ELSEVIER

EVALUATION OF FALSE ULTRASONOGRAPHIC PREGNANCY DIAGNOSES IN SOWS BY MEASURING THE CONCENTRATION OF UNCONJUGATED ESTROGENS IN FECES

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ABSTRACT

On Days 26, 28, and 30 after AI, ultrasonographic pregnancy diagnoses were performed on 207 gilts and sows by using a 3.5 MHz linear-array transducer. Fecal samples were taken from the rectum after each ultrasonographic examination, and the concentrations of unconjugated estrogens in selected samples (n=73) were measured by RIA. Fecal unconjugated estrogen concentration of 11.7 ng/g feces or higher was indicative of pregnancy. The overall sensitivity and specificity of the ultrasonographic test was 99% for farrowing sows and 73.1% for nonfarrowing sows. With one exception, sows with a false negative diagnosis by ultrasonography on Day 26 were correctly diagnosed pregnant by elevated fecal unconjugated estrogens or repeated ultrasonographic examinations on Days 28 or 30. Return to estrus around the sampling period may cause false positive results in the unconjugated estrogen assay, while early embryonic mortality can result in false positive diagnoses in both the ultrasonographic test and estrogen assay. Although there was a positive correlation between the concentrations of unconjugated estrogens in the feces and litter size at farrowing in the selected sows, it seems very unlikely that fecal estrogens can provide an accurate tool for predicting litter size.

Key words: pregnancy diagnosis, sow, ultrasonography, unconjugated estrogens, feces

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INTRODUCTION

Accurate and early detection of pregnant and nonpregnant sows may be a tool for improving the reproductive performance of a herd by reducing the number of open days lost. One of the most recent and accurate techniques for early pregnancy diagnosis in sows on the farm is B-mode ultrasonography. An accuracy of a 3.0 or 3.5 MHz linear-array or sector transducer for the detection of the pregnancy between 18 and 23 d after service has been investigated by several authors. In these studies, 2.5 to 5.7% of the farrowing animals were incorrectly diagnosed as nonpregnant and 31.8 to 47.7% of the nonfarrowing sows were incorrectly diagnosed as pregnant (9-11). Between 22 and 32 d after insemination fewer incorrect diagnoses (0 to 0.5% for false negative and 9.4 to 26.2% for false positive diagnoses) were detected (10,11,16,19) than those between Days 18 and 23 after service.

The early pig blastocyst (12) synthesizes substantial amounts of estrogens (mainly estrone and estradiol-17β, which pass through the uterine wall, where they are sulphoconjugated (5-6). These estrogen metabolites in maternal blood and urine or the deconjugated estrogens in the feces (3,13) between Days 23 and 30 after service are good indicators of the presence of live embryos. The estimation of estrogens is therefore a useful method for early pregnancy diagnosis in sows (1,3,14). Feces are more easily collected than blood or urine. According to Choi et al. (3) the concentration of estrogens in feces is highest between 27 and 29 d after service.

Because published results on early pregnancy diagnosis obtained from studies using real-time, B-mode diagnostic ultrasonography, equipped with a 3.0 to 3.5 MHz linear-array or sector transducer are still variable, it is necessary to evaluate the false diagnoses. The present study was thus undertaken to investigate to what extent pregnancy and nonpregnancy diagnoses made by ultrasonography on Days 26, 28 and 30 after AI could be confirmed by the measurement of unconjugated estrogen concentrations in feces collected on the same day.

MATERIALS AND METHODS

On Days 26, 28 and 30 after insemination 207 animals (sows and gilts) were tested on a commercial farm with a real-time, B-mode diagnostic ultrasound scanner (Type SSD-500)[a] equipped with a 3.5 MHz linear-array transducer. The animals were housed individually in cages which opened from behind. Scanning took place with the pig standing. At each examination, the operator was required to record a diagnosis of either pregnancy or nonpregnancy without reference to earlier results. A pig was considered to be pregnant when several, irregularly shaped, nonechogenic black spots, representing the allantoic fluid of conceptuses, appeared on the monitor within the uterine lumen. When a view of a pregnant uterus on both sides of the abdomen could not be accomplished, the result of the test was considered negative, as described previously (16). All ultrasonographic examinations were performed and interpreted by the same investigator. Immediately after scanning, 3 to 5 g of feces were taken from the rectum and stored in plastic vials at -20 ^{0}C until assayed. The unconjugated estrogens in the feces of randomly selected

[a] ALOKA Co. Ltd, Tokyo, Japan.

pregnant sows (n=42) and all other sows (nonpregnant and sows with false positive and negative ultrasonographic diagnoses: n=31) were measured by radioimmunoassay, as described previously (17). In addition, the concentration of unconjugated estrogens was also estimated in the feces of 20 non-pregnant sows just before weaning. The mean (± SD) concentration in these 20 samples was 7.4 ± 1.4 ng/g feces for the radioimmunoassay. From this value the limiting value (mean + 3SD) was calculated to distinguish between pregnant and nonpregnant animals. Sows with a concentration of 11.7 ng/g feces or higher in the radioimmunoassay were classified as pregnant.

Confirmation of pregnancy was based on farrowing records (date, total litter size: live plus dead piglets, and the numbers of male and female piglets) or on another observed event such as abortion. Nonpregnancy was confirmed by a return to estrus and subsequent rebreeding, by the failure to farrow at the expected time, or by an examination of the reproductive tract after slaughter.

On the basis of the final farrowing data the ultrasonographic results were grouped and calculated as follows: a) correct positive diagnosis, b) incorrect positive diagnosis, c) correct negative diagnosis and d) incorrect negative diagnosis. From these values the sensitivity [a/(a+d)x100], the specificity [c/(c+b)x100], the positive predictive value [a/(a+b)x100] and the negative predictive value [c/(c+d)x100] of the pregnancy test were calculated as described previously (16-17). Concentrations of fecal estrogens in pregnant sows were not normally distributed on Days 28 and 30 and are therefore shown as a boxplot diagram (Figure 1). The Wilcoxon Signed Rank Test was used to check for significant differences of fecal estrogen concentrations between the three days of fecal sampling. Correlations between the concentrations of unconjugated estrogens in the feces on each day and the total number of piglets born (alive plus dead), the number of males, the number of females, the ratio of males to females or the ratio of females to males were calculated with the Pearson Correlation test. All tests were carried out with the software package Sigma Stat® for Windows Version 2.0.[b]

RESULTS

The quantitative results of ultrasonographic examinations are presented in Table 1. Of the 176 farrowing sows, 172 (97.7%) were already diagnosed correctly on Day 26 after insemination. Five false negative diagnoses (4 at Day 26 and 1 at Day 30) in 4 farrowing sows were made by ultrasonography (Table 2). These sows gave birth to 2, 4, 6 and 9 piglets, respectively. Of the 31 nonfarrowing sows, 27 (87%), 20 (64.5%) and 21 (67.7%) were correctly diagnosed by ultrasonography as nonpregnant on Day 26, 28 and 30 after AI (Table 3). However, there were 13 sows with all together 25 false positive ultrasound diagnoses made on Day 26 (n=4), Day 28 (n=11) and Day 30 (n=10) after service (Table 4).

The mean (± SE) fecal unconjugated estrogen concentration of 42 randomly selected pregnant sows was 20.9 ± 1.3 ng/g feces on Day 26 and was significantly ($P<0.001$) lower than on the other two examined days. Concentrations on Day 28 showed a tendency (although

[b]Jandel Cooperation, Erkrath, Germany.

Table 1. Early pregnancy diagnosis in pigs performed by real-time B-mode ultrasonography equipped with a 3.5 MHz linear-array transducer

	Test performed during days after AI			
Grouping and evaluation	Day 26	Day 28	Day 30	Day 26 - 30
Diagnosis pregnant correct	172	176	175	523
Diagnosis pregnant incorrect	4	11	10	25
Diagnosis nonpregnant correct	27	20	21	68
Diagnosis nonpregnant incorrect	4	0	1	5
Sensitivity /%/	97.7	100	99.4	99.0
Specificity /%/	87.0	64.5	67.7	73.1
+ predictive value /%/	97.7	94.1	94.5	95.4
- predictive value /%/	87.0	100	95.4	93.1
Overall accuracy /%/	96.1	94.6	94.6	95.1

Table 2. Unconjugated estrogen concentrations (UEC) in the feces (ng/g feces) of pregnant sows diagnosed as nonpregnant (false negative diagnosis) in some cases by real-time B-mode ultrasonography (US) using a 3.5 MHz linear-array transducer

	Test performed during days after AI						
	Day 26		Day 28		Day 30		
Sow ID number	US	UEC in feces	US	UEC in feces	US	UEC in feces	Number of piglets born
2361	NP	8.7	P	8.6	P	11.0	2
3739	NP	23.4	P	24.9	NP	21.5	4
2002	NP	24.3	P	27.9	P	20.5	6
3985	NP	25.7	P	30.2	P	22.9	9

NP: non-pregnant.
P: pregnant.

Table 3. Unconjugated estrogen concentrations in the feces (ng/g feces) of nonfarrowing sows diagnosed correctly by real-time B-mode ultrasonography using a 3.5 MHz linear-array transducer

	Test performed during days after AI			
Sow ID number	Day 26	Day 28	Day 30	Remarks
1464	14.7	10.9	14.7	AI: Day 29
1655	8.5	6.9	10.1	AI: Day 28
1999	14.2	8.9	10.6	AI: Day 29
2023	30.0	41.0	45.6	
2129	33.6	16.4	7.6	AI: Day 27
2742	12.4	9.3	7.3	
3165	5.5	7.2	11.9	
3667	15.8	8.6	4.7	
3735	16.6	10.2	23.4	AI: Day 28
3909	6.0	7.7	9.0	AI: Day 77
3999	8.7	6.0	8.7	AI: Day 45
4003	3.8	13.2	6.8	
1649	10.8	8.6	9.9	
2278	14.5	7.3	9.0	
2695	7.3	5.6	7.1	
3966	6.2	7.0	9.6	
3778	7.8	4.8	6.4	
3609	8.0	10.0	11.2	
1551	13.9			
1463	12.8			
211	10.7			
2534	9.2			
3437	8.0		10.4	AI: Day 28
3398	15.8		13.1	
2939	10.0		12.1	AI: Day 28
4012	3.4	3.7		AI: Day 78
3869	3.5	7.6		AI: Day 43

not significant; $P = 0.26$) to be higher than on Day 30 (Figure 1). The positive correlation between the total number of piglets born or the number of live born piglets and the concentration of unconjugated estrogens in the feces was highest for the samples ($n=42$) taken on Day 26 ($r=0.46$ and $r=0.51$, respectively; $P<0.01$). The highest concentrations of estrogens (70; 96; 122 ng/g feces) were found in those sows which had mainly male (male to female ratio: 4; 9 and 9, respectively) piglets (all showed a litter size of 10 or more). Fecal estrogen concentration were significantly ($P<0.01$) correlated with the number of live male piglets (highest on Day 26; $r=0.53$) and the ratio of males to females (highest on Day 30; $r=0.62$; $P<0.001$).

Table 4. Unconjugated estrogen concentrations (UEC) in the feces (ng/g feces) of nonfarrowing sows diagnosed as pregnant (false positive diagnosis) at least once by real-time B-mode ultrasonography (US) using a 3.5 MHz linear-array transducer

	Test performed during days after AI						
	Day 26		Day 28		Day 30		
Sow ID number	US	UEC in feces	US	UEC in feces	US	UEC in feces	Remarks
3987	P	27.1	P	24.5	P	20.8	
2344	P	12.3	P	18.5	P	21.8	
3458	P	13.0	P	19.1	P	35.6	
4023	P	25.2	P	32.1	P	25.0	
1551			P	11.0	P	16.5	Very small conceptuses
1463			P	13.7	P	10.1	
211			P	14.1	P	10.8	
2534			P	7.2	P	16.0	
3437			P	12.4			AI: Day 28
3398			P	19.7			
2939			P	10.6			AI: Day 28
4012					P	10.8	AI: Day 78
3869					P	8.8	AI: Day 43

NP: nonpregnant.
P: pregnant.
AI: artificial insemination.

There were no significant correlations between the other parameters (the number of males, the number of females and the ratio of females to males) investigated. Among the randomly selected farrowing sows there were 5 sows in which the unconjugated estrogen concentrations did not reach the threshold level on Day 26, but with the exception of 2 sows (8.7 and 8.5 ng/g feces, with 2 and 6 piglets, respectively) they were very close (10.7, 11.3 and 11.5 ng/g feces) to it and gave birth to 4, 6 and 6 piglets, respectively.

The fecal unconjugated estrogen concentrations of the 4 sows with 5 incorrect nonpregnancy diagnoses made by ultrasonography on Day 26 (n=4) and Day 30 (n=1) after AI are presented in Table 2. With the exception of one sow, which gave birth to only 2 piglets, the concentrations of unconjugated estrogens in the feces were above the threshold level.

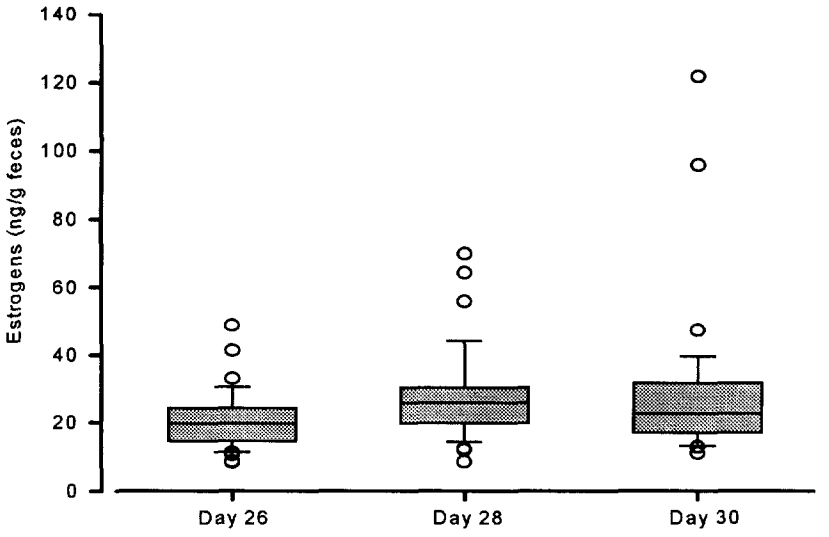

Figure 1. Boxplot of unconjugated estrogen concentration in the feces of 42 sows at early stages of pregnancy.

The fecal unconjugated estrogen concentrations of the sows with correct nonpregnancy diagnoses made by ultrasonography between Days 26 and 30 after AI are presented in Table 3. The unconjugated estrogen concentrations in the feces were below the threshold level in 16 sows on Day 26 (59.2%), 17 sows on Day 28 (85%) and in 15 sows on Day 30 (71.4%), while in some sows elevated fecal estrogen concentrations occurred around the return to estrus. On the other hand, the fecal unconjugated estrogen concentration of the other 13 nonfarrowing sows which had false positive ultrasound diagnoses was below the threshold level in 3 sows on Day 28 and in 4 sows on Day 30 after AI (Table 4).

DISCUSSION

The use of transabdominal real-time, B-mode ultrasonography for early pregnancy diagnosis in pigs has been reported by several authors in recent years (9-11,16,19). The overall accuracy has been reported to range between 93.2 and 98.9% when ultrasonography was performed between 22 and 32 d after breeding. In the present study a similar overall accuracy (95.1%) was reached.

The concentrations of unconjugated estrogens in the feces of 42 selected pregnant sows did not reach the threshold level in 5 sows on Day 26, and with the exception of 1 sow (which gave birth to 2 piglets) no errors were detected on Days 28 and 30 after service. So it can be concluded that fecal estrogens are a reliable index for the presence of live embryos in the sow, confirming earlier data published by Choi et al. (3).

In the present study, 5 false negative diagnoses were made by ultrasonography on Days 26 and 30 after AI. The concentration of unconjugated estrogens in the feces exceeded the threshold level in 3 of 4 sows during the examination period. The remaining sow gave birth only to 2 piglets. So, with the exception of that sow, on the basis of these positive estrogen tests, the false nature of some of our negative ultrasonographic diagnoses could already be confirmed during early pregnancy (20). In agreement with Botero et al. (2) the false negative ultrasonographic diagnoses were most probably due to the smaller volume of allantoic fluid, especially in the sows with small litters (all gave birth to 6 or less piglets). The condition of the sow, the method of restraint or the time taken for each individual ultrasound examination can also play an important role in the rate of false negative diagnoses (2,16). With the exception of one sow, the false negative diagnoses made on Day 26 could also be corrected by examining these sows again on Day 28 or Day 30. Thus, these results suggest that repeating the ultrasonographic examinations in sows with a negative diagnosis has to be recommended.

In the cases of correct negative diagnoses made by ultrasonography, the concentration of the unconjugated estrogens in the feces was above the threshold level in 11 (Day 26), 3 (Day 28) and in 6 (Day 30) feces samples. Four of these sows were reinseminated around the examined period between Days 27 and 29. It may be assumed that in these cases the estrogens originated from growing follicles rather than from conceptuses. There are, however, no data in the literature concerning the changes of unconjugated estrogens in the feces during the normal estrus cycle. However, it is well known that before ovulation the concentration of total estrogens in the plasma is high (7). In the present study, a false positive ultrasound diagnosis was made at least once in 13 sows. With the exception of 7 samples (3 on Day 28 and 4 on Day 30), the concentration of unconjugated estrogens in the feces taken from these sows on the day of scanning exceeded the threshold level. In fact, only 2 sows had fecal estrogen values below the threshold in each of the three samples taken. Taverne et al. (19) and Botero et al. (2) suggested that the false positive ultrasonographic diagnoses may result either from embryonic mortality or from intrauterine fluid (endometritis) other than allantoic fluid. So on the basis of the positive result of both the ultrasonographic and the RIA test, it can be assumed that most of these sows were in fact pregnant between Days 26 and 30 after service, but because of early embryonic mortality they subsequently failed to farrow. In agreement with other studies, the overall accuracy of diagnosing nonfarrowing sows at an early stage by ultrasonography (10,11,16,19) or estrogen test (1,4,19) greatly depends on the rate of the occurrence of early embryonic mortality.

Although there was a positive correlation between the concentrations of unconjugated estrogens in the feces and litter size at farrowing in the selected sows, it seems very unlikely that fecal estrogens can provide a tool to predict litter size accurately; in another study, only one third of the small litters could be predicted accurately by measuring the concentration of estrone sulphate in the serum (4,15). Sows with the highest concentrations of unconjugated estrogens in the feces gave birth primarely to male piglets in this study. By measuring the concentration of

estrone sulphate in the serum of pregnant sows, a similar significant correlation was not detected (4). However, Tarraf and Knight (18) recently reported that the placentas of male fetuses release higher concentrations of estrone on Day 50 of gestation than the placentas of female fetuses.

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