 72 hours after the GnRH treatment was high, 89%, and did not differ between the GnRH treatments (72.7% after gonadorelin, 81.8% after lecirelin at the dose of 25 μg, and 100% after lecirelin at the dose of 50 μg and buserelin, logit test, NS). Whatever the GnRH products, the ovulation occurred within 23 to 54 hours after GnRH administration for 85% of dominant follicles. It was followed by the emergence of a new follicular wave 1 to 4 days after the GnRH treatment (mean ± SD, 2.10 ± 0.58 days). An accessory CL was detected in almost all females between 2 or 4 days after the GnRH administration.

In the gonadorelin group, five dominant follicles did not ovulate, two of them showed a peripheral luteinization which did not prevent the emergence of a new follicle wave, suggesting that they had lost their dominance. Two cows that had received 25 μg of lecirelin presented a

Table 1
Pharmacodynamic parameters (mean ± standard deviation, range), $AUC_{LH0\text{-}6h}$ of LH concentrations (ng.h/mL), maximum plasma LH concentrations (C_{maxLH}, ng/mL), and time corresponding to the maximum plasma LH concentrations (T_{maxLH}, hours post-GnRH) after an intramuscular administration of gonadorelin (100 μg), lecirelin at a dose of 25 and 50 μg, and buserelin (10 μg) in 12 heifers.

LH response	GnRH treatment			
	Gonadorelin (n = 11)	Lecirelin, 25 μg (n = 11)	Lecirelin, 50 μg (n = 12)	Buserelin (n = 12)
$AUC_{LH0\text{-}6h}$ (ng.h/mL)	18.2 ± 8.20[a] (5.42–31.1)	49.7 ± 19.3[b] (24.5–92.5)	50.5 ± 16.3[b] (19.6–79.6)	47.7 ± 11.1[b] (33.3–69.4)
C_{maxLH} (ng/mL)	6.92 ± 2.72[a] (2.10–11.2)	16.9 ± 7.63[b] (6.12–32.2)	17.9 ± 5.86[b] (7.26–27.0)	16.4 ± 5.70[b] (7.91–24.7)
T_{maxLH} (h)	1.00 ± 0.55[a] (0.5–2)	2.09 ± 0.30[b] (2–3)	2.00 ± 0.00[b] (2–2)	2.25 ± 0.45[b] (2–3)

For each LH parameter, means without common superscripts were significantly different or approached significance (ANOVA, *post hoc* Tukey test). For $AUC_{LH0\text{-}6h}$ and C_{maxLH}, there is a significant effect of the interaction between sequence and treatment, and the effect of treatment was significant or approached significance for two or three sequences.

persistent follicle, one of them had two dominant follicles, one of which ovulated, and the other remained persistent. Yet again, this persistent follicle did not prevent the emergence of a new follicle wave.

4. Discussion

The present experiment shows that although the nonapeptides, buserelin and lecirelin, induced a greater stimulatory effect on LH secretion than gonadorelin treatment, all the GnRH analogs tested were equally effective at inducing the disappearance of the dominant follicle present at the time of treatment, followed by the emergence of a new follicular wave in cattle.

In our experimental design, the GnRH response was evaluated under standardized conditions, i.e., in cyclic Holstein heifers receiving the four modalities of GnRH treatments at Day 6 to 7 of the estrous cycle. Under these conditions, we showed that gonadorelin and analogs of GnRH (lecirelin and buserelin) at doses indicated to induce ovulation, or at a half dose for lecirelin, induced a rapid increase of LH concentrations, which declined within 6 hours. Irrespective of the analogs tested, the LH response

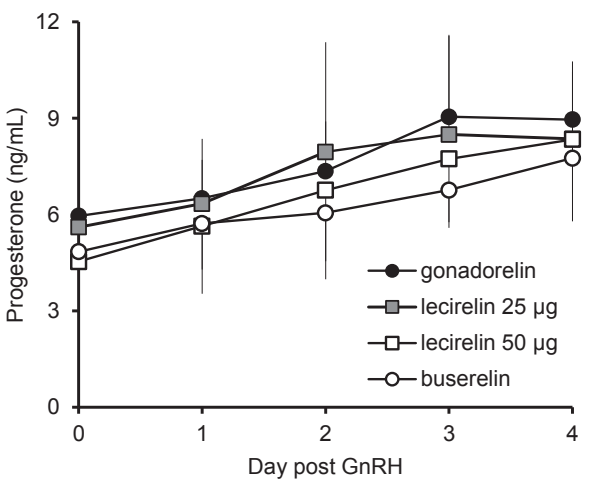

Fig. 2. Time course of mean (±standard deviation, ng/mL) plasma progesterone concentrations after an intramuscular administration of gonadorelin (100 μg), lecirelin at a dose of 25 and 50 μg, and buserelin (10 μg) in 12 heifers. Gonadotropin-releasing hormone was administered on Day 6 or 7 of the cycle.

lasted for only 6 hours, i.e., about half the duration of a natural preovulatory surge. These results are in agreement with those of Lucy and Stevenson [17] who compared, in the same trial, the spontaneous LH surge in cattle and the LH release induced by 100 μg of gonadorelin administered 72 hours after a prostaglandin treatment and showed that the duration of the LH surge in GnRH-induced animals (6.1 ± 0.8 hours) was significantly shorter than the spontaneous LH surge (11.0 ± 0.7 hours). However, under the conditions of this former investigation, it appeared that the maximum plasma LH concentration attained after gonadorelin treatment was comparable to that determined during a preovulatory surge. In our trial, the maximum plasma LH concentration in response to nonapeptide agonists (overall mean for buserelin and lecirelin, 17.1 ± 6.25 ng/mL) was greater than in gonadorelin treatment (6.92 ± 2.72 ng/mL). These results are consistent with the results obtained by Chenault et al. [6] and Nawito et al. [4] and could be explained by the prolonged duration of action of the nonapeptides compared to gonadorelin. Furthermore, the mean maximum plasma LH concentration predicted by a pharmacokinetic or pharmacodynamic model for a standard dose of gonadorelin of 100 μg was around 7 ng/mL [18] and was similar to that obtained in our study. Moreover, the estimated dose of GnRH required to achieve half the maximum possible stimulating effect on LH release was estimated at 203 μg [18]. Altogether, these data show that the maximum response of LH was not reached after an administration of gonadorelin at the dose of 100 μg during the luteal phase, as also previously observed in cyclic dairy cattle [4,19] or in suckled beef cattle [20].

The magnitude of the GnRH-induced LH release is modulated by the steroid environment. Indeed, the LH response to GnRH treatment was lower in heifers with high P4 concentrations (around 7 ng/mL) than those with low P4 levels (2 ng/mL) [12,21]. By contrast, as previously reported by Webb et al [20], we did not observe any relationship between P4 levels before GnRH administration and the LH response (data not shown). However, in our study, the luteal phase P4 concentrations observed before GnRH treatment varied between 2.01 and 8.32 ng/mL and were relatively high (mean ± SD, 5.21 ± 1.50 ng/mL).

Although the LH response to GnRH differed between the groups treated with gonadorelin and with nonapeptide analogs, the GnRH treatments were equally effective in inducing ovulation. The percentage of disappearance of the

Table 2
Pharmacodynamic parameters (mean ± standard deviation, range), $AUC_{P4D0\text{-}D4}$ (ng.h/mL) and C_{maxP4} (ng/mL) of corrected plasma progesterone concentrations and T_{maxP4} (hours post-GnRH) after an intramuscular administration of gonadorelin (100 μg), lecirelin at a dose of 25 and 50 μg, and buserelin (10 μg) in 12 heifers.

Progesterone response	GnRH treatment			
	Gonadorelin (n = 11)	Lecirelin, 25 μg (n = 11)	Lecirelin, 50 μg (n = 12)	Buserelin (n = 12)
$AUC_{P4D0\text{-}D4}$	6.54 ± 2.66 (2.73–11.1)	7.33 ± 6.61 (−2.09–21.7)	8.43 ± 4.82 (1.01–19.3)	5.46 ± 3.49 (−1.74–11.2)
C_{maxP4}	3.87 ± 1.54 (1.53–7.28)	3.96 ± 2.34 (0.83–9.08)	4.42 ± 2.18 (1.58–8.81)	3.12 ± 1.50 (1.38–7.03)
T_{maxP4}	3.45 ± 0.69 (2–4)	3.36 ± 0.67 (2–4)	3.50 ± 0.67 (2–4)	3.67 ± 0.65 (2–4)

The three pharmacodynamic parameters describing the progesterone response to GnRH were not affected by the GnRH treatment, the sequence group, or the interaction between sequence and treatment (ANOVA, $P > 0.05$).

dominant follicle of 89% observed in our experiment was consistent with previous studies reporting a high rate of formation of an additional CL after GnRH given on Days 5 to 7 of the estrous cycle (buserelin: 93% [22], 100% [23]; gonadorelin: 90% [7], 95% [8], 77% [10]). The removal of the suppressive effect of the dominant follicle in GnRH-treated cows by way of induced ovulation or luteinization creates a permissive environment, thus allowing a new follicular cohort to emerge 2 days after the treatment. Despite the limited number of animals, our data suggest that the GnRH analogs, at the doses selected, were able to trigger a sufficient LH response to guarantee the disappearance of the dominant follicle in most cows and the emergence of a new follicle wave.

Recent studies have shown that in the Ovsynch protocol, P4 concentrations at the time of the PGF2α-induced luteolysis (7 days after the first GnRH) had a substantial impact on the probability of pregnancy in dairy cows [24]. In our study, the plasma P4 concentrations increased by 70%, 4 days after the administration of GnRH (Day 11 of the estrous cycle), and were not different between the treatments. However, the possible difference in increase of P4 concentrations between GnRH treatments may have been masked by the large variations in P4 measured among cyclic heifers. Moreover, no correlation was observed between LH concentrations after GnRH treatment and subsequent luteal activity. However, during the cycle, the plasma P4 concentrations reached their maximal values between Days 11 and 16 [25,26] and the percentage of the P4 increase per day was of 68.6% on Days 3 to 6 and 9.4% on Days 6 to 11 [25]. In our study, a high P4 increase (17.5% per day) was observed between Days 7 and 11 of the estrous cycle. Despite the absence of the control group, these data suggest that the increase in P4 concentrations could be attributed at least partly to the GnRH effect. In the literature, the effect of increased GnRH-induced LH release at diestrus phase on P4 concentrations remained unclear. Twagiramungu et al. [27] did not detect any increase in plasma P4 during the 6 days after formation of an induced CL by administration of buserelin on Day 7 of a synchronized estrous cycle. By contrast, other studies reported that luteal function was enhanced from Days 11 to 16 (i.e., 6–11 days after GnRH injection) in cattle treated with buserelin on Day 5 of the estrous cycle [22,28]. In addition, the average P4 levels 7 days after a GnRH treatment were significantly higher in lactating cows with a Day-13 CL and an additional Day-7 accessory CL than those in the control cows with a Day-13 CL (5.22 vs. 3.53 ng/mL) [24]. In our study, the circulating P4 concentrations were not measured beyond 4 days after GnRH, but the plasma P4 concentrations were high (8.34 ± 1.94 ng/mL, 4.81–14.5 ng/mL) 4 days after GnRH treatment, suggesting that the increase in plasma P4 after the GnRH administration could be attributed, at least in part, to the formation of an accessory CL as a result of the acute increase of LH after administration of GnRH and/or to the hypertrophy of the luteal cells in the spontaneous CL [27,29].

In conclusion, the present experiment shows that buserelin and lecirelin induced a greater stimulatory effect on LH secretion than gonadorelin treatment. However, our data suggest that the GnRH analogs, at the posology indicated for the induction of ovulation or the half dose for lecirelin, were able to trigger a sufficient LH response to guarantee the disappearance of the dominant follicle in most cows and the emergence of a new follicle wave. In the Ovsynch protocol using mainly gonadorelin, fertirelin, and buserelin [30], the coordination of a functional dominant follicle at the time of PGF2α and subsequent final GnRH was a key determinant for a successful synchronization outcome. Our data suggest that lecirelin, even at the half dose, could be recommended in an ovulation synchronization program for cattle to synchronize the emergence of a new follicular cohort and that a presynchronization of estrus could maximize the chance of having a dominant follicle at the GnRH administration. More studies are needed to evaluate the efficacy of this protocol in terms of ovulation and fertility in both heifers and cows in field conditions.

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