Fecal Estrone Sulfate Profile of a Sow Showing Abnormal Pregnancy with Fetal Mummification

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(Received 11 May 1999/Accepted 2 November 1999)

ABSTRACT. Fecal and plasma E₁S of a sow with mummified fetuses, was compared with normal delivery cases. Fecal and plasma fluctuation patterns in E₁S were similar. In the sow with fetal mummification both fecal and plasma E₁S concentration rapidly decreased after day 80–90 compared to normal farrowing sows. This coincided with the estimated time of fetal death.—**KEY WORDS:** estrone sulfate, fetal mummification, swine.

J. Vet. Med. Sci. 62(3): 309–311, 2000

Mummification is common in the pig. Fetuses which die between days 40–90 of pregnancy are discharged together with normal ones at the time of delivery. The causes of mummification include heredity compression of the *navel obi* during gestation, damage to the placenta, infection and hormonal imbalances. To date, there has been no report on the endocrinological features of a sow with fetal mummification.

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 [12]. Robertson and King [12] suggested that E₁S concentration in maternal blood reflects the amount of estrogens synthesized and secreted by the blastocyst or feto-placental unit. Thus, early pregnancy diagnosis [1, 15, 16] may be made by measuring E₁S in maternal blood.

In a previous study [9], the authors measured E₁S in feces, which are easier to collect than blood, of sows. The study showed that changes in fecal and plasma E₁S were similar with a two day delay in fecal E₁S compared to plasma E₁S.

This report describes a rare case in swine, a sow which delivered 4 mummified fetuses and one immature piglet. The E₁S profile of this sow was compared with that of sows which delivered normal fetuses.

The sow was a hybrid (Large White × Landrace × Duroc), primiparaous and was one year six months old at the time of delivery. The sow had not been vaccinated against any diseases. It was kept in an individual stall and fed 2.4 kg per day of a commercially available compound feed for breeding sows (Power Breed®, Chubu-Siryou Ltd. Co., Japan) and had 4 l of water per day. Estrus detection was performed daily by inspecting the vulva, checking for the standing reflex when pressed on the back, and contractility of the cervix uteri by palpation per rectum. The sow was artificially inseminated three times with a separating interval of 24 hr on the days of displaying estrous signs with a commercially available liquid semen. Fecal and blood

samples were collected at intervals of two days during pregnancy. Fecal samples were processed as described by Moriyoshi *et al.* [7] and Ohtaki *et al.* [9]. Briefly, an appropriate amount of impacted feces was taken from the rectum and immediately aliquoted in 2 g amounts. The aliquots were put in 8 ml of 0.01 M phosphate buffer with 0.1% of bovine serum albumin (BSA; Sigma Chemicals Co., St. Louis, U.S.A.) and thoroughly mixed. The mixture then was centrifuged at $1,700 \times g$ for 15 min, and the supernatant was frozen at -20°C until assayed for E₁S. Blood samples were collected from the median tail vein into heparinized test tubes. Plasma was separated immediately by centrifugation ($1,700 \times g$, 15 min) and kept at -20°C until assayed for E₁S. For comparison feces and blood were collected in the same way from 5 sows with normal delivery.

The assay procedure for fecal and plasma E₁S was previously described by Ohtaki *et al.* [9], with estrone-3-sodium sulfate as a standard (provided by Dr. A. Kambegawa; Kambegawa Laboratory, Japan), estrone sulfate ammonium salt [$6.7\text{-}^3\text{H}(\text{N})$] as a tracer (prepared by Daiichi Pure Chemicals Co., Ltd., Japan), and anti-estrone-3-sulfate rabbit serum (anti-E₁S serum). 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. Cross reactivities of this antibody to estrone glucuronide, estrone, estradiol-3-sulfate, estradiol-3-glucuronide and estriol sulfate were 5.4, 4.0, 1.5, 0.8 and 0.8%, respectively. The fecal E₁S assay had a sensitivity of 8.5 pg/tube and the mean recovery rate was 95.0%. The intra-assay coefficients of variation for the lowest (260 pg/g of fecal weight) and highest E₁S concentrations (2,460 pg/g of fecal weight) were 8.9 and 8.8%, respectively. The inter-assay coefficients of variation for the lowest (258 pg/g of fecal weight) and highest E₁S concentrations (2,352 pg/g of fecal weight) were 10.7 and 14.2%, respectively.

Pregnancy diagnosis was done using ultrasonography 22 days or more after the last insemination [4, 5], and the sow

was judged pregnant. Purulent vaginal discharge was observed at 83 days of pregnancy, but the sow was not clinically ill. The sow was injected intramuscular with 10 mg dinoprost (Veterinary Pronalgon® F Injection, Pharmacia Upjohn, Ltd. Co., Japan) to induce parturition on day 116 of pregnancy, because no signs of parturition were observed through day 115. On day 117 the sow farrowed a total of 5 piglets, one immature (female, birth weight 700 g, placenta weight 89 g) and 4 mummified fetuses (two male and two female). The mummified fetuses are shown in Fig. 1, the mean crown-rump length (CRL) and weight were 18.9 cm (range: 18.2–19.5) and 348 g (range: 330–362), respectively. The mummified fetuses had eyebrows and hard teeth. The cause of the mummification was not determined.

Five control sows had normal farrows (total litter size was 8–15). Changes in fecal and plasma E_1S concentration of the normal farrowing sows and the sow with mummified fetuses are shown in Fig. 2. The data of the normal farrowing cases is the same in a previous report [9]. Mean E_1S concentration of normal farrowing cases in feces fluctuated exhibiting two peaks: the first peak was on days 28–32, and from around days 72–82 E_1S began to rise gradually reaching a second peak concentration on days 110–114. Changes in fecal E_1S showed a two-day delay compared to plasma E_1S .

As shown in Fig. 2, fluctuations in E_1S were similar in feces and plasma for the mummification case. The first peak of E_1S concentration was evident on day 28 in feces and on days 24–26 in plasma. E_1S concentration in both feces and plasma remained at baseline levels during mid-pregnancy. However, it began to rise gradually from around days 72–76 in feces, and 68–74 in plasma. At 90 days of gestation fecal E_1S decreased from 1,447 pg/g and fluctuated between 274–800 pg/g until delivery. Similarly, plasma E_1S decreased from 1,875 pg/ml on day 88 and fluctuated between 411–880 pg/ml until delivery. Fecal and plasma E_1S concentrations on and after days 80–90 were remarkably lower than those of normal farrowing sows.

The delivery of 4 mummified fetuses and one immature piglet is rare in swine. Viral infection is the major cause of mummified fetuses. When there is only one or a few stillborn piglets in an otherwise normal litter, the likely cause is at farrowing, such as large litter size, prolonged farrowing, or anoxia. When stillbirths are part of a litter that also contains mummies, an infectious agent is more likely to be involved [14]. The causes of stillborns, mummifications and abortions are often difficult to ascertain.

Mean CRL and weight of the mummified fetuses was 18.9 cm and 348 g, respectively. The fetuses probably died at day 80 of gestation because they had hair (e.g., eyebrows) and the milk teeth had callus (hardened teeth) [2, 6, 8]. Furthermore, a purulent discharge was observed at the vulva on day 83 of pregnancy, and this indicates the estimated time of fetal death. The E_1S concentrations in feces and plasma were also low around this time, especially after 90 days in this case, compared with normal parturition.

Fig. 1. Mummified fetuses after delivery (Note the similarity in size and weight).

Fig. 2. Changes in fecal (top) and plasma (bottom) estrone sulfate (E_1S) concentration during pregnancy: mummification case (○), normal (●).

A consequence of steroid synthesis in the preimplantation blastocyst is elevated concentrations of estrone and estradiol- 17β in the luminal fluid of the uterus. Although these unconjugated estrogens pass readily into the endometrium, their local tissue concentration is rapidly decreased by sulphotransferase, which results in the formation of estrogen sulphoconjugates with low biological activity [3, 10, 11]. Therefore, attempts have been made to make an early pregnancy diagnosis [1, 15, 16] and estimation of litter size [13] by measuring the E_1S concentration in blood during early pregnancy.

The time of decrease in E_1S concentration in this study, coincided with the estimated time of fetal death. This probably resulted from a remarkable decrease in placental function. The mummified fetuses died around the same

time, because their CRLs and weight were similar and they all had eyebrows and hard teeth.

In conclusion, this study showed a disparate E₁S profile in normal and mummified farrowing sows from insemination to parturition. This study is the first to report E₁S concentrations of a sow with so many mummified fetuses, though it is only one case.

We are grateful to Dr. A. Kambegawa of Kambegawa Laboratory for the generous supply of E₁S standard.

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