—Technical Note—

Early Pregnancy Diagnosis in the Sow by Fecal Gestagen Measurement Using a Bovine Milk Progesterone Qualitative Test EIA Kit

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Abstract. An attempt was made to measure the qualitative fecal gestagen concentration in the sow using a commercial bovine milk progesterone qualitative test EIA kit (qualitative kit), which can measure the gestagen concentration in approximately 10 min. Additionally, the possibility of applying this method of gestagen concentration measurement to early pregnancy diagnosis in the sow was investigated. Feces were collected from the rectum of the sow, and 0.5 g of feces was placed in 20 ml of distilled water and stirred. The supernatant was used as the fecal solution, which was subjected to measurement by the qualitative kit. The accuracy of pregnancy diagnosis at 21–25 days after last mating for 111 sows was 97.6% (80/82) for positive cases and 100% (17/17) for negative cases. The overall pregnancy diagnosis accuracy, including 12 indeterminable cases, was 87.4% (97/111). A comparison of the diagnoses based on gestagen concentrations that were measured using the qualitative kit, and the fecal gestagen concentrations of identical samples that were measured using the quantitative kit showed close agreement: 17 cases diagnosed as negative pregnancy by the qualitative kit all had a gestagen concentration of less than 200 ng/g, while 79 of 82 cases diagnosed as positive pregnancy by the qualitative kit showed a gestagen concentration of over 200 ng/g. The results of this study showed that qualitative measurement of the fecal gestagen concentration in the sow using a bovine milk progesterone qualitative test EIA kit is a practical method for early pregnancy diagnosis.

Key words: Early pregnancy diagnosis, Feces, Gestagen, Qualitative test EIA kit, Sow.

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For efficient livestock breeding, pregnancy diagnosis as soon as possible after mating is important in order to cull or rebreed farm animals that are not pregnant to minimize economic losses associated with non-pregnancy. This requires a reliable method of early pregnancy diagnosis. Various methods of early pregnancy diagnosis for sows have been reported: the rectal examination method [1–4], vaginal mucosal tissue examination

method [5, 6], ultrasonic Doppler method [7], ultrasonic echo method [8], ultrasonography [9, 10], urine estrogen measurement method [11], blood progesterone measurement method [12–14], and blood estrone sulphate measurement method [15–17]. However, all of these methods have problems in terms of simplicity, cost, and accuracy, and are not used widely in the clinical field.

The authors of this study have previously used a commercially available bovine milk progesterone quantitative test enzymeimmunoassay (EIA) kit (quantitative kit) in the sow, which can quanti-

tatively measure the gestagen concentration in feces in approximately 1.5 h and uses a measurement sample (i.e., feces) that is much easier to collect than blood is. This method is quick, requires no special equipment, and good results were obtained from the application of this method to early pregnancy diagnosis [18]. The present study consisted of an investigation of a bovine milk progesterone qualitative test EIA kit (qualitative kit) that can measure progesterone concentration in approximately 10 min, and whether or not it can be applied to the qualitative measurement of gestagen in the feces of sows and whether these results can be applied to early pregnancy diagnosis.

Materials and Methods

Animals

A total of 111 sows, including 21 Large White sows, 2 Landrace sows and 88 Large White and Landrace first-generation crossbred sows, at day 21 to day 25 after their last mating day, were selected from a swinery in Hokkaido, Japan for these experiments. These sows had been raised in individual stalls, measuring 60 cm in width $\times$ 180 cm in length $\times$ 103 cm in height. The floor of each stall was concrete and was maintained in a continuously dry state by a covering of sawdust. The feed was a commercially available compound feed for breeding sows (High Breed 80[®], Hokuren, Japan) and was fed to the sows by an automatic feeding device 3 times a day at a rate of 2.1–3.1 kg per sow. Water was freely available at all times. Generally, the quality of feces excreted by such sows is nonviscous lumps with a low water content, and there were almost no differences in the quality of feces among sows in this study.

Fecal collection and fecal solution preparation

Feces was collected and preserved according to a method described in a previous report [18]. Fecal collection was carried out while each sow remained unrestrained in her stall. A plastic glove for rectal examination was worn on the hand, which was inserted into the rectum of the sow. An appropriate amount of impacted feces was taken from the rectum and immediately weighed into 0.5 g amounts, which were each put into a plastic test tube containing 20 ml of distilled water with 0.05% of sodium azide as an antiseptic, and taken

to the laboratory. After stirring well, the solution was centrifuged at 1700 g for 15 min, and the supernatant was frozen at -20 °C until gestagen measurement.

Qualitative kit

The test kit used in this study was a bovine milk progesterone qualitative test EIA kit (Ovucheck Cowside W kit, Cambridge Veterinary Sciences Ltd., UK), which is composed of the following elements. Reagents: Three plastic drop bottles, A, B and C. Bottle A contains an enzyme conjugated compound solution; bottle B contains a substrate buffer solution, and bottle C contains a substrate solution. Standard progesterone solution: One plastic drop bottle, S, containing a standard progesterone solution prepared to a 8.6 ng/ml concentration (not used in the experiments). Microtitre plate: Two 16-well strips in which the progesterone antibody is pre-coated. Each well is fitted with a lid and contains a stabilizing buffer solution. Spot: 32 sample dropping disposable spots (3 mm in diameter $\times$ 123 mm in length). One drop from the spot is approximately 40 μ l. Because the cross reaction between the progesterone antibody used in the kit and progesterone metabolites (conjugated progesterone) included in feces was not investigated, the substances measured by this kit were expressed as gestagen (progesterone and progesterone metabolites).

Standard gestagen fecal solution preparation

Because the previous study [18] showed that the criterion for positive pregnancy diagnosis was a gestagen concentration of more than 200 ng/g of fecal weight or 5 ng/ml of fecal solution [18], a standard fecal solution was prepared with at a progesterone concentration of 5 ng/ml. The method of preparation was the same as that used in the previous study [18]. First, fecal samples were collected from 5 sows in estrus by a method described previously. The fecal solutions produced were pooled and treated by dextran-coated charcoal [19], and a gestagen-free fecal solution was made. This was then poured into a test tube containing standard progesterone that had been dried from dissolved methanol solution at 85 °C, and the standard fecal solution was made by adjusting the solution until the final progesterone concentration was 5 ng/ml.

Measurement procedure

The method of measurement is outlined in Fig. 1. Before commencing the measuring operation, the kit was left for 30 min at room temperature. The lids were removed from the wells to be used, and the stabilizing buffer solution was discarded. The residual solution in each well was then absorbed using absorption paper. One drop each of standard fecal solution and test fecal solution were placed in different wells using the spot. Four drops of A solution were added to each well and left for 5 min at room temperature (15–30 °C). After discarding the solution in each well, the wells were washed 3 times with either distilled water or tap water, and the residual solution was absorbed using absorption paper. Four drops of B solution followed by 1 drop of C solution were added to each well, and the well was lightly tapped to mix the solution. After 3–5 min, when the standard fecal solution turned blue, the fecal solution gestagen concentration was determined by comparing the color of the standard fecal solution to that of each test sample.

Criteria for early pregnancy diagnosis and confirmation of pregnancy

Early pregnancy diagnosis was made by comparing the color of the test fecal solution with that of the standard fecal solution: diagnosis was posi-

tive if the color of the test fecal solution was lighter than the standard fecal solution, and negative if the color of the test fecal solution was darker than the standard fecal solution; it was indeterminable if the colors of both solutions were similar. Final pregnancy diagnosis was made using ultrasonography after day 22 of the last mating [9, 10].

Reliability of measurements

Identical samples were also prepared for fecal gestagen measurement using the quantitative kit, and the results were compared with those using the qualitative kit. Measurements using the quantitative kit were carried out according to the method of the authors [18].

Results

Early pregnancy diagnosis using the qualitative kit

Table 1 shows the accuracy of pregnancy diagnosis determined by using qualitative measurements of the fecal solution gestagen concentration from day 21–25 of the last mating. The accuracy of positive pregnancy diagnosis based on fecal gestagen concentration was 97.6% (80/82), and the accuracy of negative pregnancy diagnosis was 100% (17/17). Of the 12 indeterminable cases, 8 cases were later determined to be positive and 4 were negative. The overall accuracy of early pregnancy diagnosis, including the indeterminable cases, was 87.4% (97/111).

Accuracy of diagnosis using the qualitative kit

Figure 2 shows the relationship between the results using the qualitative kit and the gestagen concentration values measured by the quantitative kit. The 17 cases diagnosed as negative pregnancy by the qualitative kit all had a gestagen concentration of less than 200 ng/g of fecal weight. On the other hand, 79 of the 82 cases diagnosed as positive pregnancy by the qualitative kit showed a gestagen concentration of over 200 ng/g, and the remaining 3 cases were less than 200 ng/g. Of the 12 indeterminable cases, 8 showed a gestagen concentration of over 200 ng/g and 4 were less than 200 ng/g. Thus, the results of pregnancy diagnosis determined by qualitative measurements of the gestagen concentration were almost identical to previously reported results of pregnancy diagnosis (positive for gestagen > 200 ng/g and negative

Fig. 1. Outline of the procedure for qualitative measurement of fecal gestagen in the sow using a qualitative EIA kit.

Table 1. Accuracy of pregnancy diagnosis at 21–25 days after mating based on a qualitative measurement of fecal gestagen concentration in the sow using a qualitative EIA kit

Results using qualitative kit		Results using ultrasonography ^{a)}		Accuracy of diagnosis (accurate cases/ diagnosed cases)
Diagnosis	Cases	Positive cases	Negative cases	
Positive	82	80	2	97.6% (80/82)
Negative	17	0	17	100.0% (17/17)
Indeterminable	12	8	4	
Total	111	88	23	87.4% (97/111) ^{b)}

a) After 22 days from last mating.

b) Including 12 indeterminable cases.

for gestagen < 200 ng/g) based on quantitative measurements of the gestagen concentration [18].

Discussion

To measure progesterone or estrogen levels in pigs, blood is generally collected from the cranial vena or caudal auricular vein by retention of the nose, which involves great effort and the possibility of accidents. This procedure is also thought to be very stressful for the pig. Thus, the authors used feces, which are easier to collect than blood, to measure the gestagen concentration using a bovine milk progesterone quantitative test EIA kit [18]. Previously, the changes in fecal gestagen and blood plasma progesterone concentrations in the pig during the estrous cycle were compared, and the changes in fecal gestagen concentration were similar to those in blood plasma progesterone concentration but with an approximately two-day delay. Hultén *et al.* [20] also obtained very similar results. Moreover, a 96.0% accuracy in early pregnancy (21–25 days after last mating) diagnosis of sows was obtained based on these gestagen concentration measurements [18]. The accuracy of early pregnancy (21–25 days after last mating) diagnosis of sows in the present study, which was based on qualitative measurements of the gestagen concentration using a qualitative kit, was 97.6% for positive cases, 100% for negative cases, and 87.4% (97/111) overall, including indeterminable cases. Thus, the accuracy using this method was slightly lower than that using the quantitative kit.

Among the 12 cases for which pregnancy diagnosis was indeterminable, there were some cases in which the measurement of gestagen concentra-

Fig. 2. Relationship between the results of pregnancy diagnosis based on a qualitative measurement of the fecal gestagen concentration in the sow using the qualitative kit and the measurements of fecal gestagen concentration using the quantitative kit. ○ : pregnant case, ● : non-pregnant case.

tion was much higher or much lower than 200 ng/g. The reason these cases were judged indeterminable was thought to be due to the rough measurement procedure using the qualitative kit compared to that using the quantitative kit, as well as limitations in judgment due to the visual meth-

od of determination.

The reported accuracies of the other main methods of pregnancy diagnosis for sow are: 81.3–85.2% for the rectal examination method at 20 days after mating [1–4]; 94.0–97.0% for the vaginal mucosal tissue examination method at 20 days after mating [5, 6]; 58.0–80.0% at 22 days after mating and 91.9–100% at 40–50 days after mating for the ultrasonic Doppler method [7]; 96.0–100% for the ultrasonic echo method at 30–90 days after mating [8]; and 100% for the ultrasonography at more than 22 days after mating [9, 10]. Tamamura *et al.* [17] reported 93.3% accuracy for the endocrinological method of pregnancy diagnosis at 22–27 days after mating, based on measurements of blood estrone sulphate using EIA. As for the accuracy of pregnancy diagnosis using EIA to measure the progesterone concentration, Saga *et al.* [14] reported 90.1–96.4% accuracy at 18–21 days after mating, based on measurements of the serum progesterone concentration using the double antibody EIA method. The authors [21, 22] measured the progesterone concentration in saliva using bovine milk progesterone quantitative and qualitative kits, which are the same as those used in the present study, and reported accuracies of 96.3% (258/268) based on a quantitative progesterone concentration measure-

ment and 80.4% (111/138) based on a qualitative progesterone concentration measurement in early pregnancy (17–24 days after the last mating) diagnosis. Although the period of early pregnancy diagnosis in the present study, based on qualitative measurements of the fecal gestagen concentration in sows, is slightly later than that of above methods, the method used in the present study has a high clinical value in terms of simplicity and speed.

The results of the present study have shown that a qualitative measurement of the gestagen concentration in feces using a bovine milk qualitative test EIA kit, which can be operated easily and can measure the gestagen concentration in approximately 10 min, is a sufficiently accurate and clinically useful method for early pregnancy diagnosis in the sow.

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