

Changes in Plasma Progesterone Levels in the Caudal Vena Cava and the Jugular Vein and Luteinizing Hormone Secretion Pattern After Feeding in Lactating and Non-lactating Dairy Cows

Natsumi ENDO^{1,2)}, Kiyosuke NAGAI^{1,2)}, Tomomi TANAKA^{1,2)} and Hideo KAMOMAE^{1,2)}

¹⁾Laboratory of Veterinary Reproduction, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan

²⁾United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan

Abstract. The present study was designed to assess progesterone profiles at the secreted (caudal vena cava) and circulating levels (jugular vein) and luteinizing hormone (LH) secretion pattern in lactating and non-lactating cows with reference to feeding. Four lactating and four non-lactating cycling Holstein cows were examined. Blood samples were collected simultaneously from the caudal vena cava (via a catheter inserted from the coccygeal vein) and the jugular vein every 15 min for 12 h (0500–1700 h) during the functional luteal phase. Cows were fed 50% of the daily diet 6 h after the start of blood sampling. During the 12-h sampling period, mean progesterone concentrations in the caudal vena cava did not differ between lactating and non-lactating cows (49.0 ± 2.9 and 53.3 ± 3.7 ng/ml; mean \pm SE), whereas mean progesterone concentrations in the jugular vein in lactating cows were higher than those in non-lactating cows (6.4 ± 0.1 and 5.6 ± 0.1 ng/ml, $P < 0.001$). Lactating cows had a higher frequency of LH pulses than non-lactating cows (7.0 ± 0.7 and 4.3 ± 0.9 pulses/12 h, $P < 0.05$). The influence of feeding was not observed on LH profiles but was observed on progesterone profiles in both veins. Progesterone concentrations in the caudal vena cava increased after feeding in both groups. Progesterone concentrations in the jugular vein decreased after feeding in lactating cows but not in non-lactating cows. These results indicate the difference in feeding-related changes in progesterone dynamics between lactating and non-lactating cows.

Key words: Dairy cow, Feeding, Lactation, Metabolism, Progesterone

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The main function of the corpus luteum (CL) is to synthesize and secrete progesterone, which regulates the estrous cycle length and maintains pregnancy in many species. Low circulating concentrations of progesterone during the luteal phase might reduce fertilization and/or embryo survival rates [1, 2], which can be a cause of low fertility in modern dairy cows. A basic understanding of the mechanisms involved in luteal function and progesterone dynamics is needed to develop strategies to improve fertility of dairy cows.

A recent study demonstrated that lactating cows had higher metabolic rates of steroid hormones than non-lactating cows [3]. Increased metabolic rates of progesterone caused by high milk production have been hypothesized to lower circulating concentrations of progesterone during the estrous cycles in lactating dairy cows [3, 4]. In particular, the effect of progesterone metabolism on circulating progesterone was clearly observed in association with feeding [5–7]. In a study using non-lactating cows that were treated with exogenous progesterone, Rabiee *et al.* [5] showed that there was a negative relationship between level of feed intake and plasma progesterone concentrations. Furthermore, it was reported that there was a significant decrease in circulating concentrations of progesterone during the post-feeding period [6, 7]. These changes appear to be related to the

acute change in liver blood flow and resultant increase in metabolic rates of progesterone [3, 8]. While such feeding-related changes in plasma progesterone concentrations have been well documented in animals given exogenous progesterone treatment, it has not been studied whether a difference in progesterone metabolism between lactating and non-lactating cows could influence the secretion status of progesterone by the CL during the estrous cycle.

During the functional luteal (mid-luteal) phase, progesterone exhibits negative feedback control on the hypothalamus, resulting in a reduced frequency of luteinizing hormone (LH) pulses. However, Vasconcelos *et al.* [6] reported that lactating dairy cows had a higher frequency of LH pulses than non-lactating dairy cows, despite having a mid-luteal level of circulating progesterone comparable to that in non-lactating cows. Our previous study using frequent blood sampling from the jugular vein in lactating and non-lactating cows during the estrous cycles showed that lactating cows had lower progesterone concentrations during the early luteal phase and higher progesterone concentrations during the mid-luteal phase and had a higher frequency of LH pulses during the early to mid-luteal phase than non-lactating cows [9]. Luteinizing hormone stimulates progesterone production by the CL [10], and a close association between LH pulses and secretion of progesterone has been observed during the early and mid-luteal phase in cows [11, 12]. Although the exact mechanism for the increase in LH pulses in lactating dairy cows remains unclear, it is likely that an alteration in LH secretion patterns may be associated with luteal activity of steroidogenesis.

Catheterization at a point naturally close to the ovary, for example,

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Correspondence: H Kamomae (e-mail: kamomae@cc.tuat.ac.jp)

the caudal vena cava, has been utilized to facilitate collection of blood containing high concentrations of ovarian steroid hormones before they are metabolized by the liver [11, 13–15]. Blood samples frequently collected from the caudal vena cava would provide detailed information regarding the secretion status of progesterone from the CL. Furthermore, the progesterone concentration in the circulating blood reflects the balance between secretion and metabolic status of progesterone. This prompted the idea that the metabolic status of progesterone in lactating and non-lactating cows could be inferred from the difference in progesterone concentrations at the secreted level (i.e., in the caudal vena cava) and circulating level (i.e., in the jugular vein).

The objectives of the present study were 1) to assess the progesterone profiles at the secreted level (in the caudal vena cava) and the circulating level (in the jugular vein) in lactating and non-lactating dairy cows and 2) to examine the association between LH pulses in the jugular vein and progesterone secretion patterns in the caudal vena cava, with reference to feeding.

Materials and Methods

Animals

Four lactating (two primiparous and two multiparous; 4.5 ± 1.2 [mean \pm SE] years of age; milk yield of 25.5 ± 1.8 kg/day) and four non-lactating (one primiparous and three multiparous; 6.0 ± 1.1 years of age) Holstein dairy cows maintained at the dairy farm of the Tokyo University of Agriculture and Technology were used in this study. The lactating cows (102.5 ± 7.8 days postpartum) were housed in free stalls with open-air and sheltered areas, milked twice daily at 0900 and 1700 h and fed after each milking. The non-lactating cows (570.3 ± 119.3 days postpartum) were reared in a paddock or tie stalls and spent at least one month after dry off before being subjected to this study. Diets for the lactating and non-lactating cows were formulated according to the Japanese feeding standards for dairy cattle (2006). Lactating cows were provided daily with 23.2 kg per cow of a total mixed ration that consisted of Sudan grass and alfalfa hay, corn silage, cottonseeds and concentrate mixture. The diet contained 73.1% total digestible nutrients, 14.5% CP, 3.0% ether extract and 37.3% neutral detergent fiber on a dry matter basis. Non-lactating cows were provided daily with 7.0 kg of Sudan grass and 3.0 kg of concentrate mixture per cow. The diet was divided into two equal portions and fed at about 0900 and 1700 h. At the beginning of the study, body weights were 637.5 ± 40.4 and 731.5 ± 19.5 kg for lactating and non-lactating cows, respectively ($P=0.10$), and their body condition scores based on a five-point scale [16] were 3.2 ± 0.2 and 3.6 ± 0.2 , respectively ($P=0.11$). The cows were confirmed to be clinically healthy with no genital abnormality and to have normal estrous cycles before the study. All procedures were approved by the University Committee for the Use and Care of Animals of Tokyo University of Agriculture and Technology (No. 23-40).

Experimental procedure

The study was started at the beginning of the cycle (day 0: day of ovulation) and lasted until the end of the cycle, for which subsequent ovulation was confirmed. For the analysis of follicular and luteal development and plasma progesterone, ovarian ultrasonography

and blood sampling were conducted every other day from day 0 to 14 and then daily throughout the cycle. For the analyses of progesterone in the caudal and jugular veins and LH in the jugular vein, catheterization into both veins was conducted on one day during the mid-luteal phase (day 10, 11, 12 or 13). On the day after catheterization, frequent blood sampling was conducted at 15-min intervals for 12 h (0500 to 1700 h). All cows were kept unfed for 6 h before the start of the frequent blood sampling and then fed 50% of the daily amount of diet at 6 h after the start of sampling (1100 h). The remaining 50% of the diet was fed after the end of sampling. Cows were kept in designated pens located next to the free-stall area from the day of catheterization until the end of frequent blood sampling for two days; otherwise, they were managed in the same manner as their herdmates.

Ovarian ultrasonography

Ovarian ultrasonography was performed using a B-mode scanner (Ultrasonic Scanner HS-101V, Honda Electronics, Aichi, Japan) equipped with a 5.0-MHz linear array probe. Measurements of follicles and CLs were used to calculate the mean diameter [17]. Growth rate of the dominant follicle of the first follicular wave was calculated by dividing the total change in diameter of the dominant follicle during the monitoring period by the growth period. Growth period of the dominant follicle was defined as the period between its emergence and the day on which it reached its maximal diameter.

Catheterization procedures

Catheterization into the caudal vena cava was conducted according to the method of Norman and Fields [14]. All procedures were implemented after milking in the morning. Practically, cows were restrained in a treatment stall and sedated with 20 mg of xylazine (Celactal; Bayer, Tokyo, Japan), and epidural anesthesia was achieved by injection of 4.5 ml of 2% lidocaine hydrochloride (Xylocaïne; AstraZeneca, Osaka, Japan). The coccygeal vein was punctured using a 20-gauge needle equipped with a catheter introducer kit (Radifocus® Introducer II H, Terumo, Tokyo, Japan), and a mini-wire (0.64 mm in diameter; 45 cm in length) was passed through the needle and advanced into the vein. The needle was then removed, and a sheath was passed over the mini-wire and placed into the vein. After the mini-wire was removed, a hydrophilic-coated guide wire (Radifocus® guide wire M; 0.46 mm in diameter; 150 cm in length; Terumo, Tokyo, Japan) was threaded into the lumen of the coccygeal vein through the sheath toward the caudal vena cava. Then, a catheter (Optiflash® XL; inside diameter, 1.12 mm; outside diameter, 1.35 mm; 110 cm in length; Terumo) was passed through the coccygeal vein up to the 100 cm length with the wire guide in place. Once the guide wire had been pulled out, nine blood samples (6 ml) were taken every 5 cm from 100 to 60 cm of the catheter length inserted into the vein. After the last blood sample had been taken, the catheter was reinserted up to 100 cm in length and wrapped with the tail using an adhesive bandage. Catheterization into the jugular vein was performed using a commercial catheter kit (14 gauge, 30 cm in length; Medicut Catheter Kit, Nippon Sherwood Medical Industries, Tokyo, Japan). After all procedures were accomplished, cows were released and fed their morning diet at 1100 h. Obtained blood samples were centrifuged at 3,000 rpm for 20 min at 4 °C immediately after

sampling, and plasma was harvested. Plasma concentrations of progesterone for the obtained samples were analyzed by EIA [18], for which the incubation time was shortened for rapid measurement. After the night milking, the catheter was adjusted to the length at which the progesterone concentration in the obtained blood samples showed the highest value.

Blood sampling and assays

Blood samples (10 ml) for progesterone determination were collected by jugular venipuncture into heparinized vacutainers (Venoject II, Terumo) every other day from day 0 to 14 and then daily through the cycle. Blood samples (6 ml) from the catheterized caudal and jugular veins were collected into test tubes containing 10 IU heparin at 15-min intervals for 12 h during the frequent blood sampling period. Plasma was separated by centrifugation immediately after blood collection and stored frozen at -20 C until assay. In addition, for blood chemical analyses, blood samples (10 ml) were collected at the beginning and end of frequent blood sampling (6 h before and 6 h after feeding) into vacutainers for serum separation (Venoject II, Terumo). Blood was allowed to coagulate at room temperature for 1 h and centrifuged at 3,000 rpm for 20 min at 4 C , and serum was stored at -20 C until assay.

Plasma concentration of progesterone was measured by EIA [18]. The intra- and interassay coefficients of variation were 4.3% and 9.7%, and the sensitivity was 0.14 ng/ml. Plasma concentration of LH was measured by RIA [19]. The intra- and interassay coefficients of variation were 5.4% and 8.8%, and the sensitivity was 0.09 ng/ml. Serum concentrations of total cholesterol, BUN, aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (γ -GTP) were analyzed using an automatic analyzer (DRI-CHEM 4000, Fujifilm, Tokyo, Japan). Serum concentrations of glucose and NEFA were analyzed using commercial assay kits according to the manufacturer's instructions (Wako Pure Chemical Industries, Osaka, Japan).

Statistical analyses

To assess data for follicular and luteal diameter, progesterone and LH profiles and blood chemical analysis, two-way ANOVA with repeated measures was used to determine the fixed effects of group and time and their interaction. When significant differences were detected, Tukey's post hoc follow-up test or the Student's *t*-test was used to detect significant differences among groups within time and among time within groups. To assess hourly changes in the area under the curve (AUC) of progesterone relative to feeding for each group, Dunnett's multiple comparison test was used to determine the significance of values in comparison with the value 1 h before feeding.

Pulsatile patterns of LH in the jugular vein and progesterone in the caudal vena cava were analyzed using the cluster analysis program of Veldhuis and Johnson [20] and 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; 2 points were used for the determination of a peak, and 1 point was used to establish a nadir.

Temporal relationships between pulses of LH in the jugular vein and progesterone in the caudal vena cava were determined

according to the method described by Rhodes *et al.* [15], in which the percentage of LH pulses that were followed by a progesterone pulse within 60 min of the peak of the LH pulse was calculated. In addition, the percentage of progesterone pulses that followed an LH pulse within 60 min of the peak of the LH pulse was calculated. Differences between groups (lactating vs. non-lactating) were tested using chi-squared analysis.

Measured values are presented as means \pm SE. *P* values less than 0.05 are considered to be significant, and *P* values between 0.05 and 0.1 are defined as indicating a tendency. Analyses were performed using the statistical software program SPSS for Windows version 20.0.

Results

Luteal development and progesterone concentrations during the estrous cycles

The length of the estrous cycle (interovulatory interval) did not differ between lactating and non-lactating cows (21.5 ± 0.6 and 20.8 ± 0.3 days, $P > 0.10$). Mean diameters of the CL during the mid-luteal phase on days 8–14 were greater ($P < 0.01$) in lactating cows than in non-lactating cows (25.5 ± 0.6 and 23.0 ± 0.2 mm, respectively). Mean concentrations of progesterone during the mid-luteal phase on days 8–14 were higher ($P < 0.05$) in lactating than in non-lactating cows (7.1 ± 0.5 and 5.9 ± 0.2 ng/ml, respectively), although mean concentrations of progesterone during the early luteal phase on days 0–6 did not differ between lactating and non-lactating cows (2.7 ± 0.4 and 2.4 ± 0.4 ng/ml, $P > 0.10$). There was no difference between lactating and non-lactating cows in terms of the growth rate of the first wave dominant follicles (1.2 ± 0.2 and 0.9 ± 0.1 mm/day, $P > 0.10$) and the maximal diameter of the first wave dominant follicles (14.8 ± 1.3 and 14.7 ± 1.0 mm, $P > 0.10$).

Progesterone profiles in the caudal and jugular veins

Representative profiles of progesterone concentrations in the caudal vena cava and in the jugular vein and pulsatile LH secretion in the jugular vein in one lactating (No. 6) and one non-lactating cow (No. 1) are shown in Fig. 1. In addition to LH pulses, a pulsatile pattern of progesterone (asterisks in Fig. 1) was also determined for the blood samples in the caudal vena cava. Progesterone concentrations in the caudal vena cava were ten times higher on average than those in the jugular vein (Table 1). When comparisons were made between groups, mean progesterone concentrations in the caudal vena cava during the 12-h frequent sampling period did not differ between lactating and non-lactating cows (49.0 ± 2.9 and 53.3 ± 3.7 ng/ml, $P > 0.10$). However, in the jugular vein, mean progesterone concentrations during the 12-h sampling period were higher in lactating cows than in non-lactating cows (6.4 ± 0.1 and 5.6 ± 0.1 ng/ml, $P < 0.001$). For progesterone pulses in the caudal vena cava, a total of 26 pulses were detected in all sampling periods of lactating cows. This value was the same as that of non-lactating cows (26 pulses). In addition to the frequency of progesterone pulses (6.5 ± 0.5 and 6.5 ± 0.6 pulses/12 h, $P > 0.10$; Table 1), the basal level and amplitude of progesterone pulses did not differ ($P > 0.10$) between lactating and non-lactating cows.

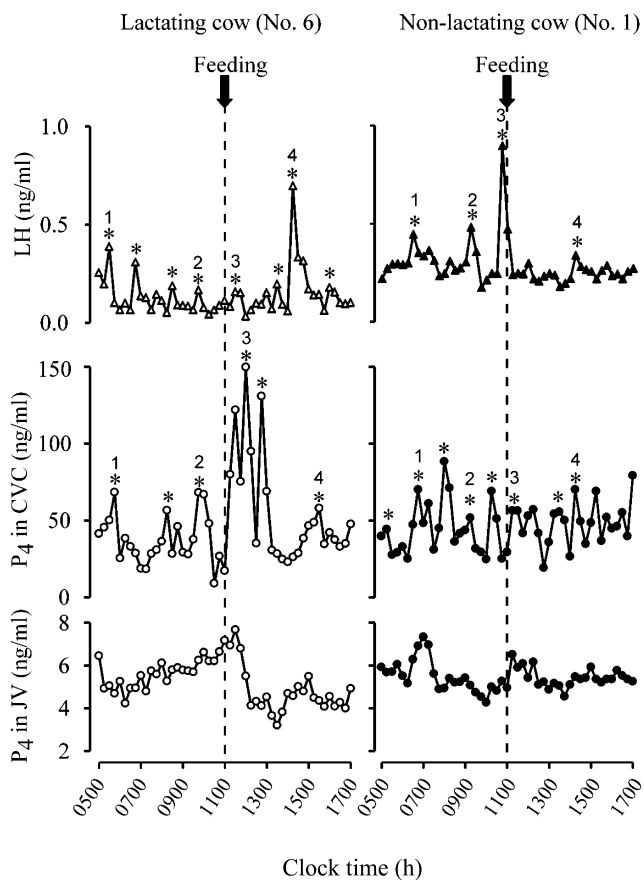


Fig. 1. Representative profiles of progesterone (P_4) concentrations in the caudal vena cava (CVC) and jugular vein (JV) and pulsatile luteinizing hormone (LH) secretion in the JV in one lactating (No. 6) and one non-lactating cow (No. 1). Asterisks indicate peaks of LH or P_4 pulses. LH and P_4 pulses with the same numbers above them are those for which a temporal association was detected. Arrows and dashed lines indicate the time when half of the daily diet was provided.

Luteinizing hormone profile in the jugular veins and its association with progesterone pulses in the caudal vena cava

For LH pulses, the total numbers of pulses detected in the whole sampling periods for the lactating and non-lactating cows were 28 and 17, respectively. The frequency of LH pulses was higher in lactating cows than in non-lactating cows (7.0 ± 0.7 and 4.3 ± 0.9 pulses/12 h, $P < 0.05$; Table 2). The other characteristics for LH secretion such as mean concentration, basal level and amplitude of LH pulses were not different ($P > 0.10$) between the groups. Most of the LH pulses were observed in association with progesterone pulses in the caudal vena cava. During the whole sampling period, the percentages of LH pulses that were followed by a pulse of progesterone were 71.4% (20/28) and 88.2% (15/17) in the lactating and non-lactating cows, respectively, with no significant difference between the groups. Also, the percentages of progesterone pulses that followed an LH pulse did not differ between lactating and non-lactating cows (73.1% (19/26) and 61.5% (16/26), respectively).

Influence of feeding on progesterone and LH profiles

To examine the influence of feeding on progesterone and LH profiles, mean concentration, pulse frequency, pulse amplitude and basal concentrations during the 6-h pre-feeding period were compared with those during the 6-h post-feeding period. In the caudal vena cava, the mean concentration and pulse amplitude of progesterone were higher ($P < 0.05$) during the 6-h post-feeding period than during the 6-h pre-feeding period for both lactating and non-lactating cows (Table 1), but the basal level and pulse frequency did not change significantly. In the jugular vein, however, the influence of feeding on progesterone concentrations differed between lactating and non-lactating cows. In lactating cows, the mean progesterone concentration in the jugular vein was lower ($P < 0.05$) during the 6-h post-feeding period than during the 6-h pre-feeding period. Conversely, in non-lactating cows, the mean progesterone concentration in the jugular vein tended to be higher ($P = 0.054$) during the 6-h post-feeding period than during the 6-h pre-feeding period. Consequently, the mean progesterone concentration during the 6-h

Table 1. Progesterone (P_4) profiles in the caudal vena cava (CVC) and jugular vein (JV) during the whole 12 h and the 6-h pre- and post-feeding sampling periods in lactating (L; $n=4$) and non-lactating (N; $n=4$) cows

| Vein | P_4 characteristic | Group | Whole 12 h | 6 h Pre-feeding | 6 h Post-feeding |
|--------------------------------------|----------------------------|-----------------|-------------------|--------------------|------------------|
| CVC | Mean concentration (ng/ml) | L | 49.0 ± 2.9 | 41.8 ± 2.2^x | 56.5 ± 3.4^y |
| | | N | 53.3 ± 3.7 | 46.1 ± 4.1^x | 60.8 ± 6.1^y |
| | Basal level (ng/ml) | L | 32.8 ± 3.4 | 32.5 ± 4.6 | 33.1 ± 5.1 |
| | | N | 31.1 ± 3.2 | 26.2 ± 2.7 | 36.3 ± 5.7 |
| Pulse frequency (pulses/12 h or 6 h) | L | 6.5 ± 0.5 | 3.0 ± 0.4 | 3.5 ± 0.3 | |
| | N | 6.5 ± 0.6 | 3.3 ± 0.8 | 3.3 ± 0.6 | |
| Pulse amplitude (ng/ml) | L | 57.3 ± 6.5 | 38.3 ± 6.0^x | 67.0 ± 9.8^y | |
| | N | 57.4 ± 14.1 | 38.1 ± 13.1^x | 78.3 ± 24.9^y | |
| JV | Mean concentration (ng/ml) | L | 6.4 ± 0.1^a | 6.7 ± 0.2^{ax} | 6.1 ± 0.2^y |
| | | N | 5.6 ± 0.1^b | $5.4 \pm 0.1^{b†}$ | $5.8 \pm 0.1^†$ |

Values are means and SE. ^{a, b} Different superscripts within a column indicate significant differences ($P < 0.05$) between the L and N groups. ^{x, y} Different superscripts within a row indicate significant differences ($P < 0.05$) between the 6-h pre- and post-feeding periods. [†] There was a tendency ($P = 0.054$) for a difference between the 6-h pre- and post-feeding periods within a row.

Table 2. Luteinizing hormone (LH) profiles in the jugular vein during the whole 12 h and the 6-h pre- and post-feeding sampling periods in lactating (L; n=4) and non-lactating (N; n=4) cows

| LH characteristic | Group | Whole 12 h | 6 h Pre-feeding | 6 h Post-feeding |
|--------------------------------------|-------|------------------------|-----------------|------------------|
| Mean concentration (ng/ml) | L | 0.34 ± 0.07 | 0.32 ± 0.10 | 0.36 ± 0.10 |
| | N | 0.41 ± 0.05 | 0.40 ± 0.09 | 0.42 ± 0.06 |
| Basal level (ng/ml) | L | 0.28 ± 0.07 | 0.26 ± 0.08 | 0.31 ± 0.12 |
| | N | 0.33 ± 0.05 | 0.32 ± 0.07 | 0.33 ± 0.07 |
| Pulse frequency (pulses/12 h or 6 h) | L | 7.0 ± 0.7 ^a | 3.8 ± 0.3 | 3.3 ± 0.5 |
| | N | 4.3 ± 0.9 ^b | 1.8 ± 0.6 | 2.5 ± 1.0 |
| Pulse amplitude (ng/ml) | L | 0.24 ± 0.03 | 0.27 ± 0.06 | 0.21 ± 0.03 |
| | N | 0.37 ± 0.10 | 0.34 ± 0.15 | 0.40 ± 0.14 |

Values are means and SE. ^{a, b} Different superscripts within a column indicate significant differences (P<0.05) between the L and N groups.

post-feeding period did not differ between the groups, although the mean progesterone concentration during the 6-h pre-feeding period was higher (P<0.05) in lactating cows than in non-lactating cows.

Hourly changes in progesterone AUC relative to feeding in the caudal and jugular veins are shown in Fig. 2. In lactating cows, the progesterone AUC in the caudal vena cava was greater (P<0.05) during the period from 0 to 2 h after feeding than the value at 1 h before feeding. Conversely, the progesterone levels in the jugular vein were lower (P<0.05) during the period from 1 to 4 and 5 to 6 h after feeding than the value at 1 h before feeding. In non-lactating cows, no significant differences were detected in the progesterone AUC throughout the sampling period in both caudal and jugular veins compared with the value at 1 h before feeding.

Profiles of LH secretion were not influenced by feeding; pulse frequency, mean concentration, pulse amplitude and basal level of LH did not differ (P>0.10) between the 6-h pre-feeding and 6-h post-feeding periods for both groups (Table 2).

Blood chemical profiles

There was no difference (P>0.10) in the concentrations of any parameters between the samples collected 6 h before and 6 h after feeding for both groups. Therefore, values for the two samples were combined, and the averages are presented (Table 3). A significant difference between lactating and non-lactating cows was observed for the concentrations of total cholesterol (224.5 ± 18.5 and 79.9 ± 3.7 mg/dl, P<0.01). In addition, the AST value was significantly higher in lactating cows than in non-lactating cows (74.6 ± 2.2 and 50.0 ± 2.5 U/l, P<0.01), but all the individual values for both lactating and non-lactating cows (ranges: 65–85 and 40–61 U/l, respectively) were below the suggested reference value, <132 U/l [21]. No significant difference was observed between the groups for the concentrations of glucose, NEFA, BUN and γ-GTP.

Discussion

The lactating cows had greater progesterone concentrations in blood samples collected from the jugular vein on days 8–14 of the estrous cycle and had larger CLs than the non-lactating cows, which is in agreement with our previous study [9]. The frequency of LH pulses was higher in the lactating cows (mean: 7.0 pulses/12 h)

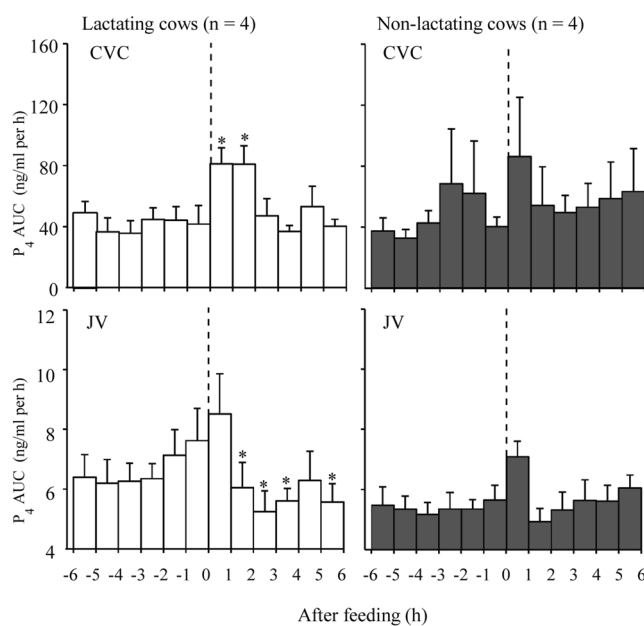


Fig. 2. Changes in area under the curve of progesterone (P₄ AUC) in the caudal vena cava (CVC) and jugular vein (JV) after feeding. Asterisks indicate a difference (P<0.05) compared with the value at -1 to 0 h after feeding. The dashed line indicates the time when half of the daily diet was provided.

Table 3. Blood chemical profiles in lactating and non-lactating cows on the day of frequent blood sampling

| Item | Lactating (n = 4) | Non-lactating (n = 4) |
|---------------------------|---------------------------|-------------------------|
| Glucose (mg/dl) | 67.2 ± 1.5 | 71.4 ± 2.3 |
| NEFA (μEq/l) | 93.1 ± 22.7 | 83.6 ± 12.1 |
| Total cholesterol (mg/dl) | 224.5 ± 18.5 ^c | 79.9 ± 3.7 ^d |
| BUN (mg/dl) | 11.6 ± 1.1 | 10.4 ± 1.3 |
| AST (U/l) ^{a)} | 74.6 ± 2.2 ^c | 50.0 ± 2.5 ^d |
| γ-GTP (U/l) ^{b)} | 33.0 ± 2.5 | 36.4 ± 1.3 |

Values are means and SE. ^{a)} AST: aspartate aminotransferase. ^{b)} γ-GTP: gamma glutamyl transpeptidase. ^{c, d} Different superscripts within a row indicate significant differences (P<0.01).

than in the non-lactating cows (4.3 pulses/12 h) or the previously described observation in non-lactating cows (3.6 pulses/12 h [11]). Peripheral progesterone concentrations are considered as a net result of secretion and metabolism. A possible explanation for the higher circulating concentrations of progesterone in the lactating cows is as follows. It is likely that greater amounts of progesterone were secreted from the CL in the lactating cows than in the non-lactating cows, even though lactating cows have a higher metabolic rate of progesterone than non-lactating cows [3, 6]. The greater CL diameter of the lactating cows seems to be one of the main factors involved in the greater progesterone production, because luteal tissue volume has been found to be correlated with the circulating progesterone concentration in some studies [22, 23].

To assess the secreted level of progesterone in this experiment, catheters were adjusted to the respective length for each cow by measuring progesterone concentrations in vena cava blood collected by 5-cm gradation of the inserted catheter length, as proposed by Benoit and Dailey [13]. The progesterone concentrations in the caudal vena cava blood were approximately ten times higher than those in the jugular blood and sufficiently fulfilled the criteria that blood samples from the caudal vena cava have at least three times higher concentrations of progesterone than peripheral blood [11, 13, 14]. However, we could not detect any difference between the two groups for progesterone profiles from the caudal blood samples. This might be partially due to the wider fluctuations of progesterone concentrations throughout the frequent sampling period in the caudal vena cava than in the jugular vein, the short sampling period and the small number of animals examined in this study.

Besides, although blood collected from the caudal vena cava contained a greater amount of ovarian steroids before they were metabolized by the liver, the hormonal concentration might be influenced by blood flow rate at the sampling point. It is well recognized that the onset of lactation after calving is accompanied by increases in the blood volume, cardiac output, mammary blood flow [24] and blood flow through the gastrointestinal tract and liver [25, 26] in order to provide the udder with nutrients and hormones for regulation of milk synthesis [27]. In addition, feeding causes rapid changes in the blood circulatory system, such as increases in heart rate and blood pressure [28] and in blood flow to the portal and hepatic veins [26, 29]. These changes indicate the possibility that the concentrations of ovarian hormones at the sampling point within the caudal vena cava could vary apparently when changes in blood flow occur. In the present study, feeding 50% of the daily amount of diet caused a significant increase in the progesterone concentrations in the caudal vena cava for both groups. Such a post-feeding increase in progesterone concentrations in the caudal vena cava has been reported in a study on early pregnant gilts, and the authors speculated about the presence of a direct effect of a metabolic mediator (such as insulin) on the ovaries [30]. In our study, a significant increase in the progesterone AUC in the caudal vena cava was found from 0 to 2 h after feeding in the lactating cows. This increase seemed to occur in association with the period when the lactating cows were eating their diets. In contrast, such a significant increase in progesterone AUC was not detected in the non-lactating cows. It is possible that, in addition to differences in feed (amount, energy density or nutrient composition), physiological responses to feeding

such as intake and digestibility of feeds, changes in blood flow to the splanchnic circulation and metabolic and hormonal responses could differ between lactating and non-lactating cows.

Comparison of pulsatile patterns of progesterone in the caudal vena cava between the pre- and post-feeding periods showed that feeding influenced the pulse amplitude but not the basal level and pulse frequency in both groups. These results indicate that the increased pulse amplitude of progesterone could mainly contribute to the increase in mean progesterone concentrations during the post-feeding period. During the frequent sampling period, a high proportion of LH pulses was followed by pulses of progesterone in the caudal vena cava (71.4 and 88.2% in the lactating and non-lactating cows), suggesting a close association between LH pulses and secretion of progesterone [11, 12]. We supposed that the increased frequency of LH pulses in lactating cows would stimulate progesterone secretion by the CL, which could alter progesterone profiles in the caudal vena cava. However, further differences between the groups were not found in the association of LH pulses with the progesterone secretion patterns in the caudal vena cava throughout the pre- and post-feeding periods. Alternatively, we noticed that a low proportion of progesterone pulses occurred in the absence of an LH pulse and that feeding increased the progesterone concentrations in the caudal vena cava without apparent alterations in the LH pulse pattern. While progesterone secretion by small luteal cells is stimulated by LH in a dose-dependent manner [31], more than 80% of the progesterone secreted by the CL is produced by large luteal cells, which seems to be independent of LH stimulation [10]. It is possible that other hormonal or nonhormonal factors in addition to LH may be involved in luteal function in cows. Further studies are required to clarify the mechanisms concerning how feeding causes such a change in progesterone profiles in the caudal vena cava, including the measurement of blood flow at the sampling point.

On the other hand, feeding 50% of the daily amount of diet decreased circulating progesterone concentrations in the lactating cows. This finding concurs with the previous observation that provision of 100 or 50% of the TMR to pregnant lactating cows decreased the circulating progesterone by 1 h after feeding [6]. However, such a decrease was not observed in the non-lactating cows in the present study. In fact, the mean progesterone concentrations in the jugular vein in the non-lactating cows tended to be higher during the 6-h post-feeding period than during the 6-h pre-feeding period, which might reflect the increase in the progesterone concentrations in the caudal vena cava after feeding. It is suggested that high feed intake results in increased liver blood flow and metabolic clearance of progesterone, which decreases the progesterone concentration in the plasma in sheep [8], pigs [32] and cows [3, 4]. Particularly in lactating dairy cows, a continuous high plane of nutrition appears to result in chronic elevation of liver blood flow and metabolic clearance rate of progesterone [3]. Therefore, differences in metabolic clearance rates of progesterone between lactating and non-lactating cows could bring about different profiles of circulating progesterone in response to feeding. In lactating cows, the circulating progesterone concentration may be greatly influenced by the elevated progesterone metabolism, which could negate the post-feeding increase in the progesterone concentration in the caudal vena cava. In non-lactating cows, however, if the metabolic rate of progesterone was increased

after feeding, the effect would have little influence on circulating progesterone concentrations.

Metabolic and nutritional status has major impacts on ovarian function and fertility in dairy cows [33]. In early postpartum cows, negative energy balance resulting from increased milk production can reduce follicular and luteal activity by decreasing IGF-1 concentrations in blood [34, 35]. The lactating cows used in the present study were in the mid-lactation period (102.5 ± 7.8 days postpartum) and maintained their body weights and body condition scores within normal ranges. As a result, no significant difference between lactating and non-lactating cows was observed in blood chemical profiles except for AST and total cholesterol. Although there was a significant difference in AST values among the groups, all of the individual values for both lactating and non-lactating cows were within the normal range [21]. The higher AST values in lactating cows than in non-lactating cows were interpreted not as a pathological condition but rather as an adjustment of liver function to the increased metabolic requirements for milk production [36]. Regarding total cholesterol, a great difference was found between lactating and non-lactating cows (224.5 ± 18.5 and 79.9 ± 3.7 mg/dl). The higher concentrations of total cholesterol in the lactating cows compared with those in the non-lactating cows may reflect greater feed intake and alterations in lipid metabolism to support lactation [37]. From these points, the cows used in the study might not have nutritional problems related to subnormal luteal function. This is also supported by the finding that lactating cows had greater circulating progesterone and greater developed CLs during the mid-luteal phase than non-lactating cows. Therefore, the post-feeding decrease in the circulating progesterone observed only in the lactating cows is likely the result of elevated steroid metabolism associated with lactating status [3, 4]. In addition, previous studies on cattle [38, 39] indicated that dietary energy positively influenced LH pulse frequency and plasma concentrations of LH by affecting the hypothalamic mechanisms that control the release of luteinizing hormone-releasing hormone. This may account for the greater frequency of LH pulses in lactating cows observed in the present and previous studies [6, 9].

In conclusion, lactating cows maintained well-developed CLs, higher progesterone concentrations in the jugular vein and greater frequency of LH pulses during the mid-luteal phase than non-lactating cows. However, progesterone concentrations at the secreted level (in the caudal vena cava) did not differ between lactating and non-lactating cows. Although profiles of LH secretion were not influenced by feeding, progesterone profiles in the caudal vena cava and the jugular vein changed in relation to feeding, and the patterns of these changes differed between lactating and non-lactating cows. Progesterone concentrations in the caudal vena cava increased after feeding in both groups of cows, whereas progesterone concentrations in the jugular vein were decreased in lactating cows but not in non-lactating cows. These results indicate the difference in feeding-related changes in progesterone dynamics between lactating and non-lactating cows.

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