Seasonal Differences in the Parameters of Luteinizing Hormone Release to Exogenous Gonadotropin Releasing Hormone in Prepubertal Holstein Heifers in Sapporo

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Abstract. Stress due to summer heat has adverse effects on reproduction in Holstein dairy cattle. Summer suppression of reproduction of Holsteins can pose an important economic problem, even in Hokkaido prefecture located in the northern region of Japan. Hokkaido is one of the most important dairy farming areas of Japan. This study is an attempt to clarify the seasonal differences in the parameters of luteinizing hormone (LH) response to exogenous gonadotropin releasing hormone (GnRH) in Sapporo, Hokkaido, Japan. A total of 12 prepubertal heifers received an injection with GnRH analogue intramuscularly in either May (n=4, May group), July (n=4, July group), or November (n=4, November group), and serial blood samples were collected to analyze the parameters of the LH response curve after GnRH injection. The parameters were as follows: the basal LH concentration, peak LH concentration, duration from the time of GnRH injection to the time of the peak LH concentration, and area under the LH response curve (AUC). There were no significant differences in the basal and peak LH concentrations or the AUC among the three groups. The July group reached the LH peak significantly (P<0.05) faster than the May group, but there was no significant difference with the November group. Therefore, the results of the present study do not demonstrate an effect of summer heat on the LH response to the exogenous GnRH in Holstein heifers.

Key words: Heat stress, Luteinizing hormone, Pituitary response

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problem, even in Hokkaido, which is in the northern region of Japan [3]. Hokkaido is the most important dairy farming area of Japan. This region has experienced unusual summer weather in the past, with hot summer weather lasting about a month, although people living in Hokkaido believe that the hot summer weather of normal years usually lasts only a few weeks (from last week of July to the first week of August). However, words related to temperature and season, e.g. “summer weather”, “heat stress”, “cool temperature”, and “summer suppression” are subjective parameters, and the scientific definitions for these words have not been well established in Holsteins. Berman [4] recently reported that no clear criteria currently exist for conditions in which heat stress relief is needed for Holsteins. Furthermore, summer suppression of pulsatile LH release and preovulatory LH surges have only been reported in studies conducted in warm areas of outside Japan, such as Arizona and Florida in the U.S., Cuba, and Israel [5–8], where temperatures 29 °C or more are considered to cause heat stress by scientists in California [8]. Additionally, we need to speculate about the effects of summer heat from past studies under various natural conditions because of insufficiently large experiment facilities controlling targeted environment factors without changes in other factors. Thus, the effect of summer heat is poorly understood in Holsteins.

The present study is an attempt to clarify the seasonal differences in the parameters of LH response to exogenous GnRH in Holstein heifers in Sapporo, Hokkaido, Japan. Prepubertal heifers were used in this experiment to avoid potential confounding effects of stage of estrous cycle on LH release because the existence of blood progesterone secreted from the ovary seems to have a strong effect on LH response to GnRH [9]. Furthermore, estrous synchronization for cyclic females requires approximately two weeks and precise weather forecast for the day after synchronization is impossible.

**Materials and Methods**

*Animals and treatment*

All procedures used in this experiment were approved by the Animal Care and Use Committee of the Hokkaido National Agricultural Experiment Station (Sapporo, Japan). A total of 12 prepubertal Holstein heifers were housed in a tie-stall barn without fans or sprinklers at the Animal Center of the Hokkaido National Agricultural Experiment Station (42°58’ N, 141°23’ E). They were provided with free access to food (grass hay; 83.7% DM, 1.89 Mcal/kg of ME, and 7.6 %CP) to meet their growing requirements according to the Japanese Feeding Standard [10]. The precise feed intake was not measured in this study, but daily observation confirmed their normal feed consumption based on the amount of feed offered and refused. Water and mineral blocks (Koen-S; Nippon Zenyaku Kogyo, Fukushima, Japan) were provided ad libitum.

All heifers were used for the trials with GnRH on either 27th July (n=4, July group; 245 ± 10 kg, 7.5 ± 0.2 months old) 1995, 5th November (n=4, November group; 246 ± 10 kg, 7.6 ± 0.2 months old) 1995, or 31st May (n=4, May group; 244 ± 10 kg, 7.4 ± 0.2 months old) 1996. The decisions to conduct the trials on these respective days were made according to the weather forecast of the previous day. There was no rain or snow on these days. The highest daytime temperature, the lowest nighttime temperature, and the average humidity measured in the animal house on 27th July 1995, 5th November 1995, and 31st May 1996 were 31.6 °C, 19.6 °C and 79%, 8.8 °C, –1.5 °C and 66%, and 16.8 °C, 5.6 °C and 70%, respectively. Only on 26th, 27th and 28th, July 1995, the highest day time temperatures measured in the animal house were equal to or more than 29 °C. The trial on 5th November 1995 was four days before the first snowfall that year. All snow melted by mid-April 1996.

On the day preceding GnRH injection, all heifers were fitted with an indwelling jugular vein catheter (Medicut Catheter Kit 14G; Argyle, Tokyo, Japan) connected to an extension tube (LX1-L100; Top, Tokyo, Japan). The external end of the extension tube was fitted with a control valve. The tube was adhered to the skin of the heifer with glue and tape. The catheter and tube were flushed with saline containing 25 IU/ml heparin sodium. At 1330 h on the day of the GnRH trial, blood samples were collected for analysis of the basal LH concentration. Heifers received an intramuscular injection of 50 µg of GnRH analogue (Fertirelin acetate; Takeda Pharmaceutical, Osaka, Japan) at 1400 h (time=0). Blood samples were collected at 30-min intervals for eight hours after the injection. Blood was collected into heparinized syringes and centrifuged...
at 12,000 × g for 30 min at 4 C, and the plasma samples were harvested and stored at −30 C until analysis.

**Assay**

Plasma LH was assayed in duplicate by double antibody radioimmunoassay using bovine LH as the standard, 125I-labeled bovine LH as the tracer, and anti-ovine LH-antiserum (AFP11743B and AFP192279; National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, CA, USA). The limit of detection was 0.40 ng/ml, and the intra- and inter-assay coefficients of variation were 3.9% and 6.9% at 0.51 ng/ml.

**Statistical analyses**

The maximum concentration observed among the serial samples during eight hours (the peak LH concentration) and the duration from the GnRH injection to the time of the samples containing the peak LH concentration (duration to the LH peak) were calculated in order to analyze the peak-shaped LH responses after the GnRH injection. As an index of the amount of LH released in response to GnRH injection, the area under the LH response curve (AUC) for the eight hours after GnRH injection was also calculated [11]. The basal LH concentration, the peak LH concentration, the duration to the LH peak, and the AUC were subjected to one-way analysis of variance (ANOVA) with post hoc comparisons using Fisher’s PLSD test in order to evaluate the differences between the 3 groups. All results are presented as means ± standard error of the means (SEM).

**Results and Discussion**

Fig. 1 shows the changes in the blood LH concentrations of the three groups. All heifers in the all groups showed an LH response to the GnRH injection. There were no significant (P > 0.1) differences in the basal LH concentrations (Fig. 2a), the peak LH concentrations (Fig. 2b), and the AUCs among the three groups (Fig. 2d). The July group (113 ± 8 min) reached the LH peak significantly (P < 0.05) faster than the May group (203 ± 33 min), but the difference with the November group (165 ± 29 min) did not become significant (Fig. 2c).

Compared to the other groups, the July group reached the LH peak in a shorter period of time. This may suggest both the acute heat stress and the altered steps in the mechanism controlling the LH release to GnRH, for example, upregulated GnRH receptors on the cell surface of pituitaries in the July group. Secretion of luteinizing hormone is pulsatile in prepubertal heifers [12], and only one blood sample was collected before the GnRH injection from each heifer to evaluate the basal LH concentration in this study. Thus, further work is needed to determine the seasonal effect on pulsatile LH release in Holsteins in Hokkaido (Japan).

This study did not find significant differences in the peak LH concentrations or the AUCs among the groups. Three possible reasons may account for this observation. First, although prepubertal noncyclic heifers were used in this study, there was large variance in the parameters due to large individual differences in each group and the ANOVA could not detect significant difference in the parameters of the LH response curve among the groups. Thus, there may have been the suppressed LH release in some of the heifers of the July group. Second, because summer suppression of LH response to the GnRH injection has been reported in warm areas outside Japan [5–8], longer durations of high temperatures during the day and at night may be required to suppress pituitary LH response to GnRH injection. Third, non-lactating heifers may have more resistance to heat stress than lactating cows because lactating cows consume more feed and produce more heat than non-lactating heifers [4]. Therefore, although the results in this study do not demonstrate the significant effect of summer heat on LH response to exogenous GnRH in Holstein heifers, the necessity of the strategy of using hormonally-induced timed artificial insemination [1] against heat stress during the summer requires further investigation in Holstein lactating cows in cool regions like Hokkaido. No clear criteria exist for conditions in which heat stress relief is needed in Holsteins at the moment [4]. In dairy cows in Arizona, USA, vaginal temperatures, and respiratory rates, but not heart rates, were significantly higher during the hot weather season than during the cool weather season [13]. However, Berman [4] recently suggested the necessity of radiant, convective, skin evaporative, respiratory heat loss, hair coat depth, and exposed body surface in order to estimate heat
stress. Thus, although these parameters could not be measured in this study, these parameters should be considered important in future study of summer heat stress in Holsteins in cool regions.

Photoperiod is another potential factor inducing seasonal changes in LH secretion, because the serum LH concentration is the highest in ovariectomized cows in Nebraska around the spring equinox, decreases gradually until the autumn equinox, and then increases and reaches its peak again during the following spring equinox [14]. In ovariectomized heifers in Wisconsin, the LH concentration is the highest during the winter solstice period and lowest during the summer solstice period [15]. However, the heifers used in this study were housed indoors, and the contribution of the effect of photoperiod could not be evaluated.

In conclusion, the results of the present study do not demonstrate a significant effect of summer heat on LH response to exogenous GnRH in Holstein heifers.

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References