Fecal Estrone Sulfate Profile in Sows during Gestation

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ABSTRACT. The aim of this study was to establish radioimmunoassay (RIA) for fecal estrone sulfate (E1S) and to elucidate changes in fecal E1S during pregnancy in the sow. Fecal E1S was extracted on a commercially available solid phase column, and the E1S fraction obtained was subjected to RIA. The sensitivity of the RIA was 8.5 pg/tube. The intra- and inter-assay coefficients of variation were 8.8–8.9% and 10.7–14.2%, respectively. Mean recovery for E1S added to fecal samples was as high as 95.0%. A significant positive correlation (r=0.904, n=147 p<0.001) existed between fecal and plasma E1S concentrations. Mean E1S concentration in feces and plasma fluctuated exhibiting two peaks. The first peak of E1S concentration was evident on days 28–32 in feces and on days 26–30 in plasma. The E1S concentration in both feces and plasma remained at baseline levels during mid-pregnancy, but began to rise gradually around days 72–82 and 70–80, in feces and plasma respectively, and reached a peak concentration on days 110–114. Following parturition, the concentration of E1S in plasma declined rapidly, but there was a two-day delay before a decline in fecal E1S. Apart from this two-day delay, changes in fecal E1S were similar to those in plasma E1S. The study indicates that the measurement of E1S in feces could be a useful tool for early pregnancy diagnosis and for monitoring fetal development in sows and gilts.—KEY WORDS: estrone sulfate, feces, pregnancy, radioimmunoassay, sow.


In pregnant sows, estrone sulfate (E1S) in peripheral blood is first detectable around day 16 of pregnancy and fluctuates with 2 peaks, one on days 23–30 and the other within a few days before parturition [18]. Robertson and King [18] suggested that the E1S concentration in the maternal blood reflects the amount of estrogens synthesized and secreted by the blastocyst or fetoplacental unit. Thus early pregnancy diagnosis [4, 23, 24] and the diagnosis of normal or abnormal fetal development [5] may be made in terms of maternal blood E1S concentrations.

In a previous study [15], the authors measured the E1S concentration in the saliva of sows, which is easier to collect than blood. In recent years, the measurement of the steroid hormone concentration in feces has been reported to be a useful diagnostic tool for wild animals [8, 10, 12, 19, 21, 26] and pigs [1, 2, 7, 13, 20, 25], blood collection from which is especially difficult.

Fecal estrogen has been tested in pigs [1, 2, 25]. Choi et al. [2] measured unconjugated estrogen in fecal samples, Vos [25] measured conjugated and unconjugated concentrations of estrone, estradiol and estriol, and Bamberg et al. [1] demonstrated the presence of estrone and estradiol, but little is known about the profile of fecal E1S in sows during gestation.

The present study was therefore undertaken to measure fecal concentrations of E1S by radioimmunoassay (RIA) and to correlate the changing pattern of fecal E1S with that of plasma E1S in pregnant sows.

MATERIALS AND METHODS

Collection of fecal and blood samples: Five hybrid sows, 5 castrated boars and a total of 147 pregnant sows raised in our department were used for the present study. The sows were artificially inseminated on the first day of estrus. Fecal samples were collected from each sow at intervals of two days during pregnancy, as described previously by Moriyoshi et al. [13]. Briefly, a plastic glove for rectal examination was worn on the hand which was inserted into the rectum of sows. An appropriate amount of impacted feces was taken from the rectum and immediately aliquoted in 2 g amounts. The aliquots were put into plastic test tubes each containing 8 ml of 0.01 M phosphate buffer with 0.1% of bovine serum albumin (BSA; albumin, bovine fraction V, Sigma-Chemicals Co., St. Louis, U.S.A.), and then taken back to the laboratory. After thoroughly shaking on a Direct Mix TS-50 (Thermal Kagaku Sangyo Co., Ltd., Japan), the solution was centrifuged at 1,700 × g for 15 min, and the supernatant was frozen at −20°C as the fecal solution until assayed for E1S. Immediately after fecal collection, blood samples were also collected from the median tail vein into heparinized test tubes. Plasma was separated by immediate centrifugation (1,700 × g, 15 min) and kept frozen at −20°C until assayed for E1S. In order to use as E1S standard for fecal samples, E1S-free feces were collected from 5 castrated boars. The E1S-free fecal solutions prepared as described above were pooled.

Preparation of standard fecal E1S solution: Fecal samples were collected from 5 castrated boars and the fecal solutions prepared were pooled as described above. These were used for the preparation of the standard fecal solutions as an E1S-free fecal solution. This was added to a standard E1S methanol solution, and the standard E1S fecal solutions were prepared by adjusting the solutions to final E1S concentrations of 0–5,000 pg/ml.

Extraction of E1S: E1S was extracted from fecal solution
(standard or test fecal solutions) and plasma E1S by a slight modification of the method described in the previous report [15]. Briefly, fecal solutions and plasma samples (0.5–2 ml) were diluted with 2 ml of 0.02 M phosphate buffer, and then poured into a 10 ml disposable syringe attached to a solid phase column (Sep-PakPLUS C18 Cartridge, Waters Co., Milford, Massachusetts, U.S.A.). The sample solutions were allowed to flow into the columns at the rate of 0.2–1 ml per min. The columns were then washed with 4 ml of distilled water and with 3 ml diethylether successively, and the effluents were discarded. Finally, five ml of acetone was poured into the columns, and the resultant effluent (E1S fraction) was recovered into test tubes. These were each put in 0.5–1 ml small test tubes. The effluents were evaporated to dryness at 50°C under a stream of nitrogen gas, and the residues were dissolved in 200 μl of borate buffer (0.2 M, pH 8.0) containing 0.05% BSA and 0.06% bovine γ-globulin (BSA buffer). The same extraction work was done for the standard fecal solution. It was then divided into test tubes so that the final concentration were 0–1,000 pg/tube and it was dried by the same procedures as used for the fecal samples. BSA buffer (200 μl) was then added.

**RIA for E1S**: E1S in fecal solution and plasma was quantified by a modification of the RIA described in the previous report [15], with estrone-3-sodium sulfate as a standard, estrone sulfate ammonium salt [6.7–3H(N)] (3H-E1S; specific activity, 1.48–2.22 TBq/mM) as a tracer and anti-estrone-3-sulfate rabbit serum (anti-E1S serum). The standard E1S preparation was provided by Dr. A. Kambegawa (Kambegawa Laboratory, Japan) and 3H-E1S was prepared by Daitech Pure Chemicals Co., Ltd., Japan. The anti-E1S serum was generated by Cosmo Bio Co., Ltd., Japan with anti 6-oxo-estrone-3-sulfate-6-CMO-BSA as the antigen. The cross-reactivities of the anti-E1S serum with E1S, estrone glucuronide, estrone, estradiol-3-sulfate, estradiol-3-glucuronide and estriol sulfate were 100, 5.4, 4.0, 1.5, 0.8 and 0.8%, respectively. 3H-E1S was diluted with BSA buffer at 250,000 cpm/ml. Anti-E1S serum was 1:50,000 for fecal samples and 1:25,000–36,000 for plasma samples.

Standard E1S, fecal solution or plasma extracts dissolved in 200 μl BSA buffer was each mixed with 100 μl 3H-E1S solution. Then, 100 μl of anti-E1S serum solution was added and the mixture allowed to react at 4°C for 12–24 hr. The incubated solutions were mixed with 250 μl of dextran-coated charcoal solution [0.2 M borate buffer solution containing 0.5% charcoal (Norit A; Kishida Chemical, Co., Ltd., Japan) and 0.05% dextran (Dextran770; Pharmacia Fine Chemicals AB Uppsala, Sweden), pH 8.0], left at 4°C for 10 min and subsequently centrifuged at 4°C for 15 min (1,700 x g). Two hundred μl of the supernatant was emulsified with 3 ml scintillator (Scintisol® EX-H, Wako Pure Chemical Industries, Ltd., Japan), and the radioactivity was assayed in a scintillation counter (Liquid Scintillation Counter LSC-703, Aloka Co., Ltd., Japan). The quantities of E1S in fecal and plasma samples were estimated by interpolation from a standard curve.

**Statistical Analysis**: Data were analyzed with a StatView program for Macintosh ver. J 4.02 (Abacus Concepts Inc., California, U.S.A.). Correlations between fecal and plasma E1S concentrations were examined by means of Pearson's correlation coefficients. Moreover, based on the excretion patterns, correlations were determined with fecal values lag corresponding to the plasma sample collected two days earlier.

**RESULTS**

**Parameter for reliability of RIA for E1S**: The standard curve obtained by the assay of fecal standard solution in quantities ranging from E1S of 3.9 to 1,000 pg is shown in Fig. 1. The sensitivity estimated was 8.5 pg/tube. The rate of recovery of E1S in swine fecal solutions was determined by adding 500, 1,000 or 1,500 pg E1S to 1 ml of fecal solution, as shown in Table 1. The mean recovery rate ranged from 94.2 to 96.2% and the average from a total of the 15 measurements was 95.0%.

The intra- and inter-assay coefficients of variation were each calculated from quintuplicate assays on 2 pooled fecal

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**Table 1. Recovery of varying amounts of estrone sulfate (E1S) added to swine fecal samples**

<table>
<thead>
<tr>
<th>Added E1S (pg/ml)</th>
<th>No. of assays</th>
<th>Assay value (pg/ml)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>228 ± 30*</td>
<td>-</td>
</tr>
<tr>
<td>500</td>
<td>5</td>
<td>701 ± 37</td>
<td>94.5</td>
</tr>
<tr>
<td>1,000</td>
<td>5</td>
<td>1,170 ± 65</td>
<td>94.2</td>
</tr>
<tr>
<td>1,500</td>
<td>5</td>
<td>1,672 ± 15</td>
<td>96.2</td>
</tr>
</tbody>
</table>

Total mean 95.0

* Mean ± S.E.
solutions with different mean E: S concentrations. The intra-assay coefficient of variation was 8.9% for the solution with the lowest E: S concentration (260 pg/g of fecal weight), and the solution with the highest E: S concentration (2,460 pg/g of fecal weight) was 8.8%. The inter-assay coefficient of variation for the solution with the lowest E: S concentration (258 pg/g of fecal weight) was 10.7%, and 14.2% for the solution with the highest E: S concentration (2,352 pg/g of fecal weight).

Correlation between fecal and plasma E: S: The correlation between fecal and plasma E: S concentrations was examined in a total of 147 pregnant sows. The regression line obtained from a plot of the two parameters is given in Fig. 2. A significant positive correlation existed between feces and plasma E: S concentrations. The correlation coefficient was 0.904 (p<0.0001).

Changes in fecal and plasma E: S concentrations during pregnancy: As shown in Fig. 3, mean E: S concentrations in feces and plasma fluctuated with two peaks. The first peak in the E: S concentration was evident on days 28–32 in feces and on days 26–30 in plasma. Therefore, the steroid in both feces and plasma remained at baseline levels during mid-pregnancy. Feces and plasma E: S, however, began to rise gradually around days 72–82 and days 70–80, respectively, and reached a peak concentration (the second peak) on days 110–114 (within a few days before parturition). Following parturition, the concentration of E: S in plasma declined rapidly. On the other hand, there was a two-day delay in the decline of fecal E: S. The changes in the fecal E: S levels were therefore paralleled by those of plasma E: S, except that there was a two-day delay in the fecal E: S level changes compared to the plasma E: S level changes. A significantly high correlation was found between the concentrations of E: S in plasma and those in feces collected two-days later (r=0.940, n=144, p<0.0001, data not shown).

DISCUSSION

In pigs, blood is generally collected from the cranial vena cava or caudalauricular vein under nose restraint. Restraint of the pig involves great effort and risk of accident, and may also be very stressful for the pig.

In a previous study [15], the authors measured the E: S concentration in the saliva of sows, which is easier to collect than blood. It was noted that the E: S concentration in saliva was much lower than in plasma [15]. A few reports
indicated that steroid concentrations in feces were higher than those in saliva [1, 2, 9, 11, 25]. Therefore, the present study was carried out to establish the RIA for E;S in feces and to demonstrate correlation between fecal and plasma E;S in pregnant sows.

Variations in individual recovery rates for E;S added to fecal solution were in a small range, and the mean recovery for steroid was very high (95.0%). The intra- and inter-assay coefficients of variation obtained from RIA for fecal E;S in the present study were low and comparable to the values reported for plasma E;S assay in cows [14], humans [3] and sows [15]. A high correlation was also observed between the fecal E;S concentration and the plasma E;S concentration in this study. The sensitivity of the assay (8.5 pg/tube) was high enough to measure fecal E;S concentrations. Thus, the assay used in this study was reliable.

Two peaks of plasma E;S concentrations were evident in the present study, the first peak around day 30 and the second peak within a few days before parturition. This was in agreement with the findings by Robertson and King [18], who carried out serial estimations of plasma E;S only in a single gilt at implantation, throughout pregnancy and at parturition, and with our previous study [15].

Vos [25] has previously reported E;S concentrations in the feces of the sows. In his report, however, E;S concentrations were measured only at the early stage of pregnancy in the pig. The present study is the first to describe changes in fecal E;S concentrations throughout pregnancy in the same sows.

Hultén et al. [7] and Moriyoshi et al. [13] compared the changes in concentrations of fecal gestagen and blood progesterone in the sow during the estrous cycle, and described that the changes in the fecal concentration were similar to those in the blood concentration, but with delay of an approximately two days. Shideler et al. [21] found a time-lag among the serum, urinary and fecal hormones by injecting 14C-estradiol or 14C-progesterone in monkeys. The present findings are in accord with those of Choi et al. [2], who monitored the changes in the estrogen level in the sow during the first trimester of pregnancy, and reported that the changes in the estrogen levels in feces were similar to that of the estrogen level in blood serum, but with a two-day delay. Thus, many workers found that the concentration of hormones in feces, changes in parallel with that in blood but the profile of the former follows that of the latter. In the present study, the changing pattern of fecal E;S was correlated with that of plasma E;S in pregnant sows. The changes in the concentration of E;S in feces followed those in blood with a delay by two days. This was confirmed by the significant, high correlation between E;S in the feces and E;S in the blood collected with an interval of two days. The steroid hormones in feces derives from the blood steroid hormones, which are metabolized by the liver and released into the digestive tract as metabolites or are transferred directly from peripheral blood in alimentary blood vessels of the digestive tract into peptic juice or mucus (secretion fluid). These hormones are contained in decomposed food in the digestive tract, and it is a general agreement that it may take approximately two days for the hormones to be finally excreted from the body into the feces. This point must be taken into consideration when clinical application of measured fecal E;S concentration is carried out.

A consequence of steroid synthesis in the preimplantation blastocyst is a high concentration of estrone and estradiol-17β in luminal fluid in the uterine environment. Although these unconjugated estrogens pass readily into the endometrium, their local tissue concentration is rapidly decreased by sulfotransferase which results in the formation of estrogen sulphoneconjugates with low biological activity [6, 16, 17]. Therefore, attempts have been made for an early pregnancy diagnosis [4, 23, 24] and estimation of litter size [22] by measuring the E;S concentration in blood during early pregnancy.

Jurke et al. [8] observed an increase in fecal estrone conjugate (E;C) at 50 days postestrus that was influenced by placental unit in pregnant female pygmy loris, and suggested that pregnancies can be confirmed as early as 50 days postestrus and E;C measurements in this species may predict single or twin births by mid-term.

In the present study, the concentrations of fecal E;S exhibited two peaks and were low during mid-pregnancy (Fig. 3). Therefore, a pregnancy diagnosis or the estimation of the growth conditions of fetus (diagnosis of abnormal fetal development) may be possible by measuring fecal E;S concentrations on 28–32 days when the first peak appears or after 72–82 days of pregnancy when the concentration increases again towards the second peak.

Wasser et al. [26] showed a variation in the measured values, depending on which part of the feces was used; i.e. the subsampling variance from the same wet fecal sample was highest on the outside compared to the inside. However, they found that the change was small when they used a well-mixed, dried fecal powder taken from premixed wet samples. Therefore, to increase precision for the measurement, a well-mixed, dried fecal powder taken from premixed wet samples should be used. In this study, although the wet-sample was not homogenized, the change of the measured values was very small and thus the results obtained were reliable.

The present study established and validated a RIA for E;S in feces and indicates that the measurement of E;S could be a useful tool for early pregnancy diagnosis and for monitoring fetal development in sows and gilts.

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REFERENCES


