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—Full Paper—

Changes in Plasma Metabolic Hormone Concentrations During the Ovarian Cycles of Japanese Black and Holstein Cattle

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Abstract. The aim of the present study was to investigate the changing profiles of plasma metabolic hormones during the ovarian cycles of beef and dairy cattle. We used 16 non-pregnant, non-lactating Japanese Black beef cattle (6 heifers and 10 cows; parity=2.3 ± 0.8) and 12 multiparous Holstein dairy cows (parity=3.0 ± 0.3). Blood samples for hormonal analysis (growth hormone, GH; insulin-like growth factor-I, IGF-1; insulin; and progesterone, P4) were obtained twice weekly for 40 days before artificial insemination for Japanese Black cattle and from 50 to 100 days postpartum for Holstein cows. Luteal phases were considered normal if the P4 concentrations for at least 3 time points over the course of 7 days remained above 1 ng/ml and at least 2 of the time points were above 2 ng/ml. The patterns of the ovarian cycles were classified into two types (normal or abnormal, such as having prolonged luteal phase and cessation of cyclicity) on the basis of the plasma P4 profiles. The plasma concentrations of IGF-1 in both breeds increased transiently during the preovulatory period when the P4 levels were low and decreased to lower levels during the luteal phase when the P4 levels were high. The plasma concentrations of insulin in the 3rd week of normal ovarian cycles when the plasma P4 concentration dropped to less than 1 ng/ml were higher than those at other time points in the Japanese Black cattle, but not in the Holstein cows. The plasma concentrations of GH did not change during the ovarian cycle in either breed. In conclusion, the present study indicates that the plasma IGF-1 concentration increases during the follicular phase (low P4 levels) and decreases during the luteal phase (high P4 levels) in non-lactating Japanese Black and lactating Holstein cattle. The results suggest that ovarian steroids, rather than nutrient status, may be related to the cyclic changes in IGF-1 secretion from the liver in cattle.

Key words: Cattle, Insulin-like growth factor-I, Ovarian cycle, Progesterone

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Metabolic control of nutrients is quite different between lactating dairy cows, such as the Holstein, and beef cows, such as the Japanese Black [1]. Holstein cows experience a severe negative energy balance during the early lactation period that is characterized by loss of body weight and mobilization of body fat stores due to increased energy requirements under limited amounts of feed intake [2, 3]. Consequently, Holstein cows

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show the catabolic effects of higher levels of growth hormone (GH) and lower insulin-like growth factor-I (IGF-1) and insulin levels during the postpartum period [4, 5]. In contrast, in Japanese Black cows that produce only one-tenth of the total milk production of Holstein cows, the changes in GH and insulin secretions remained essentially unchanged between the lactation and non-lactation period [1].

It is accepted that changes in metabolic hormones affect reproductive function in cattle. Insulin and IGF-1 stimulate estradiol (E2) production in granulosa cells [6–9] and the proliferation of follicular cells [10, 11]. We have previously shown that the ovulatory dominant follicle at the first follicular wave postpartum is stimulated by high IGF-1 and insulin levels during the growth and final maturation periods, respectively [5]. Moreover, the expression of mRNA for GH receptor within the hypothalamus, the pituitary, the corpus luteum (CL) and follicles [12] suggested that GH has a direct effect on various reproductive cells. On the other hand, sex steroids are known to participate in metabolic regulation. In ovariectomized goats, an injection of E2 increased GH secretion, whereas progesterone (P4) implants decreased it [13]. Moreover, circulating IGF-1 concentrations during the luteal phase are lower than those in the follicular phase in ovariectomized goats [13]. Therefore, P4 during ovarian cycles may have a strong relationship to metabolic hormone secretion. However, whether plasma metabolic hormone concentrations are changed by the ovarian cycles in cattle and whether the possible changes in metabolic hormones during the ovarian cycle have different profiles in beef and dairy cattle remain unknown. Thus, the aim of the present study was to determine the changes in metabolic hormone levels during the ovarian cycles of non-lactating Japanese Black and lactating Holstein cattle.

Materials and Methods

Ethical approval

The experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro University.

Animals

This experiment was carried out at the Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine. We used 16 non-lactating Japanese Black cattle (6 heifers and 10 cows, parity; 2.3 ± 0.8) and 12 multiparous Holstein cows (parity; 3.0 ± 0.3) from days 50 to 100 postpartum. The Japanese Black cattle were housed in a paddock and offered timothy hay (54.1%DM for TDN and 12.5%DM for CP). The Holstein cows were housed in a free-stall barn throughout the experimental period and offered a total mixed ration consisting of grass, corn silage, and concentrate (70.6%DM for TDN and 14.8%DM for CP). Milking of the Holstein cows was performed twice daily (0600 and 1800 h), and the average 305-day milk yield was approximately 10589 ± 698 kg.

Sampling

For the Japanese Black cattle, blood samples for hormonal analysis were obtained by jugular venipuncture twice weekly for 40 days before artificial insemination. For the Holstein cows, blood samples for hormonal analysis were obtained by caudal venipuncture twice weekly from days 50 to 100 postpartum. We used sterile 10 ml tubes containing 200 μl of stabilizer solution (0.3 M EDTA, 1% acetylsalicylic acid, pH 7.4) for sampling. These tubes were centrifuged at 3000 rpm for 20 min at 4 C, and the plasma samples were kept at –30 C until hormonal analyses.

Definition of ovulation cycles

Cows were confirmed as having luteal activity when their plasma P4 concentration increased to more than 1 ng/ml [14]. Luteal phases were considered normal if the P4 concentrations for at least 3 time points over the course of 7 days remained above 1 ng/ml and at least 2 of the time points were above 2 ng/ml. The patterns of ovarian cycles were classified into the following two types (normal and abnormal) on the basis of P4 profiles. Ovarian cycles followed by a luteal phase of normal length were considered to be normal ovarian cycles. Ovarian cycles that were abnormal, such as those having a prolonged luteal phase (a luteal phase of more than 22 days) or cessation of cyclicity (no or weak luteal activity for at least 14 days), were considered to be abnormal ovarian cycles.

Measurement of P4, GH, IGF-1, and insulin

Determination of plasma P4 concentrations was performed by enzyme immunoassay (EIA) after extraction using diethyl ether as described previously [15]; the extraction efficiency was 93%. The standard curve ranged from 0.05 to 50 ng/ml, and the ED50 of the assay was 3.2 ng/ml. The mean intra- and interassay coefficients of variation (CVs) were 6.7 and 7.2%, respectively.

Determination of the plasma GH, IGF-1, and insulin concentrations was performed by EIA using the biotin-streptavidin amplification technique.

The GH concentration was measured by the EIA described by Roh *et al.* with slight modification [16]. A diluted rabbit antibody to bovine GH (100 μl, × 150,000, D. Schams) was distributed into all wells of a microplate coated with anti-rabbit γ-globulin antiserum. The plate was incubated for 24 h at room temperature and then decanted. After 1% chicken serum in assay buffer (42 mM Na_2HPO_4, 8 mM KH_2PO_4, 20 mM NaCl, 4.8 mM EDTA, 0.05 % BSA, pH 7.5; 100 μl) was added to each well, 15 μl of GH standards (0.78 to 100 ng/ml, NIDDK-bGH, AFP-9984C) was dissolved in the assay buffer or plasma and incubated in the wells for 24 h. After decanting the plate, biotin-labeled GH was distributed into each well and then incubated for 3 h. Finally, colorimetric treatments were carried out. The intra- and interassay CVs were 8.1 and 8.5%, respectively, and the ED50 of this assay system was 6.2 ng/ml.

Determination of the plasma total IGF-1 concentration was performed by EIA after protein extraction using acid-ethanol (87.5% ethanol and 12.5% 2 N hydrochloric acid) to obtain IGF-1 free from the binding proteins [17]. Thirty μl of human IGF-1 standard (0.39 to 50 ng/ml; Roche, Indianapolis, IN, USA) dissolved in assay buffer or sample was added to each well coated with anti-rabbit γ-globulin antiserum. In addition, 100 μl of biotin-labeled hIGF-1 (× 10,000) and rabbit anti-hIGF-1 (× 40,000, NIDDK, AFP18111298) diluted in assay buffer were distributed into all wells, and then the plate was incubated for 72 h at 4 C. Finally, colorimetric treatments were carried out. The intra- and interassay CVs were 5.7% and 6.6%, respectively, and the ED50 of this assay system was 2.5 ng/ml.

Determination of the insulin concentrations was carried out by EIA. Insulin standard (Sigma, St. Louis, MO, USA) was diluted with charcoal-treated serum (insulin-free) during preparation. Thirty μl of insulin standard (39 to 5,000 pg/ml) or plasma was added to each well coated with anti-guinea pig goat γ-globulin antiserum. In addition, 100 μl of anti-bovine guinea pig insulin (× 150,000; D. Schams) dissolved in assay buffer was distributed into all wells, and the plate was then incubated for 24 h at 4 C. After decanting the plate, 100 μl of biotin-labeled bovine insulin (× 50,000) was distributed into all wells, and the plate was then incubated for 2 h at 4 C. Finally, colorimetric treatments were carried out. The intra- and interassay CVs were 9.7 and 14.5%, respectively, and the ED50 of this assay system was 800 pg/ml.

Statistical analysis

In normal ovarian cycles, the week when the P4 concentration become less than 1 ng/ml was defined as week zero of the estrous cycle. We analyzed the data for cows with normal ovarian cycles from –0.5 to 3 weeks for each breed. The data for cows with abnormal ovarian cycles was arranged as a sampling period for each cow. Statistical analysis of abnormal ovarian cycles could not be conducted due to a limited amount of data (prolonged luteal phase, n=1; cessation of cyclicity, n=2).

There were no outliers for any of the variables (Grubb's test). The D'Agostino-Peason K^2 and Kolomogorov-Smirnov test were used for statistical testing of normality. The P4 values in both breeds were abnormally distributed. The significance of the effect of time was evaluated for the plasma concentrations of each hormone by repeated measures ANOVA. The statistical significance of differences in the concentrations of IGF-1 of both breeds and insulin in the beef cows during the estrous cycle was calculated using the Tukey-Kramer test. The statistical significance of differences in the concentrations of P4 for both breeds during the estrous cycle was calculated using the Steel-Dwass test. Results were expressed as mean ± standard error of mean (SEM). Differences of $P<0.05$ were considered significant.

Results

Changes in the metabolic hormone levels of the Japanese Black cattle during the ovarian cycle

Sixteen normal ovarian cycles were observed in

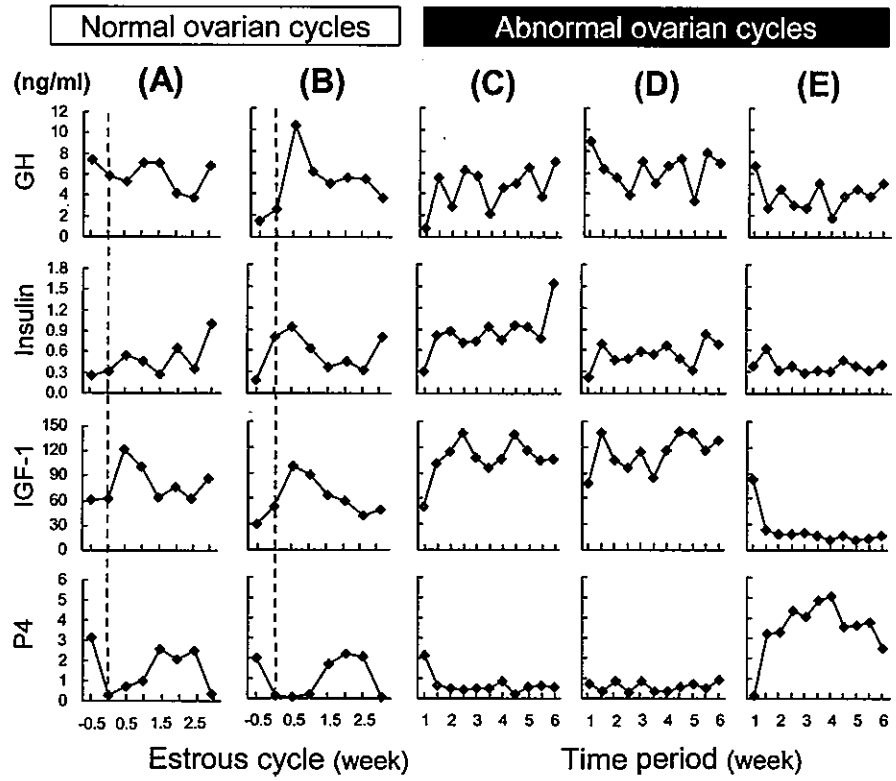

Fig. 1. Representative changes in the plasma concentrations of GH, IGF-1, insulin and P4 during the ovarian cycle in 5 Japanese Black cattle. The week when the P4 concentrations became less than 1 ng/ml was defined as week zero of the estrous cycle. A and B show normal estrous cycles, C and D show cessation of cyclicity, and E shows a prolonged luteal phase (C, D and E were considered abnormal estrous cycles).

13 of the Japanese Black cattle. Three abnormal ovarian cycles were observed in the remaining 3 Japanese Black cattle: 2 cows had cessation of cyclicity and one had a prolonged luteal phase.

Representatives changes in metabolic hormones in individual cattle during ovarian cycles are shown in Fig. 1. A and B indicate normal ovarian cycles, C and D show cessation of cyclicity, and E indicates a prolonged luteal phase. The plasma concentrations of IGF-1 increased during the follicular phase when the P4 levels were low and decreased to lower levels during the luteal phase when the P4 levels were high for both normal and abnormal ovarian cycles. In normal ovarian cycles, the changes in the plasma concentrations of insulin were similar to the changes in the IGF-1 concentrations. The plasma concentrations of GH were not changed by the ovarian cycle.

The changes in metabolic hormones during normal ovarian cycles are shown in Fig. 2. The plasma concentrations of IGF-1 increased transiently during the preovulatory period when the P4 levels were low and decreased to lower levels during the luteal phase when the P4 levels were high. The plasma concentrations of insulin in the 3rd week of normal ovarian cycles when plasma P4 concentrations dropped to less than 1 ng/ml were higher than those at other time points. The plasma concentrations of GH did not change during the normal ovarian cycle.

Changes in the metabolic hormone levels of the Holstein cows during the estrous cycle

Thirteen normal ovarian cycles were observed in the 12 Holstein cows. Representatives changes in metabolic hormones during two normal ovarian cycles are depicted in Fig. 3, and the changes in metabolic hormones during the normal ovarian cycles of the 12 cows are shown in Fig. 4. Similar to beef cows, the plasma concentrations of IGF-1

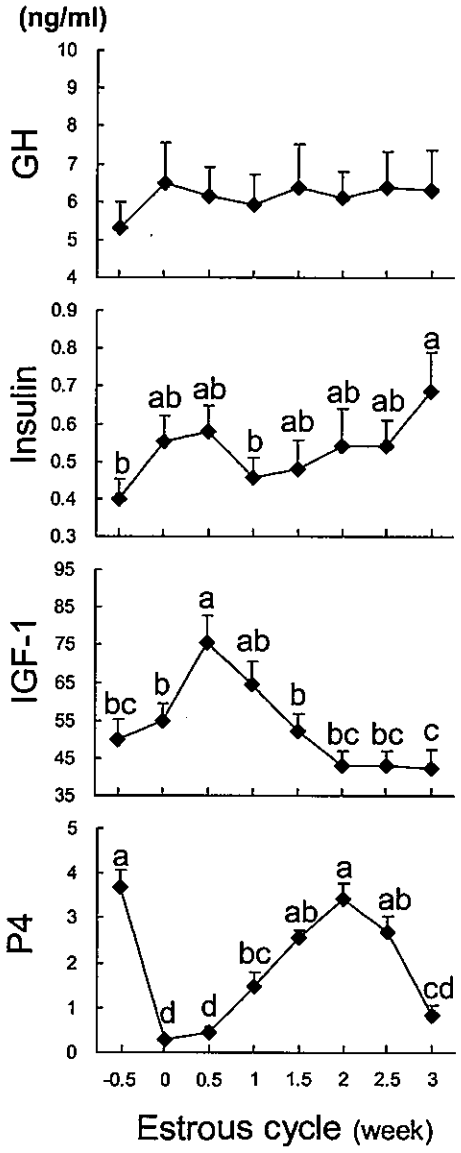

Fig. 2. Plasma concentrations of GH, IGF-1, insulin and P4 during the normal estrous cycle (n=16, mean ± sem) in Japanese Black cattle. The week when the P4 concentrations became less than 1 ng/ml was defined as week zero of the estrous cycle. a, b, c, d and e indicate differences of $P<0.05$ for each metabolic hormones.

increased transiently during the preovulatory period when the P4 levels were low and decreased to lower levels during the luteal phase when the P4 levels were high (Figs. 3 and 4). There were no significant differences in the plasma concentrations of GH and insulin during the ovarian cycles.

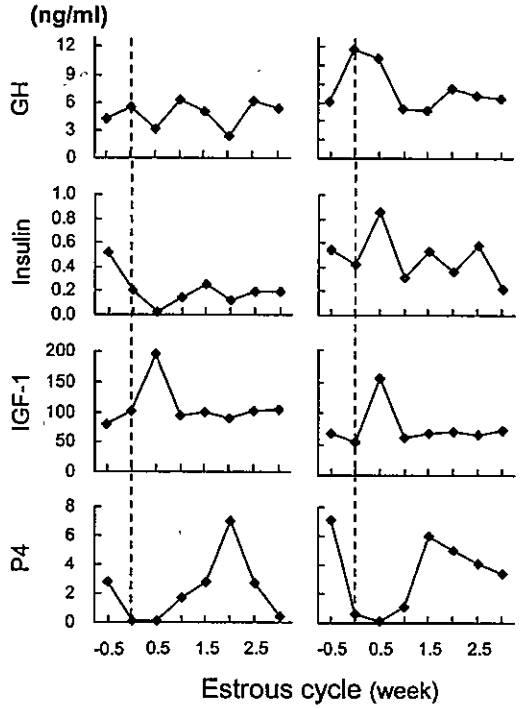

Fig. 3. Representative changes in the plasma concentrations of GH, IGF-1, insulin and P4 during the normal estrous cycle in 2 dairy cows. The date when the P4 concentrations became less than 1 ng/ml was defined as week zero of the estrous cycle.

Discussion

In Japanese Black cattle, the plasma metabolic hormone levels remain essentially unchanged during lactation and non-lactation periods [1], while the metabolic conditions of Holstein cattle during the lactation period shifts toward to catabolic metabolism [18–20]. Thus, although the actual feed intake was not measured in the present study, we believe that the metabolic statuses of Japanese Black and Holstein cattle are basically different. However, the present data showed that the plasma IGF-1 concentrations were changed in a similar manner by the estrous cycle in both breeds. The plasma concentrations of IGF-1 are positively correlated with the level of feed intake [6], and the circulating IGF-1 level decreases during both the periparturient period and acute feed restriction [21–23]. On the other hand, the increase in plasma IGF-1 concentrations is synchronized with estrus [6]. Therefore, nutritional status may not be the

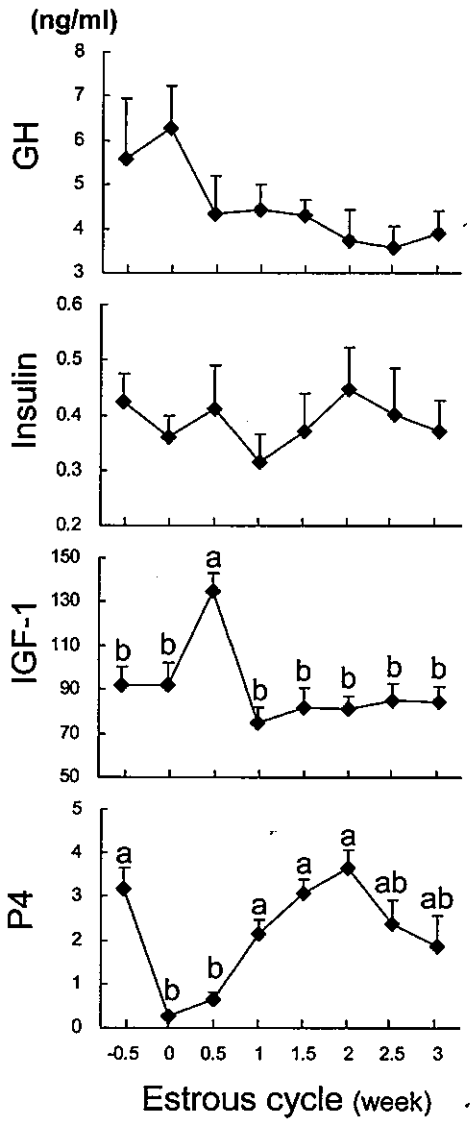

Fig. 4. Plasma concentrations of GH, IGF-1, insulin and P4 during the normal estrous cycle (n=13, mean ± sem) in dairy cows. The week when the P4 concentrations became less than 1 ng/ml was defined as week zero of the estrous cycle. a, b, c and d indicate differences of $P<0.05$ for each metabolic hormone.

factor that regulates the plasma IGF-1 levels during the estrous cycle.

In cattle, GH exerts galactopoietics and lipolytic effects, leading to a preferential partition of nutrients to mammary glands and enhancement of milk yield [24]. The GH released from the pituitary regulates IGF-1 production in the liver [6, 25] to synthesize the protein in muscle [26]. In addition, P4 implants in ovariectomized goats decreased the GH pulse amplitude and area under the curve [27]. The level of plasma GH in ewes increases transiently at estrus [28]. We recently showed that the basal GH levels and GH pulse amplitude during the first follicular wave with CL formation are lower than during the first follicular wave without a CL [13]. These findings suggest that plasma P4 has a negative relationship with GH pulse secretion. Since P4 receptor mRNA is not expressed in the bovine liver [29, our unpublished data], we hypothesize that the decrease of plasma IGF-1 in the present study may have been induced through P4 inhibition of GH pulses. In the present study, however, we did not examine GH pulse secretion because blood sampling was only performed twice per week. Further studies are necessary to determine the effect of P4 on the mechanism of GH pulse generation and the relationship between GH pulse generation and IGF-1 production in cows.

We observed that the plasma insulin concentrations change during the estrous cycle in Japanese Black cattle, but not in Holstein cows in the present study. A previous study demonstrated that plasma insulin levels change in parallel with E2 during development of the dominant follicle [6]. In addition, E2 secreted from follicles also enhances insulin secretion from the pancreas in rat [30]. Thus, it is likely that plasma insulin concentrations change during the estrous cycle under the influence of E2. However, the plasma concentrations of glucose, which are related to insulin secretion from the pancreas, during early lactation in Holstein cows are much lower than those in Japanese Black cows due to severe negative energy status [1]. In this context, the insulin secretion of the Holstein cows in this study may have been influenced by higher milk production during the estrous cycle, which may have overcome the effects of ovarian steroids on insulin secretion, and therefore the plasma insulin levels of the lactating Holstein cows remained unchanged.

Taken together, the present study indicates that plasma IGF-1 concentrations increase transiently during the follicular phase and decrease during the luteal phase of the ovarian cycle in both non-lactating Japanese Black and lactating Holstein cattle. The results suggest that ovarian steroids, rather than the nutrient status, may be related to the cyclic changes in IGF-1 secretion from the liver

in cattle.

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