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—Original Article—

Progesterone Profiles in the Caudal Vena Cava and Jugular Vein in Response to Pulsatile Luteinizing Hormone Stimulation Induced by GnRH Treatment During the Mid-luteal Phase in Lactating Dairy Cows

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Abstract. The aim of this study was to examine whether increased frequency of luteinizing hormone (LH) pulses influences luteal progesterone (P_4) secretion by measuring progesterone concentrations at the secreted (caudal vena cava) and circulating levels (jugular vein) in lactating dairy cows. Cows received six intravenous administrations of 2.5 μg of GnRH (gonadorelin acetate, n=4) or 2 ml saline (n=3) at 1-h intervals on 12.4 ± 0.4 (mean ± SE) days after ovulation. Blood samples were collected from the caudal vena cava and jugular vein every 12 min for 12 h (6 h before and after treatment). During the 6 h after treatment, frequency of LH pulses (5.3 ± 0.3 and 3.0 ± 0.0 pulses/6 h) and mean LH concentration (0.50 ± 0.06 and 0.38 ± 0.05 ng/ml) were greater ($P<0.05$) in GnRH-treated cows than in saline-treated cows. Mean P_4 concentration and amplitude of P_4 pulses in the caudal vena cava during the 6 h after treatment were greater ($P<0.05$) in GnRH-treated cows than in saline-treated cows, but the frequency of P_4 pulses was not different between the groups. Mean P_4 concentration in the jugular vein during the 6 h after treatment was also higher ($P<0.05$) in GnRH-treated cows than in saline-treated cows (7.0 ± 1.3 and 5.4 ± 0.9 ng/ml). These results indicate that the increased frequency of LH pulses stimulates progesterone secretion from the functional corpus luteum and brings about higher P_4 concentrations in the circulating blood in lactating dairy cows.
Key words: Corpus luteum, Dairy cow, GnRH, LH pulse, Progesterone

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Luteinizing hormone (LH) is released in a pulsatile manner from the anterior pituitary gland during the estrous cycle. It is widely accepted that pulsatile LH secretion is necessary for both structural and functional development of the corpus luteum (CL) [1–3]. We recently reported that lactating dairy cows had a greater frequency of LH pulses, higher progesterone (P_4) concentrations in the circulating blood and greater CL size during the mid-luteal phase than non-lactating cows [4]. A plausible interpretation of our findings is that the increased frequency of LH pulses in lactating dairy cows could enhance P_4 secretion by the CL. However, it became experimentally important to determine the physiological role of the LH pulses in the regulation of circulating P_4 levels in lactating cows. When the CL is fully developed, the subsequent role of LH pulses remains to be fully determined. According to previous reports, pulsatile stimulation of LH in the fully functional CL has been considered not to be essential [2] or even of minor importance [2, 5, 6]. Peters *et al.* [2] reported that blockade of pulsatile LH release by treatment with a GnRH antagonist from days 12 through 17 of the estrous cycle did not influence the circulating P_4 concentrations. In contrast, a recent study showed that blockade of pulsatile LH release by treatment with a GnRH antagonist during the mid-luteal phase in heifers decreased the circulating P_4 concentrations as well as pulsatile P_4 release by the CL [7].

Therefore, the present study investigated the direct relationship between frequency of LH pulses and luteal P_4 secretion in lactating dairy cows. For this purpose, low-dose pulsatile injections of GnRH were employed in order to induce pulsatile release of LH of physiological magnitude in cows [8]. To assess the secretion patterns of P_4, blood samples were collected at a site close to the ovary (in the caudal vena cava) in addition to the jugular vein. This sampling procedure has been utilized in several studies [9–11], in which distinct pulsatile patterns of ovarian steroids were described.

Materials and Methods

Animals

Five lactating Holstein dairy cows (5.1 ± 2.1 [mean ± SE] years of age; 95.1 ± 12.8 days postpartum; 680 ± 22.4 kg body weights; 27.7 ± 2.1 kg daily milk yield) maintained at the dairy farm of the Tokyo University of Agriculture and Technology were used in this study. They were housed in a free-stall barn, milked twice daily at 0900 and 1700 h and fed after each milking. The cows were provided a total mixed ration (TMR) that consisted of Sudan grass and alfalfa hay, corn silage, cottonseeds and concentrate mixture, according to Japanese feeding standards for dairy cattle [12]. The cows were clinically healthy and confirmed to have normal genital tracts and estrous cycles before the experiment. All experiments were conducted after approval by the University Committee for the Use and Care of Animals of Tokyo University of Agriculture and Technology (No. 23–88).

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Experimental procedure

Prior to the experiment, ovaries were monitored by rectal palpation and transrectal ultrasonography every other day or daily to determine the time of spontaneous ovulation (day 0). Thereafter, the cows were subjected to the experiment, and ovaries were monitored every other day from day 0 to 14 and then daily until the following ovulation. The cows were treated with GnRH (GnRH group; n=4) or saline (saline group; n=3) during the experiment. Two of three cows in the saline group were assigned to the GnRH group after they underwent at least one untreated estrous cycle before the GnRH-treatment cycle. Transrectal ultrasonography was performed using a B-mode scanner (HS-101V Ultrasonic Scanner, Honda Electronics, Aichi, Japan). Follicular and luteal diameters were measured using three cross-sectional images with maximal areas.

The cows were catheterized in the caudal vena cava via the coccygeal vein and jugular vein on one day during the mid-luteal phase (day 10, 11, 12 or 13). Catheterization into the caudal vena cava was conducted according to the method of Norman and Fields [13] with some modifications [14]. On the day after catheterization (on day 12.4 ± 0.4), the cows were treated six times with GnRH (2.5 μg of gonadorelin acetate; LH-RH injection, Tanabe Seiyaku, Osaka, Japan) in 2 ml of sterile 0.9% saline or 2 ml of saline at 1-h intervals via the jugular catheter, beginning at 1100 h. The dose of GnRH was determined in a preliminary experiment with reference to some previous studies [8, 15, 16] to induce a pulsatile release of LH of similar amplitude to that of spontaneous LH pulses during the mid-luteal phase in cows. Blood samples (6 ml) for LH and P_4 determinations were collected from the caudal vena cava and jugular vein at 12-min intervals for 12 h (0500 to 1700 h) (6 h before treatment and 6 h after treatment). Additionally, blood samples (10 ml) for P_4 determination were collected by jugular venipuncture every other day from day 0 to 14 and then daily during the cycle. The blood samples were collected in heparinized test tubes placed on ice, and centrifuged immediately at 1,750×*g* for 20 min at 4 C. Plasma was separated and stored at –20 C until assay. To exclude the possible influence of feed intake on parameters measured during the frequent blood sampling, residual feed was removed before the beginning of blood sampling, and 25% of the daily amount of TMR was provided at 3 h and 9 h after the beginning of blood sampling (0800 and 1400 h).

Hormone assays

Plasma P_4 concentration was measured by EIA after diethyl ether extraction [17]. The intra- and interassay coefficients of variation were 3.4% and 7.4%, and the sensitivity was 0.03 ng/ml. Plasma LH concentration was measured by RIA as described previously [18]. The intra- and interassay coefficients of variation were 6.1% and 11.9%, and the sensitivity was 0.09 ng/ml.

Statistical analyses

In one cow in the GnRH group, blood samples could not be obtained from the caudal vena cava, so the other obtained samples were used for data analysis. Data for LH and P_4 profiles were evaluated by repeated measures ANOVA using the GLM procedure of SPSS software version 20.0 for Windows. The ANOVA model included the fixed effects of treatment group (GnRH, saline) and period (before treatment, after treatment) and their interaction. When a significant difference was detected, differences between groups within each period or between periods within each group were analyzed by Tukey's post hoc follow-up test or the Student's *t*-test. Pulsatile patterns of LH in the jugular vein and P_4 in the caudal vena cava and jugular vein were analyzed using the cluster analysis program of Veldhuis and Johnson [19], and characteristics of pulsatile pattern are described in terms of mean concentration, pulse frequency, pulse amplitude and basal concentrations (after subtraction of the pulses). The cluster algorithm searched for significant increases and decreases among data points in a series via pooled *t*-tests, with 2 points being used for the determination of a peak and 1 point being used to establish a nadir. The temporal relationships between LH pulses in the jugular vein and P_4 pulses in the caudal vena cava and jugular vein were determined according to the method described by Rhodes *et al*. [11], in which the proportion of P_4 pulses that followed a LH pulse within 60 min of the peak of the LH pulse was calculated. Differences between groups were tested using Fisher's exact test. Measured values are presented as means ± SE. P values less than 0.05 are considered to be significant.

Results

There was no significant difference ($P \geq 0.05$) between the GnRH and saline groups in the length of the estrous cycle subjected to the experiment (22.5 ± 1.3 and 22.3 ± 0.7 days) or in the mean CL diameter during the mid-luteal phase of days 8 to 14 (24.2 ± 1.0 and 24.8 ± 0.3 mm). The day of luteolysis, as determined by the decrease in CL diameter and P_4 concentration [4], did not differ significantly between the GnRH and saline groups (day 17.3 ± 0.9 and day 17.5 ± 1.2).

Representative profiles of LH in the jugular vein and P_4 in the caudal vena cava for cows treated with GnRH or saline are shown in Fig. 1. Hourly injections of GnRH induced 5.3 ± 0.3 LH pulses during the 6 h after treatment (Table 1), which was greater ($P<0.05$) than those during the 6 h before treatment (3.0 ± 0.4 pulses/6 h) and in the saline group (3.0 ± 0.0 pulses/6 h). The peak of an LH pulse was detected within 12 or 24 min (mean, 18.6 ± 1.4 min) after each GnRH injection, and the amplitude of LH pulses during the GnRH treatment was similar to that in the saline group.

For P_4 profiles in the caudal vena cava, although the frequency of P_4 pulses was not changed by GnRH treatment, amplitude of P_4 pulses and mean P_4 concentrations were increased ($P<0.05$) by GnRH treatment (Table 2). Basal P_4 concentrations in the GnRH group were greater ($P<0.05$) during the 6 h after treatment than during the 6 h before treatment, but did not differ significantly from those in the saline group.

For P_4 profiles in the jugular vein, mean P_4 concentrations during the 6 h after treatment in the GnRH group were increased by GnRH treatment (Table 2). However, pulse frequency, pulse amplitude and basal concentrations of P_4 in the jugular vein were not changed by GnRH treatment. Furthermore, P_4 concentrations in the jugular vein at 24, 48 and 72 h after treatment did not differ significantly between the GnRH and saline groups (mean of the three samples, 6.6 ± 0.5 and 6.2 ± 0.4 ng/ml).

The proportions of P_4 pulses that followed an LH pulse within

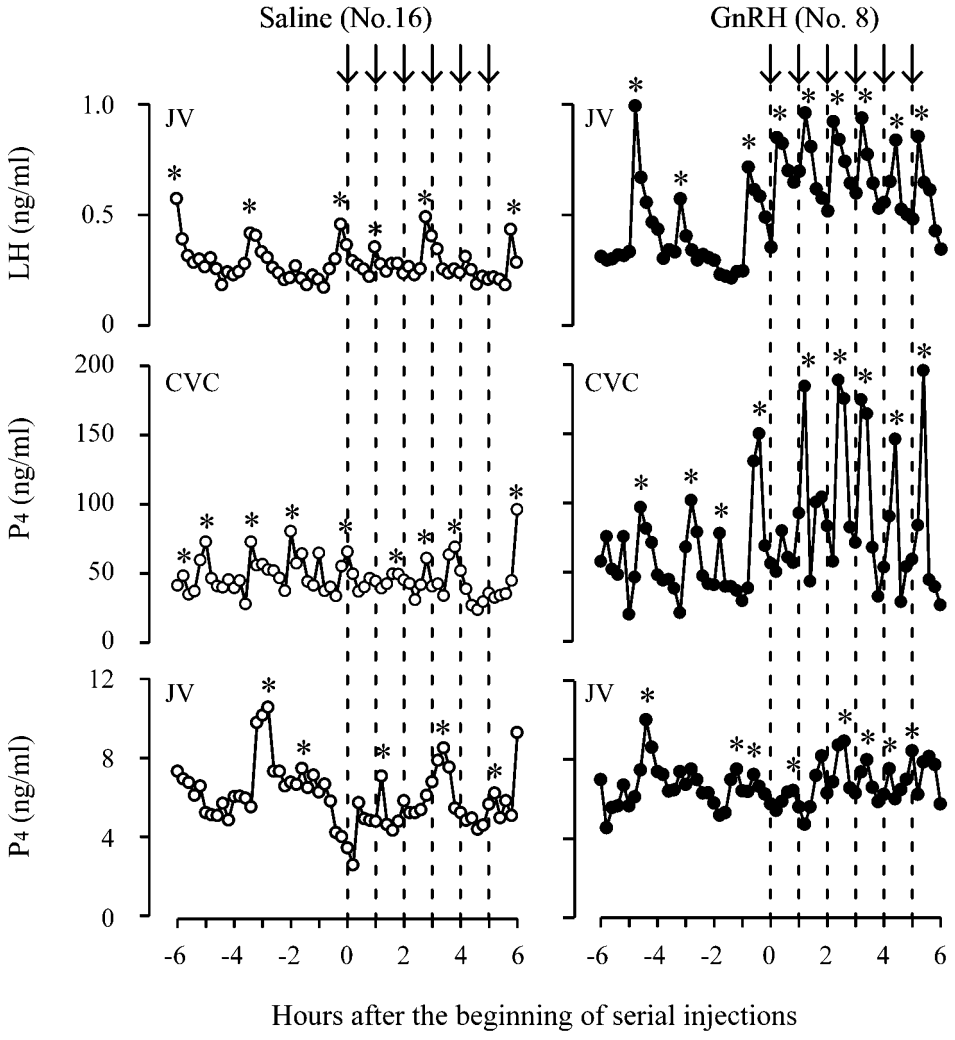

Fig. 1. Representative profiles of LH in the jugular vein (JV) and progesterone (P_4) in the caudal vena cava (CVC) and JV for cows treated with 2 ml of saline or 2.5 μg of GnRH intravenously six times at 1-h intervals on a single day during the mid-luteal phase (on day 11 after ovulation in both cows). An asterisk indicates the peak of an LH or P_4 pulse. Arrows and dotted lines indicate times of saline or GnRH injections.

Table 1. Pulsatile patterns of LH in the jugular vein for cows treated with saline or GnRH during the mid-luteal phase

LH characteristic	Group	6 h before treatment	6 h after treatment
Mean concentration (ng/ml)	Saline (n=3)	0.38 ± 0.05	0.38 ± 0.05
	GnRH (n=4)	0.32 ± 0.03	0.50 ± 0.06*†
Basal concentration (ng/ml)	Saline (n=3)	0.31 ± 0.01	0.33 ± 0.01
	GnRH (n=4)	0.23 ± 0.02	0.38 ± 0.03†
Pulse frequency (pulses/6 h)	Saline (n=3)	4.0 ± 0.6	3.0 ± 0.0
	GnRH (n=4)	3.0 ± 0.4	5.3 ± 0.3*†
Pulse amplitude (ng/ml)	Saline (n=3)	0.31 ± 0.05	0.44 ± 0.07
	GnRH (n=4)	0.29 ± 0.04	0.30 ± 0.06

Values are means ± SE. * Different ($P<0.05$) *vs.* saline group. † Different ($P<0.05$) *vs.* 6 h before treatment.

Table 2. Pulsatile patterns of progesterone (P_4) in the caudal vena cava (CVC) and jugular vein (JV) for cows treated with saline or GnRH during the mid-luteal phase

Veins	P_4 characteristic	Group	6 h before treatment	6 h after treatment
CVC	Mean concentration (ng/ml)	Saline (n=3)	46.6 ± 6.3	45.3 ± 2.6
		GnRH (n=3)	48.3 ± 9.7	74.8 ± 17.2*†
	Basal concentration (ng/ml)	Saline (n=3)	34.8 ± 2.7	31.3 ± 2.5
		GnRH (n=3)	29.1 ± 3.2	43.6 ± 6.0†
	Pulse frequency (pulses/6 h)	Saline (n=3)	5.3 ± 0.3	3.3 ± 0.3
		GnRH (n=3)	4.3 ± 0.8	4.3 ± 0.3
	Pulse amplitude (ng/ml)	Saline (n=3)	31.7 ± 4.5	47.3 ± 9.8
		GnRH (n=3)	51.8 ± 9.4	97.7 ± 16.9*†
JV	Mean concentration (ng/ml)	Saline (n=3)	5.7 ± 1.2	5.4 ± 0.9
		GnRH (n=4)	6.0 ± 1.0	7.0 ± 1.3*†
	Basal concentration (ng/ml)	Saline (n=3)	4.3 ± 0.9	4.3 ± 0.5
		GnRH (n=4)	4.7 ± 0.3	5.0 ± 0.4
	Pulse frequency (pulses/6 h)	Saline (n=3)	4.7 ± 1.5	3.3 ± 0.3
		GnRH (n=4)	3.5 ± 0.5	4.0 ± 0.4
	Pulse amplitude (ng/ml)	Saline (n=3)	3.6 ± 0.3	3.1 ± 0.3
		GnRH (n=4)	3.0 ± 0.3	3.6 ± 1.2

Values are means ± SE. * Different (P<0.05) *vs.* saline group. † Different (P<0.05) *vs.* 6 h before treatment.

Table 3. Association of LH pulses with progesterone (P_4) pulses in the caudal vena cava (CVC) and jugular vein (JV) for cows treated with saline or GnRH during the mid-luteal phase

Veins	Characteristic	Group	6 h before treatment	6 h after treatment
CVC	LH-associated P_4 pulses [a] (%)	Saline (n=3)	11/16 [b] (68.8)	7/10 (70.0)
		GnRH (n=3)	8/13 (61.5)	12/13 (92.3†)
	LH peak to P_4 peak [c] (min)	Saline (n=3)	12.0 ± 2.8	16.0 ± 5.1
		GnRH (n=3)	7.5 ± 3.9	11.0 ± 3.8
JV	LH-associated P_4 pulses (%)	Saline (n=3)	9/14 (64.3)	6/10 (60.0)
		GnRH (n=4)	9/14 (64.3)	13/16 (81.3)
	LH peak to P_4 peak (min)	Saline (n=3)	18.7 ± 4.5	30.0 ± 5.1
		GnRH (n=4)	14.7 ± 3.3	18.5 ± 3.7

[a] P_4 pulses that followed an LH pulse within 60 min of the peak of the LH pulse. [b] P_4 pulses that followed an LH pulse within 60 min of the peak of the LH pulse / total number of P_4 pulses (%). [c] Intervals from the peak of an LH pulse to the peak of a P_4 pulse (mean ± SE). † Different (P<0.05) *vs.* 6 h before treatment.

60 min of the peak of the LH pulse (i.e., LH-associated P_4 pulses) were calculated in the blood samples collected from the caudal vena cava and jugular vein (Table 3). In the caudal vena cava, the proportion of LH-associated P_4 pulses during the 6 h after treatment in the GnRH group (12/13; 92.3%) was not different from that in the saline group (7/10; 70.0%) but was greater (P<0.05) than that during the 6 h before treatment in the GnRH group (8/13; 61.5%). The interval from the peak of an LH pulse to the peak of a P_4 pulse was similar between the GnRH and saline groups. In the jugular vein, the proportion of LH-associated P_4 pulses and interval from the peak of an LH pulse to the peak of a P_4 pulse were not changed by GnRH or saline treatment. Data of all cows in the saline and GnRH groups were combined and compared between the caudal vena cava and jugular vein in terms of these observations. Of 52 pulses of P_4 detected in the caudal vena cava in all cows examined (three cows in the saline group and three cows in the GnRH group), 73.1% (38/52) were associated with LH pulses. Regarding P_4 pulses in the jugular vein, of 54 pulses in all cows examined (three cows in the saline group and four cows in the GnRH group), 68.5% (37/54) were associated with LH pulses, and no significant difference was detected compared with that in the caudal vena cava. However, the interval from the peak of an LH pulse to the peak of a P_4 pulse in the jugular vein was longer than that in the caudal vena cava (19.5 ± 2.1 and 11.4 ± 1.9 min, P<0.05).

Discussion

The hourly pulsatile treatment with low-dose GnRH in lactating dairy cows induced pulsatile LH secretion and consequential pulsatile P_4 secretion from the functional CL, as indicated by the increase in plasma P_4 concentrations in both the caudal vena cava and jugular vein. The GnRH treatment would result in the secretion of LH and FSH. The luteotropic role of LH is well established by *in vivo* and *in vitro* studies [20–22]. On the other hand, to our knowledge, the

luteotropic role of FSH has not been reported previously in cattle, although several studies have reported that FSH stimulates P_4 synthesis by hamster luteal cells during the estrous cycle and pregnancy [23] and porcine luteal cells at the early luteal phase [24], and receptors for FSH have been found in the bovine CL [25]. Therefore, the rapid increase in P_4 secretion following GnRH injection is probably due to the direct action of LH rather than FSH. In the present study, the frequency of LH pulses in the GnRH group (around one pulse every 1 h) was approximately double that in the saline group (around one pulse every 2 h) and was comparable to that detected during the early luteal phase in cows [26]. The dose of GnRH (2.5 μg of gonadorelin acetate) in the present study, which were determined in a preliminary experiment with reference to some previous studies [8, 15, 16], induced pulsatile LH secretion that had a similar amplitude to that of spontaneously released LH pulses in the saline-treated cows. The obtained findings suggest that increased frequency of LH pulses promoted blood levels of P_4 in both the caudal vena cava and jugular vein in lactating dairy cows. It supports our interpretation that the higher frequency of LH pulses in lactating dairy cows during the mid-luteal phase than in non-lactating cows [4] could contribute to the increase in P_4 concentrations in the circulating blood.

Catheterization into the caudal vena cava is a relatively simple and effective method for collecting blood containing high concentrations of ovarian hormones before they are metabolized by the liver [10]. A distinct pulsatile pattern of P_4 was detected in the caudal vena cava blood in the present study, which was similar to previous studies [9, 14]. The luteal response to the LH pulses induced by the pulsatile GnRH treatment was well assessed by analyzing the patterns of P_4 pulses in the caudal vena cava blood. The frequency of P_4 pulses was not changed by the pulsatile GnRH treatment, but the proportion of P_4 pulses that occurred in association with LH pulses increased from 61.5% during the 6 h before treatment up to 92.3% during the 6 h after treatment. This indicates that almost all P_4 pulses were released in association with LH pulses during the GnRH-treatment period. Further, the approximately twofold increase in the amplitude of P_4 pulses during the GnRH treatment implies that pulsatile LH stimulation can cause a prominent increase in P_4 secretion from the bovine CL. Taken together, it seems that increased frequency of LH pulses causes an increase in the amplitude of P_4 pulses, which results in a significant increase in the mean P_4 concentration during the GnRH treatment.

Progesterone concentrations in the jugular vein also increased in response to the increased LH pulses, but the increase was smaller than in the caudal vena cava. We calculated that a twofold increase in the frequency of LH pulses led to 1.5- and 1.3-fold increases in the mean P_4 concentration in the caudal vena cava and jugular vein, respectively. The pulse profile of P_4 in the jugular vein showed that the frequency of P_4 pulses in the jugular vein was similar to that in the caudal vena cava, and was not changed by GnRH or saline treatment. In addition, although most P_4 pulses in both the jugular vein and caudal vena cava were found to be associated with LH pulses, the interval from LH peak to P_4 peak was longer in the jugular vein than in the caudal vena cava (19.5 and 11.4 min, respectively). In regard to the pulse amplitude of P_4 in the jugular vein, no significant change was observed during the GnRH treatment, in contrast to the marked increase in the caudal vena cava. Therefore, while pulsatile patterns of P_4 concentration can be seen in both the caudal vena cava and jugular vein, blood samples in the caudal vena cava may be more favorable for examining P_4 changes in the secretion pattern (i.e., pulse amplitude) and identifying the peak position of a pulse. The less clear P_4 pulses and the smaller increase in P_4 concentrations in the jugular vein in response to the increased LH pulses could be explained not only by the peripheral hemodilution of secreted P_4 [8] but also by the elevated metabolic rates of P_4 in lactating dairy cows [27, 28]. Indeed, the P_4 concentrations in the caudal vena cava were approximately tenfold higher than in the jugular vein in the present study and our previous study [14].

Administration of GnRH or its agonists has been shown to enhance luteal P_4 secretion through the release of LH in cattle [29–31], but the effect appears to depend on the dose and timing of treatment during the estrous cycle. It is commonly recognized that the luteotropic effect of LH induced by a single injection of GnRH or its agonist is short term, in which increases in P_4 concentrations in blood are sustained for 0.5–6.0 h after injection of GnRH [21, 30, 32]. Similarly, the present study found that the increased P_4 concentration was not sustained for the remaining luteal phase after the serial GnRH treatment. Additionally, the increased frequency of LH pulses caused by serial GnRH treatment did not influence the CL size and day of luteolysis. The stimulation of LH pulses within a physiological level (frequency and amplitude) during the mid-luteal phase could enhance steroidogenic activity of the CL within a comparable period and might not have long-term effects such as stimulation of CL growth or retarded luteolysis. This assumption is supported by a previous study showing that treatment with a GnRH agonist, azagly-nafarelin, from days 3 to 21 of the estrous cycle increased both the luteal size and plasma P_4 concentration, whereas treatment with GnRH agonist from days 12 to 21 of the estrous cycle increased the plasma P_4 concentration without any alteration in the luteal size compared with those in the controls [31].

In summary, the increased frequency of LH pulses led to increases in P_4 concentration in both the caudal vena cava and jugular vein in lactating dairy cows. Most P_4 pulses in the caudal vena cava and jugular vein occurred in association with LH pulses. Although it cannot be determined from the present study whether LH pulses are essential for the fully developed CL, our results suggest that an increase in the frequency of LH pulses could contribute to the increase in circulating P_4 concentration during the mid-luteal phase in lactating dairy cows.

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