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

Masaharu MORIYOSHI, Masayuki TAMAKI, Toshihiko NAKAO, and Keiichiro KAWATA

Department of Veterinary Obstetrics and Gynecology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan

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ABSTRACT. We attempted to measure the qualitative saliva progesterone concentration in the sow using a commercial bovine milk progesterone qualitative test EIA kit (qualitative kit), which can measure the progesterone concentration in approximately 10 min, and investigated the possibility of applying this method of progesterone concentration measurement to early pregnancy diagnosis in the sow. The accuracy of pregnancy diagnosis at 17–24 days after last mating for 138 sows was 91.3% (105/115) for positive cases and 100% (6/6) for negative cases. The overall pregnancy diagnosis accuracy, including 17 indeterminable cases, was 80.4% (111/138). A comparison of the diagnoses based on progesterone concentrations measured by the qualitative kit and the saliva progesterone concentrations of identical samples measured by the quantitative kit showed close agreement: 6 cases diagnosed as negative pregnancy by the qualitative kit all had a progesterone concentration of less than 5 *ng*/m*l*, while 111 out of 115 cases diagnosed as positive pregnancy by the qualitative kit all showed a progesterone concentration over 5 *ng*/m*l*. Thus, the results of this study show that qualitative measurement of the saliva progesterone 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, progesterone, qualitative test EIA kit, saliva, sow.

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Although there are many different methods of pregnancy diagnosis for the sow [1–12, 15, 19–21, 23–25], a widely accepted method in the clinical field has not yet been established due to problems of simplicity, accuracy and cost, as well as the high level of technical skill required.

Recently, the enzyme immunoassay (EIA) has been developed and applied to the determination of some steroids [13, 16–18, 20–23]. This assay does not have the limitations of, or require such special equipment as is needed in the conventional radioimmunoassay. Although EIA is used to measure various steroids in the body fluid of domestic animals, the measurement of progesterone in the body fluid by EIA has recently come to be used clinically for endocrine examination. Saga *et al.* [20] reported on the applicability of the double antibody EIA of progesterone in the blood serum to early pregnancy diagnosis in the sow. However, the relative difficulty of blood sampling, in the case of the sow, was a problem for the application of this method.

The authors previously reported [14] good results from the use of saliva progesterone measurement for early pregnancy diagnosis in the sow. Saliva progesterone was measured using a commercial bovine milk progesterone quantitative test EIA kit (quantitative kit), which has a measuring time of approximately 2 hr. The advantage of using saliva compared to blood was that it was easy and safe to collect, and did not give any pain or stress to the animal. In the present study, we investigated whether a bovine milk progesterone qualitative test EIA kit (qualitative kit), which can measure the progesterone concentration in approximately 10 min, could be applied to the qualitative measurement of progesterone in the saliva of sows, and whether these results could be applied to early pregnancy diagnosis.

MATERIALS AND METHODS

Qualitative kit: The test kit was a bovine milk progesterone qualitative test EIA kit (Ovucheck Cowside W kit, Cambridge Veterimary 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*/m*l* 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 stabilising buffer solution.

Spot: 32 sample dropping disposable spots (3 mm in diameter × 123 mm in length). One drop from the spot is approximately 40 *μl*.

Standard saliva preparation: As the previous study showed that the criterion for positive pregnancy diagnosis was a progesterone concentration of more than 5 *ng*/m*l* [14], preparation of standard saliva was made at a progesterone concentration of 5 *ng*/m*l*. The method of preparation was the same as that used in the previous study [14]. First, saliva samples collected from 5 lactating sows by a method described later were pooled and treated by dextran coated charcoal [13], and a progesterone-free saliva solution was made. This was then poured into a test tube containing a standard progesterone which had been dried up from dissolved methanol solution at 85°C, and the standard saliva was made by adjusting the solution until the final progesterone concentration became 5 *ng*/m*l*.

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

Collection and preservation of saliva: Saliva was collected and preserved according to the method described in the previous report [14]. The instrument for collecting saliva was made by attaching 2 g of commercial absorbent cotton on to the tip of a wooden chopstick (approx. 20 cm long). This was inserted into the mouth of the sow for 1–3 min, and saliva was absorbed by the absorbent cotton. The absorbent cotton was then placed into a 10 m*l* or 20 m*l* disposable syringe, and compressed to extract the saliva. Sodium azide (0.05%) was added to the saliva as an antiseptic, and the saliva was frozen at −20°C until progesterone measurement.

Early pregnancy diagnosis: For this study, 138 sows, which mated between September 1991 and September 1992, were selected from sows raised at a nearby pig farm for cross-breeding. Saliva collection was carried out from day 17 to day 24 after mating (last mating day = 0). Pregnancy diagnosis was determined by comparing the color of the test saliva with that of the standard saliva; positive if the color of the test saliva was lighter than the standard saliva, indeterminable if the colors were similar, and negative if the color of the test saliva was darker than the standard saliva. Final pregnancy diagnoses were clarified by ultrasonography after day 22 of mating [9, 10]. Identical samples were also prepared for saliva progesterone 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 [14].

RESULTS

Early pregnancy diagnosis using the qualitative kit: Table 1 shows the accuracy of pregnancy diagnosis determined by qualitative measurements of the saliva progesterone concentration from day 17–24 of the last mating. The accuracy of cases diagnosed positive by the saliva progesterone concentration was 91.3% (105/115), and the accuracy of cases diagnosed negative was 100% (6/6). Out of the 17 indeterminable cases, 10 cases were later determined to be positive and 7 were negative. The total accuracy of the early pregnancy diagnosis, including the indeterminable cases, was 80.4% (111/138).

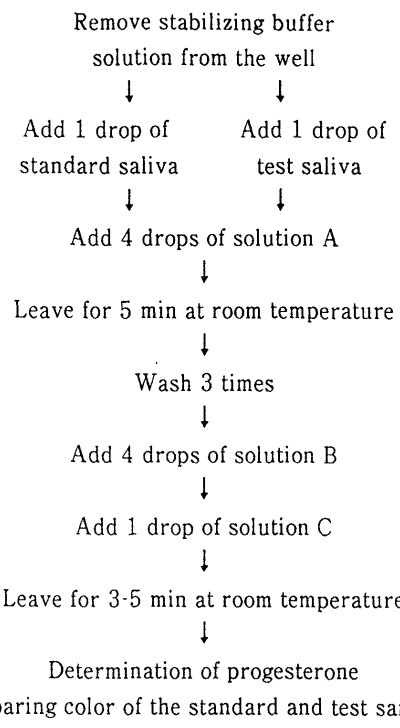

Fig. 1. Outline of the procedure for qualitative measurement of saliva progesterone in the sow using a qualitative EIA kit.

Accuracy of diagnosis using the qualitative kit: Figure 2 shows the relationship between the results using the qualitative kit and the progesterone concentration values measured by the quantitative kit. The 6 cases diagnosed as negative by the qualitative kit all had a progesterone concentration of less than 5 *n*g/m*l*. On the other hand, the 111 out of 115 cases diagnosed as positive by the qualitative kit all showed a progesterone concentration over 5 *n*g/m*l*, and the remaining 4 cases were less than 5 *n*g/m*l*. Out of the 17 indeterminable cases, 14 cases showed a progesterone concentration over 5 *n*g/m*l* and 3 cases less than 5 *n*g/m*l*. Thus, the results of positive or negative pregnancy diagnosis determined by qualitative measurements of the progesterone concentration were almost identical to the previously reported results of pregnancy diagnosis (positive for progesterone $\geqq 5$ *n*g/m*l* and negative for progesterone <5 *n*g/m*l*) based on quantitative measurements of the progesterone concentration [14].

DISCUSSION

In the past, blood samples have generally been used to measure the progesterone concentration in pigs for the purpose of assessing the luteal function. In the case of pigs, blood is generally collected from the cranial vena cava or

Table 1. Accuracy of pregnancy diagnosis at 17–24 days after mating, based on qualitative measurement of saliva progesterone 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	115	105	10	91.3% (105/115)
Negative	6	0	6	100.0% (6/6)
Indeterminable	17	10	7	
Total	138	115	23	80.4% (111/138)[b]

a) After 22 days of last mating.
b) Including 17 indeterminable cases.

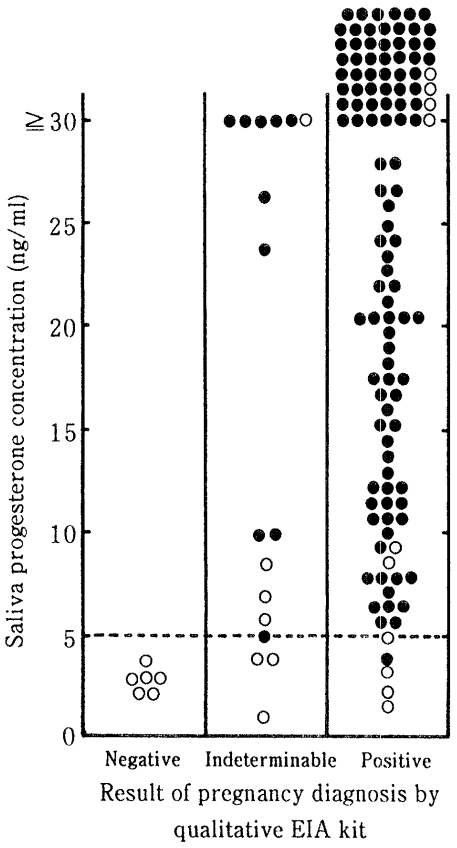

Fig. 2. Relationship between the results of pregnancy diagnosis based on qualitative measurement of the saliva progesterone concentration in the sow using the qualitative kit and the measurements of saliva progesterone concentration using the quantitative kit. ●: pregnant case, ○: non-pregnant case.

caudalauricular 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 saliva, which is easier to collect than blood, to measure the progesterone concentration by a bovine milk progesterone quantitative test EIA kit [14]. Previously, both saliva and plasma samples were collected, and a comparison of the progesterone concentrations in the two samples showed that in a low progesterone concentration range, the progesterone concentration was higher in saliva, while in a high progesterone concentration range, the progesterone concentration was higher in plasma, with a strong correlation between the two groups ($r=0.872$, $p<0.01$) [14]. Also, we were able to attain 96.9% accuracy in early pregnancy (17–24 days after last mating) diagnosis of sows, based on these progesterone concentration measurements [14]. The accuracy of early pregnancy (17–24 days after last mating) diagnosis of sows in the present study, which was based on qualitative measurements of the progesterone concentration using a qualitative kit, was 91.3% for positive cases, 100% for negative cases, and 80.4% (111/138) overall including the indeterminable cases. Thus, the accuracy using this method was slightly lower than that using the quantitative kit.

Among the 17 cases for which pregnancy diagnosis was indeterminable, there were some cases in which the measurement of progesterone concentration was much higher or much lower than 5 *ng/ml*. As for this, 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 method of determination. Also, although it is not clear why the 10 cases diagnosed as positive by the quantitative kit were shown to be negative by ultrasonography, the authors believe that some of these cases may have shown a high progesterone concentration despite negative pregnancy due to shortening or prolongation of the estrous cycle, or some cases may have actually been pregnant at the time of progesterone measurement but later showed negative in ultrasonography due to early embryo death.

The reported accuracies of the other main methods of pregnancy diagnosis for the sow are: 81.3–85.2% for the rectal examination method at 20 days after mating [1, 2, 6, 11]; 94–97% for the vaginal mucosal tissue examination at 20 days after mating [4, 15]; 58–80% at 22 days after mating and 91.9–100% at 40–50 days after mating for the ultrasonic Doppler method [24]; 96–100% for the ultrasonic echo method at 30–90 days after mating [12]; and 100% for

ultrasonography at more than 22 days after mating [9, 10]. Also, as an endocrinological method for pregnancy diagnosis, Tamamura *et al.* [23] reported 93.3% accuracy at 22–27 days after mating, based on measurements of blood estrone sulphate by EIA. As for the accuracy of pregnancy diagnosis using EIA to measure the progesterone concentration, Saga *et al.* [20] 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. Glossop *et al.* [7] measured the progesterone concentration in whole blood using an Ovucheck Sowside kit (Cambridge Veterinary Sciences Ltd., UK), which is similar to the quantitative kit used in the present study, and reported accuracies of 87.1% based on quantitative progesterone concentration measurement and 87.3% based on qualitative progesterone concentration measurement in early pregnancy (17–20 days after mating) diagnosis. Although the accuracy of early pregnancy diagnosis in the present study, based on qualitative measurements of the saliva progesterone concentration in sows is slightly lower than the accuracies reported using the above methods, the method used in the present study is considered to have high clinical value in terms of simplicity and speed.

The results of this study which showed 6 non-pregnant cases with a progesterone concentration of 5–10 *ng*/m*l*, indicate that the diagnosis criterion should have a range including 10 *ng*/m*l* as well as 5 *ng*/m*l*. Also, it is recommended that for indeterminable cases still within 17–24 days after mating, saliva should be collected again after 1–2 days and retested by the same method.

The results of the present study have shown that qualitative measurement of the progesterone concentration in saliva, which can be easily collected without the requirement of any special instruments, using a bovine milk qualitative test EIA kit, which can be operated easily and can measure the progesterone concentration in approximately 10 min, is a sufficiently accurate and clinically useful method for early pregnancy diagnosis in the sow.

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