Plasma Concentration of Estrone Sulfate during Pregnancy in Different Breeds of Japanese Beef Cattle

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Abstract. Plasma concentrations of estrone sulfate in different breeds of Japanese beef cattle and the relationship between those concentrations and feto-placental growth were examined in order to assess the possibility of monitoring abnormal growth of the fetus. Blood samples were obtained from cows from day 90 of gestation to parturition. The plasma concentration of estrone sulfate was measured by direct enzyme immunoassay. From day 180 of gestation, the mean concentration of estrone sulfate increased gradually and it was drastically elevated after day 240 of gestation with the maximum at day 285. Plasma concentrations of estrone sulfate on day 240 of gestation was significantly increased in F1 cows (Holstein Friesian and Japanese Black) compared with those in other breeds of cow. From day 270 to 278 of gestation, estrone sulfate concentrations of Holstein Friesian cows inseminated by Holstein Friesian differed from those inseminated by Japanese Black. In the cow with retained placenta, the plasma concentration of estrone sulfate reached plateau at day 240 of gestation and did not increase thereafter. There was no significant relationship between estrone sulfate concentration and duration of gestation, calf birth weight, weight of placenta or viability of newborn calves. These results indicate that changes of plasma estrone sulfate concentration in Japanese beef cattle are very similar to those in Holstein dairy cattle. They also suggest that the plasma concentration of estrone sulfate is associated with the breed of pregnant cow and that its concentration is also affected by calf birth weight depending on the breed of bull. It seems possible to predict the incidence of retained placenta but not the calf birth weight and viability of newborn calves in Japanese beef cattle.

Key words: Estrone sulfate, Feto-placenta, Gestation, Japanese beef cattle

Newborn calves of Japanese Black cattle show weakness and low birth weight in a high proportion, leading to economic loss. Among neonatal deaths in Japanese Black cattle, 65% are due to weakness [1]. The neonatal viability was positively correlated to the plasma concentration of steroid hormone that was produced by the feto-placental unit in Holstein cows [2]. It is speculated that neonatal death in Japanese beef cattle is due to delay of fetal growth and/or abnormal placental function, although these relationships remain to be elucidated.

Estrone sulfate (E1S) as conjugated estrogen, is synthesized in the fetal or cotyledonary portion of the placentome [3]. Since E1S can be detected in the plasma and milk of pregnant cows, its
concentration has been used to predict the developmental status of feto-placental function [2, 4, 5]. Robertson and King [6] reported that the plasma concentration of E1S increased at the end of the gestation period in Holstein cows. In Jersey and Holstein Friesian cattle, E1S concentrations in milk also increased dramatically after 128 days of gestation and the E1S levels continued to be elevated until 240 days of gestation [7]. Zhang et al. [2] found that plasma concentrations of E1S were positively correlated with calf birth weight from days 210 of gestation to 1 day prepartum and with the neonatal viability after day 195 of pregnancy.

If significant relationships between E1S concentration and feto-placental growth could be detected in Japanese beef cattle, the developmental status of the feto-placental unit could be monitored by measuring the maternal concentration of E1S, although such experiment is not undertaken. Recently, the semen of Japanese Black cattle have been inseminated into other breeds of cow, such as Japanese Polled and Japanese Short Horn, and crossbred between Holstein Friesian and Japanese Black, to generate calves having a high ability to produce meat. However, it is not clear whether such crossbreeds between Japanese Black and other breeds have original developmental patterns of feto-placenta.

In the present study we investigated the plasma concentration of E1S in different breeds of Japanese beef cattle. The relationship between plasma E1S concentration in Japanese beef cattle and feto-placental growth was explored in order to detect the possibility of monitoring the abnormal growth of the fetus, which possibly results in low calf birth weight and weakness of new born calves. So far, radioimmunoassay has been used for measurement of E1S concentration [7–10], although the enzyme immunoassay technique has also been experimentally validated. We developed a direct enzyme immunoassay of E1S measurement without extraction [11], and this method was employed to measure E1S concentrations in plasma in the present study.

Materials and Methods

Animals

Thirty cows kept on the Hiroshima University farm and calved between 2000 and 2001, and 2 cows kept on the farm of Saijo Agricultural High School and calved between Oct. and Nov. 2000 were used. Frozen-thawed semen from a Japanese Black (JB) bull were inseminated to 20 various breed cows, such as JB, crossbred (JB and Holstein Friesian (Hol): F1), crossbred JB and F1, crossbred JB and Japanese Shorthorn (JS), crossbred JB and Japanese Polled (JP), Hol, and semen from a Hol bull were inseminated to 6 Hol cows (Table 1). Embryos derived from JB were transferred to 6 JP cows. Animals were fed in accordance with the regulations of Hiroshima University.

Collection and storage of plasma

Blood samples (10 ml) were obtained from the tail vein using 21-gauge needles and heparinized vacuum tubes every month from day 90 to 270 of

Table 1. Duration of gestation, calf birth weight, weight of placenta, duration of placenta expulsion, score of calf viability and incidence of retained placenta in various breeds of cows

<table>
<thead>
<tr>
<th>Bull</th>
<th>Cow</th>
<th>Number of cows examined</th>
<th>Duration of gestation (days)</th>
<th>Calf birth weight (kg)</th>
<th>Weight of placenta (kg)</th>
<th>Duration of placenta expulsion (min)</th>
<th>Score of calf viability</th>
<th>Number of cows with retained placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB</td>
<td>JB</td>
<td>2</td>
<td>288.0d</td>
<td>32.5d</td>
<td>3.0d</td>
<td>140d</td>
<td>8.0d</td>
<td>0</td>
</tr>
<tr>
<td>JB (ET)*</td>
<td>JP</td>
<td>6</td>
<td>283.7bc</td>
<td>33.8bc</td>
<td>3.6bc</td>
<td>157bc</td>
<td>7.8bc</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>JB×Hol(F1)</td>
<td>3</td>
<td>285.0ghi</td>
<td>29.0ghi</td>
<td>4.6ghi</td>
<td>157ghi</td>
<td>8.0ghi</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>JB×F1</td>
<td>3</td>
<td>287.3w</td>
<td>25.3i</td>
<td>3.3i</td>
<td>ND</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>JB×JS</td>
<td>2</td>
<td>286.5wh</td>
<td>27.3id</td>
<td>3.5</td>
<td>ND</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>JB×JP</td>
<td>1</td>
<td>287.0</td>
<td>24.0</td>
<td>ND</td>
<td>ND</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>Hol</td>
<td>9</td>
<td>285.0ghi</td>
<td>36.0ghi</td>
<td>4.5ghi</td>
<td>264ghi</td>
<td>8.0ghi</td>
<td>1</td>
</tr>
<tr>
<td>Hol</td>
<td>Hol</td>
<td>6</td>
<td>280.5</td>
<td>44.1i</td>
<td>6.0</td>
<td>305i</td>
<td>7.5i</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 32, 1

JB, Japanese Black; JP, Japanese Polled; Hol, Holstein Friesian; JS, Japanese Shorthorn. ND: No data. * All JP cows were transferred embryos of JB. a-f: Values with different letters within column are significantly different (P<0.05).
gestation and every week from day 270 to parturition. The blood was immediately centrifuged (1700 × g, 15 min). Plasma for the supernatant was stored at −30 degrees C in plastic tubes until assay.

E1S assay in the plasma

Plasma concentrations of E1S were measured by a direct EIA as described by Isobe and Nakao [11] with some modifications. Briefly, plasma was diluted 20 times with assay buffer: 0.05 M borate buffer containing 0.2% BSA (fraction V, SIGMA ALDRICH, Tokyo, Japan) and 0.1 mg/ml thimerosal (SIGMA ALDRICH). All standard solutions were supplemented with 5% plasma that was derived from non-pregnant cows to omit the possible effect of plasma on the antibody-antigen reaction. Standard E1S or diluted sample plasma were applied to the wells of plates that were previously coated with goat anti-rabbit IgG antibody (ICN Biomedical, Aurora, OH). Following dilution of horseradish peroxidase (HRP) -labeled estrone-3- carboxymethylxime (Kambegawa Institute, Tokyo, Japan) and Anti-estrone-3- glucuronide-BSA IgG (Kambegawa Institute), 60,000 and 200,000 times, respectively, both were added to the wells. The plate was incubated at room temperature for 2 h and then washed with PBS three times. Substrate solution containing 4 mg/ml o-phenylenediamine, 0.2 M citric acid and 0.02% H2O2 was added to the wells followed by incubation for 30 min at room temperature. Reaction was stopped by adding 6 N H2SO4. The optical density was measured for its absorbance at 492 nm, using a microplate reader (MPR-A4i, TOSOH, Tokyo).

Data sampling around parturition

The newborn calves and placenta were weighed. Intervals from parturition to placenta expulsion were recorded. Calf viability was scored by Apgar’s index with some modification [12].

Statistical analysis

Assays for E1S were conducted in duplicate. After the conversion from optical density to concentration of E1S, the mean of two values was considered as the E1S concentration of a sample. Differences in E1S concentration and other physical data among groups were analyzed by one-way ANOVA followed by Duncan’s multiple range test [13]. The correlation coefficient between E1S concentrations and calf birth weight, weight of placenta, duration of pregnancy, viability of newborn calves and interval from parturition to placenta expulsion were calculated and the probability were assessed by Student’s t test. P<0.05 was considered a significant result.

Results

The duration of gestation in Hol cows inseminated with Hol semen was significantly shorter than breeds of other groups (Table 1). The calf birth weights of the Hol cows were significantly higher than breeds of other cows. Among the Hol cows there was a significant difference in calf birth weight between calves produced with Hol and JB semen. The Hol cows inseminated with Hol semen had significantly greater weight of placenta than other groups. A significantly longer time was required for placenta expulsion in Hol cows than Japanese beef cattle. The lowest score of calf viability was found in calves of Hol cows inseminated with Hol semen. One Hol cow inseminated with JB semen showed retained placenta.

E1S concentrations in the plasma of various breeds of cow inseminated with JB semen are shown in Table 2. From day 180 of gestation, the mean concentration of EIS increased gradually and it was markedly elevated after day 240 of gestation with the maximum on day 285. On days 240 and 270 of gestation, JB and F1 cows had significantly higher plasma concentrations of E1S than JP, crossbred and Hol (P<0.05). The concentrations of E1S on days 240 and 278 in F1 cows were significantly higher than any other group (P<0.05). Plasma concentrations of E1S decreased after day 278 of gestation in the JB and F1 cows and after day 285 in the JP cows, whereas no decrease was shown in the Hol and crossbred prepartum.

Concentrations of plasma E1S in Hol cows inseminated artificially with semen derived from Hol or JB bulls are shown in Fig. 1. Plasma concentrations of E1S on days 270 and 278 of gestation were significantly higher in cows inseminated with Hol than JB semen. The average weights of cows was not different between cows inseminated with Hol and JB semen (750 vs. 719 kg).
There was no significant relationship between maternal plasma concentrations of E1S on days 270 and 278 of gestation and birth weight of calves, weight of placenta and gestation length in any breed. However, when JB semen was inseminated, the E1S concentration in the maternal plasma was negatively correlated to the interval from parturition to placental expulsion ($r=-0.58$, $P<0.002$).

Plasma E1S concentrations during gestation in the cows with retained placenta and lack of udder enlargement being excluded. In the cow with retained placenta, plasma E1S concentration was not increased after day 240 of gestation and the levels at days 270 and 278 were markedly lower than those in the normal cows. The birth weight of the calf calved by the cow with retained placenta was 32 kg, which was not very different from those of normal cows (35 kg). The cow without udder enlargement prepartum had the same E1S concentrations in the plasma as normal cows until day 278 of gestation, but showed a higher level of E1S than normal cows on day 285.

**Discussion**

Concentrations of E1S increased gradually up to day 240 of gestation. Thereafter a marked increase was observed from days 240 to 285 of gestation. This result is comparable to those of other reports [2, 4, 5, 11, 14, 15].
Changes of plasma concentration of E1S were compared among the different breeds of cows inseminated with JB semen. Plasma E1S concentrations were significantly increased in F1 cows compared with those in JB and crossbred (JB and JP, JS or F1) cows, whereas E1S concentrations in JP and Hol cows were significantly lower than those in JB and crossbred cows on days 240 and 270. Since there was a positive correlation between calf birth weight and E1S concentrations of prepartum cows [14], the difference in the E1S concentrations among the breeds of cows may depend on the difference in the calf weight. However, F1 cows with high E1S level had calves with low weight and, conversely, Hol cows had low E1S levels but high weight calves. Abdo et al. [16] measured E1S concentration in three different breeds of cows (Sweden Jersey Breed, Swedish Red and White, Swedish Lowland Breed) inseminated with semen derived from each breed of bull and found significant differences in the concentrations among the breeds. In the present study, we compared the E1S concentrations in different cows inseminated with only JB semen and significant differences in the E1S concentrations among cows were obtained. This result suggests that the level of E1S in plasma of pregnant Japanese beef cattle is influenced by the breed of cow.

In JB, JP, F1 cows, reductions of E1S concentrations were seen after day 278 of gestation, although crossbred and Hol cows did not show such a reduction. There was no case of retained placenta among JB, JP and F1 cows. These results may indicate that JB, JP and F1 cows possess the characteristic of reduction of plasma E1S concentration prepartum.

From day 270 to 278 of gestation, E1S concentrations of Hol cows inseminated with Hol semen differed from those inseminated with JB semen. There was no significant difference in the weight of cows between the two groups, suggesting that the difference in E1S concentrations is possibly attributable to the difference in breed of bull used for the semen collection. It was also reported that when Hol cows were inseminated with semen from Hol, Brahman and Angus, E1S concentrations in the maternal plasma varied according to the breeds of bull [17]. Significantly heavier calf birth weights were observed in the Hol cows inseminated with Hol than JB semen. Since the calf birth weight has been shown to be correlated to the E1S concentration in maternal plasma [14], this result suggests that calf birth weight depends on the breed of bull, which also influences the difference in the maternal E1S concentration.

Plasma E1S concentrations of cows inseminated with JB semen were negatively correlated to the interval from parturition to placental expulsion. Retained placenta occurred in one Hol cow inseminated with JB semen. In this cow, plasma concentration of E1S reached a plateau on day 240 of gestation. These results suggest the possibility that plasma E1S concentration may be an indicator for predicting retained placenta. Similarly, long gestation periods resulted in retained placenta and reduction of E1S concentrations in Hol cows [2]. It was also reported that E1S concentrations were much lower in cows whose calves died during parturition and in cows with retained fetal membrane than in normal cows [14, 16]. A high level of estrogen is a prerequisite for the maturation of placenta, leading to the undisturbed expulsion of placenta postpartum [18]. The calf birth weights were not much different between cows with and without retained placenta. It is unclear whether the low concentration of E1S in the cow with retained placenta is attributable to the deficient growth of the fetus.

One of the main purposes of the present study was to examine whether or not we can predict low calf birth weight and weakness of newborn calves. There was no significant relationship between E1S concentration and calf birth weight, weight of placenta or viability of newborn calves. Therefore, the present results do not suggest E1S concentrations are able to predict the calf birth weight and weakness of newborn calves in Japanese beef cattle.

In conclusion, the present results indicate that changes of plasma E1S concentration in Japanese beef cattle and crossbreeds between dairy and Japanese beef cattle are very similar to those in Holstein dairy cattle. The results also suggest that plasma concentration of E1S is associated with the breed of pregnant cow as well as bull, and it seems possible to predict the incidence of retained placenta but not the calf birth weight and viability of newborn calves in Japanese beef cattle.
References