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Changes in Plasma Estrogen Concentrations during the First Trimester of Gestation in Dairy Cows: Comparison with the Origin of Embryos and Fetal Number

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Abstract

The objectives of this study were to determine the profiles of plasma estrogen concentrations during the first trimester of gestation and to correlate them with the origin of the embryos and fetal number. Pregnancies were induced either by artificial insemination (AI) on the day of estrus (day 0) or by transfer of 1 or 2 blastocysts produced *in vitro* (IVF-ET) on day 7. Five singleton-bearing cows impregnated by AI, and 6 singleton and 6 twin pregnancies induced by IVF-ET were used in the experiment. Blood was collected by jugular venipuncture at 2 to 5-day intervals until day 100. The concentrations of plasma estrone, estradiol-17β and estrone sulfate were measured by using a radioimmunoassay. Concentrations of all the estrogens fluctuated at low levels until day 50 in all the groups. Thereafter, the concentration of estrone sulfate gradually increased, whereas those of estrone and estradiol-17β remained at basal levels until day 80. The concentration of estrone sulfate after day 50 was affected by the day of gestation and the number of fetuses. After day 80, the concentration of estrone sulfate increased drastically, coinciding with the increases in the estrone and estradiol-17β concentrations. The rate of increase in the concentration of estrone sulfate during days 80 to 100 was the highest among all the estrogens. In the singleton pregnancies, the concentrations of estrone and estrone sulfate between days 80 and 100 were higher in the artificially inseminated cows than in the IVF-ET cows. In the pregnancies induced by IVF-ET, estrone sulfate levels were higher in the twin-bearing cows than in the singleton-bearing cows during days 50 to 80 and 80 to 100. These results suggest that the concentrations of estrogens in bovine peripheral blood plasma could be applied for monitoring fetoplacental development in the first trimester of gestation.

Discipline: Animal industry
Additional key words: estradiol-17β, estrone, estrone sulfate, *in vitro* fertilization and multiple pregnancies

Introduction

Estrogen is mainly produced in the placenta during gestation in cattle[16]. It plays an essential role in the maintenance of pregnancy[19] and the initiation of parturition[6,18]. The concentrations of estrone and estradiol-17β in bovine blood plasma remain low during the early period of gestation and increase gradually throughout mid- to late gestation[10,14,15]. Estrone sulfate is a sulfo-conjugate of estrogen and is mainly derived from the conceptus during gestation in cattle[13]. Its concentrations in maternal blood

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and milk increase gradually throughout gestation in the same way as those of free estrogens, and its level at each stage of gestation is the highest among all the estrogens, especially during the mid- to late periods of gestation[7,9,15]. Therefore, it is considered to be a practical indicator of placental function and has been used in pregnancy diagnosis[9,10] and in predicting the number of fetuses[1,17,20]. The objectives of this study were to characterize the peripheral plasma concentrations of estrone, estradiol-17β and estrone sulfate during the first trimester of gestation in dairy cows using a sensitive assay system, and to correlate the results with the origin of the embryos and fetal number.

Materials and methods

1. Animals

Regularly cycling Holstein cows were impregnated either by artificial insemination (AI) with Holstein semen on the day of estrus (day 0) or by nonsurgical transfer of 1 or 2 Japanese Black embryos produced *in vitro* on day 7. The procedures for *in vitro* maturation, fertilization and culture of transferred embryos have been described in detail elsewhere[12]. Pregnancy and fetal number were diagnosed around days 30, 45, 60 and 90 by transrectal ultrasonography and confirmed at parturition. Five singleton pregnancies induced by AI, 6 singleton and 6 twin pregnancies induced by *in vitro* fertilization and embryo transfer (IVF-ET) were examined in this experiment.

2. Sample collection

Fifty milliliter of blood was collected into a polypropylene tube containing 50 IU heparin by jugular venipuncture at 2 to 5-day intervals until day 100. Blood was immediately cooled down to 0°C in ice water and centrifuged at 1,800 × g within 1 h of collection. Following 1 h centrifugation at 4°C, separated plasma was stored at –35°C until analysis.

3. Solid-phase extraction

Solid-phase extraction of estrogens was previously described in detail[4,5]. Briefly, a reversed phase cartridge (SEP-PAK plus C_{18}, Waters, Milford, USA) was activated with 5 mL of methanol (Special grade, Wako Pure Chemical Industries, Osaka, Japan), followed by 10 mL of purified water. A 5 mL aliquot of bovine plasma each was applied in duplicate to the cartridge following centrifugation at 1,800 × g for 1 h. Estrone sulfate retained in the cartridge was eluted with 3 mL of 40% ($^{v}/_{v}$) methanol (HPLC grade, Wako Pure Chemical Industries, Osaka, Japan) following a 10 mL water wash. Then, estrone and estradiol-17β were eluted with 3 mL of 75% ($^{v}/_{v}$) methanol (HPLC grade). Each eluent recovered in a siliconized glass tube (12 × 75 mm, Iwaki Glass, Tokyo, Japan) was evaporated with a vacuum centrifugal concentrator (SpeedVac System, Savant Instruments, Holbrook, USA) and reconstituted in 0.5 mL of 10 mM phosphate buffered saline (pH 7.2) containing 1% bovine serum albumin (BSA, RIA grade, Sigma-Aldrich, St. Louis, USA) and 0.05% NaN_3 (Wako Pure Chemical Industries, Osaka, Japan).

4. Radioimmunoassay

The concentrations of estrone and estradiol-17β were measured using validated radioimmunoassays with 0.2 mL of the reconstituted solutions of 75% methanol eluent. Antibodies against estrone (anti- estrone- 6- carboxymethyloxime (CMO)- BSA rabbit serum) and estradiol-17β (anti- estradiol-17β- 6- CMO- BSA rabbit serum) were purchased from Cosmo Bio (Tokyo, Japan). The concentration of estrone sulfate was measured using a competitive radioimmunoassay with [6,7-^{3}H(N)]-estrone sulfate (PerkinElmer Life Sciences, Boston, USA) and a specific antibody (anti- estrone- 3- sulfate- 6-CMO- BSA rabbit serum (Lot 465)[11]) with 0.2 mL of the reconstituted solution of 40% methanol eluent. Assay procedures of all the estrogens were previously described in detail[5]. The mean value in duplicate analysis was calculated as the concentration of each estrogen. Intra- and inter- assay coefficients of variation of the concentrations of estrone, estradiol-17β and estrone sulfate were 9.2 and 14%, 7.5 and 12%, and 10 and 16%, respectively. Assay sensitivities of estrone, estradiol-17β and estrone sulfate were 0.97, 0.94 and 1.8 pg/mL, respectively.

5. Statistical analysis

Changes in the plasma concentrations of estrone, estradiol-17β and estrone sulfate were divided into 3 different stages, days 0 to 50, days 50 to 80 and days 80 to 100. Differences in the concentrations of these estrogens between the groups of AI and IVF-ET singleton-bearing cows, and IVF-ET singleton- and twin-bearing cows within a stage, and between stages within a group were subjected to repeated measures of ANOVA (StatView, SAS Institute, Cary, USA). Hormone profiles during a stage were analyzed by linear regression, and differences in the rates of daily increase (regression coefficient) in concentrations among estrogens, stages and groups were subjected to one factor ANCOVA (StatView).

Results

Changes in the concentrations of estrone, estradiol-17β and estrone sulfate in peripheral blood plasma during

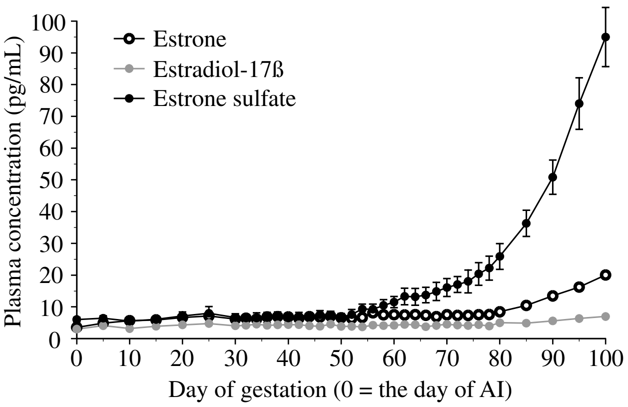

Fig. 1. Changes in estrone, estradiol-17β and estrone sulfate concentrations in peripheral blood plasma during the first trimester of gestation in Holstein cows impregnated by AI
Mean ± SEM, n = 5.

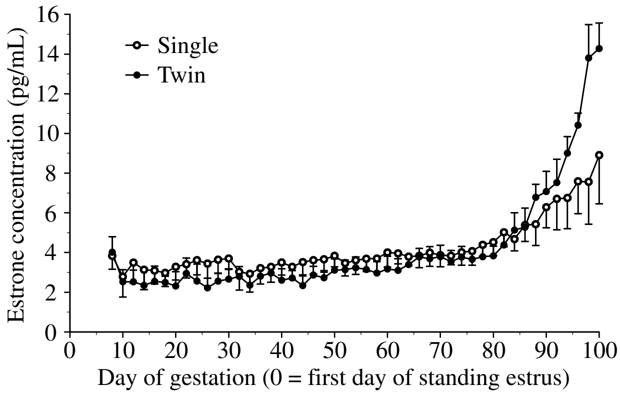

Fig. 2. Changes in estrone concentrations in peripheral blood plasma during the first trimester of gestation in Holstein cows with singleton and twin pregnancies induced by IVF-ET
Mean ± SEM, n = 6.

the first trimester of gestation in the AI singleton-bearing cows are shown in Fig. 1. Concentrations of all the estrogens fluctuated at low levels up to day 50. Estrone sulfate level began to increase around day 50 and drastically increased after day 80. Between days 50 and 80, the concentration of estrone sulfate was significantly correlated with the day of gestation (P<0.0001), whereas the estrone and estradiol-17β concentrations remained at basal levels and were not correlated with the day of gestation. After day 80, the estrone and estradiol-17β concentrations increased following the increase in the concentration of estrone sulfate. Concentrations of conjugated and free estrogens were significantly correlated with the day of gestation between days 80 and 100 (estrone and estrone sulfate, P<0.0001; estradiol-17β, P<0.01). However, the estrone sulfate concentration showed the highest rate of increase from days 80 to 100 compared with the concentrations of free estrogens (P<0.01).

Changes in the plasma estrone, estradiol-17β and estrone sulfate concentrations in the IVF-ET singleton- and twin-bearing cows are shown in Figs. 2, 3 and 4, respectively. Similarly to the changes in AI cows, the concentrations of all the estrogens fluctuated at low levels until day 50, regardless of the day of gestation or fetal number. Estrone sulfate concentrations began to increase on day 50 in both singleton- and twin-bearing cows (Fig. 4), whereas the concentrations of estrone and estradiol-17β remained at basal levels until day 80 (Figs. 2 and 3). After day 50, the estrone sulfate concentration steadily increased, depending on the day of gestation (P<0.0001) and fetal number (P<0.01). Concentrations of estrone sulfate drastically increased after day 80 in both singleton- and twin-bearing cows (Fig. 4). However, the con-

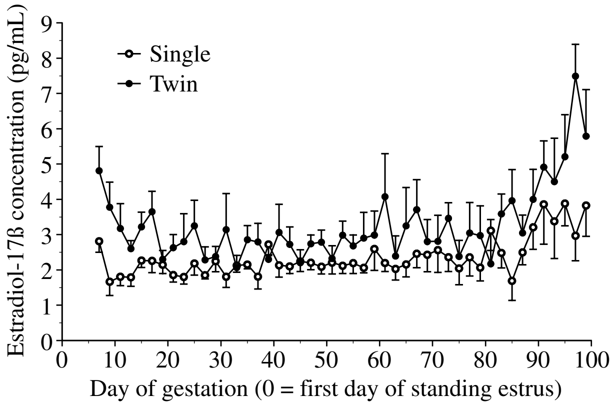

Fig. 3. Changes in estradiol-17β concentrations in peripheral blood plasma during the first trimester of gestation in Holstein cows with singleton and twin pregnancies induced by IVF-ET
Mean ± SEM, n = 6.

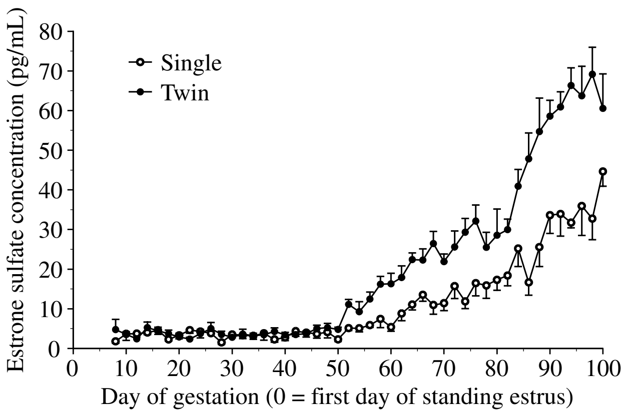

Fig. 4. Changes in estrone sulfate concentrations in peripheral blood plasma during the first trimester of gestation in Holstein cows with singleton and twin pregnancies induced by IVF-ET
Mean ± SEM, n = 6.

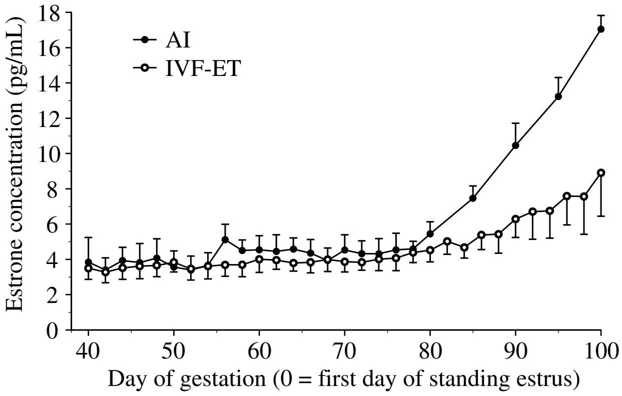

Fig. 5. Comparison of changes in estrone concentrations in peripheral blood plasma during days 40 to 100 in Holstein cows impregnated by AI and IVF-ET
Mean ± SEM.

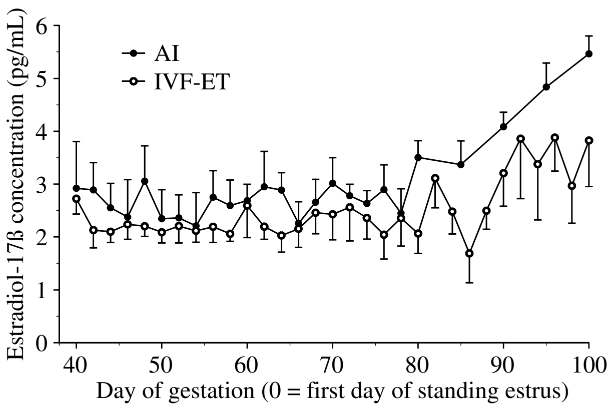

Fig. 6. Comparison of changes in estradiol-17β concentrations in peripheral blood plasma during days 40 to 100 in Holstein cows impregnated by AI and IVF-ET
Mean ± SEM.

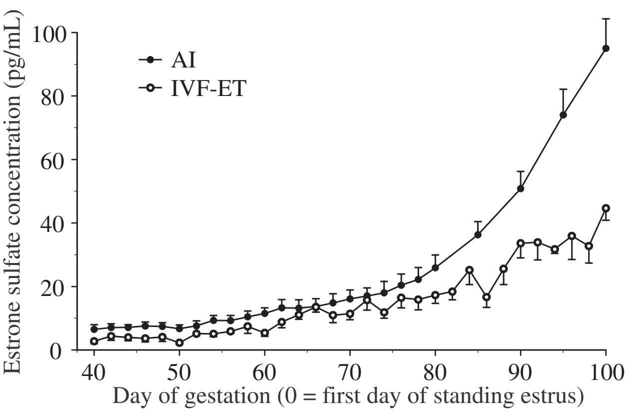

Fig. 7. Comparison of changes in estrone sulfate concentrations in peripheral blood plasma during days 40 to 100 in Holstein cows impregnated by AI and IVF-ET
Mean ± SEM.

centrations were significantly higher in the twin- bearing cows than in the singleton-bearing cows during days 50 to 80 ($P<0.01$), and 80 to 100 ($P<0.001$). Estrone and estradiol-17β concentrations started to increase around day 80 in both singleton- and twin-bearing cows (Figs. 2 and 3). After day 80, the concentrations were affected significantly by the day of gestation ($P<0.001$), but not by the number of fetuses ($P = 0.070$ and 0.14, respectively). The rate of increase in estrone concentrations during days 80 to 100 was significantly higher in the twin-bearing cows than in the singleton-bearing cows ($P<0.05$). Nevertheless, the rate of increase in plasma concentrations during days 80 to 100 was the highest for estrone sulfate among all the estrogens in both singleton- and twin-bearing cows ($P<0.05$).

In order to compare estrogen concentrations between AI and IVF-ET cows, changes in the concentrations of plasma estrone, estradiol-17β and estrone sulfate in the AI and IVF-ET singleton-bearing cows during days 40 to 100 are shown in Figs. 5, 6 and 7, respectively. Until day 80, the plasma concentration of each estrogen was similar in both groups. During days 80 to 100, the estrone and estrone sulfate levels were significantly higher in the AI cows than in the IVF-ET cows ($P<0.01$), whereas the estradiol-17β levels were similar in both groups. Furthermore, the rates of increase in the plasma concentrations of estrone and estrone sulfate during days 80 to 100 were higher in the AI cows than in the IVF-ET cows ($P<0.05$).

Discussion

In this experiment, the concentration of estrone sulfate began to increase around day 50 independently of those of free estrogens and gradually increased until day 80. Thereafter, it showed a drastic increase that coincided with the increase in the concentrations of free estrogens. These patterns of increase in the concentrations of conjugated and free estrogens were similar in the 3 groups. These results suggest that placentation in the bovine conceptus advances with the progression of pregnancy, regardless of the origin of the embryos and the number of fetuses being carried.

It has been reported that the plasma estrone sulfate concentrations were higher in the mid- to late gestation period in twin-bearing cows than in singleton-bearing cows[1,7,20]. However, this difference had not been observed during the first trimester of gestation because of the limited sensitivity of previous assay systems[1,17]. In the present experiment, the estrone sulfate concentrations in bovine peripheral blood plasma were significantly higher in the twin-bearing cows than in the singleton-bearing cows between days 50 and 80 ($P<0.01$), and 80 and 100 ($P<0.001$), whereas the estrone and estradiol-17β concentrations showed a weak correlation with the fetal number throughout the first trimester of gestation. These results suggest that the peripheral plasma concentration of estrone sulfate would, more than those of any other estrogens, reflect the mass of the conceptus after day 50, and specifically the mass of the placenta after day 80, in the first, as well as the second and the third trimesters of gestation.

The rates of increase in the concentrations of estrone and estrone sulfate after day 80 were moderate in the IVF-ET singleton-bearing cows compared with the changes in the AI cows, although the changes were parallel until day 80. The dams in the 2 groups were carrying embryos (fetuses) of disparate breeds, Holstein embryos produced by AI and Japanese Black embryos produced by *in vitro* maturation, fertilization and culture. These results suggest that the origin of embryos might affect the production of estrone and estrone sulfate in the placenta.

Changes in the estrone sulfate concentration in bovine milk during the first half of gestation has been reported to be an indicator of pregnancy status[2,3,8]. The sensitivity of these assay systems was approximately 30 pg/mL and the results were consistent. It was reported that the estrone sulfate concentration in bovine milk during gestation was lower than the detectable level in the first 80 to 90-day period and showed a sharp rise after day 100, which was consistent with the plasma concentration of estrone sulfate at the same gestational stage[1,10,15]. Based on the results of these reports and the present experiment, estrone sulfate can be detected in bovine milk before day 100.

In conclusion, the concentration of estrone sulfate in bovine peripheral blood began to increase around day 50, whereas those of estrone and estradiol-17β remained at basal levels until day 80. The rate of increase in the concentration was the highest in the case of estrone sulfate among all the estrogens after day 80. Peripheral plasma concentration of each estrogen began to increase in the same period of gestation, regardless of the origin of the embryos and the number of fetuses. The concentration of estrone sulfate was significantly affected by the day of gestation and the number of fetuses after day 50, whereas the concentrations of free estrogens showed a weak correlation with the fetal number until day 100. Estrone and estrone sulfate concentrations were significantly affected by the origin of the embryos after day 80. These results suggest that the concentration of estrogens in bovine peripheral blood could be applied for monitoring fetoplacental development in the first trimester of gestation.

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