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Assessment of diagnostic accuracy and effectiveness of trans-abdominal real-time ultrasound imaging for pregnancy diagnosis in breeding sows under intensive management

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Abstract

Early and accurate determination of pregnancy is critical to optimum reproductive performance in pigs and enables farmers to early rebreed or cull non-pregnant animals. Most of the conventional diagnostic methods are unsuitable for systematic application under practical conditions. The advent of real-time ultrasonography has made it possible to establish relatively more reliable pregnancy diagnosis. The present study was carried out to evaluate the diagnostic accuracy and effectiveness of trans-abdominal real-time ultrasound (RTU) imaging vis-à-vis pregnancy status in sows reared under intensive management. Trans-abdominal ultrasonographic examinations were performed using a mechanical sector array transducer and portable ultrasound system in crossbred sows from 20 days post-insemination for up to next 40 days. Animals were followed up for subsequent reproductive performance with farrowing data used as the definitive test for deriving predictive values. Accuracy for diagnosis was determined by diagnostic accuracy measures like sensitivity, specificity, predictive values, and likelihood ratios. Before 30 days of breeding, RTU imaging had 84.21% sensitivity and 75% specificity. Relatively higher false diagnosis rates were obtained in animals checked at or before 55 days after AI than in animals checked after 55 days (21.73% versus 9.09%). Negative pregnancy rate was low with 29.16% (7/24) false positives. Overall sensitivity and specificity, using farrowing history as the gold standard, were 94.74% and 70.83% respectively. The sensitivity of testing tended to be slightly lower in sows with litter size of less than 8 total born piglets, compared to sows with 8 or more piglets. Overall positive likelihood ratio was 3.25 while negative likelihood ratio was 0.07. The results indicate that pregnancy in swine herds can be reliably detected earlier in gestation by 30 days post-insemination using trans-abdominal RTU imaging. This non-invasive technique with portable imaging system can be used as an integral part of reproductive monitoring and sound management practices for profitable swine production systems.

Keywords Real-time B-mode · Ultrasound imaging · Pregnancy diagnosis · Accuracy · Sow

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Introduction

Early and correct detection of pregnant and open female animals is important for efficient pig production system as it has the potential to increase reproductive efficiency and profitability by reducing non-productive days per animal per year. Estrus detection and non-return rate alone will not be able to accurately identify all non-pregnant pigs as they may fail to show subsequent estrous activities due to multiple factors viz. sub-optimal nutrition, climatic stress, delayed cycle lengths, silent estrus, anestrus, or cystic ovarian disorders. The cost of any pregnancy diagnostic test other than non-return rate depends upon a relatively small portion of all anestrus females that need to be diagnosed for pregnancy. Therefore, in order to be a profitable piggery enterprise, pregnancy diagnosis methods in breeding herd should be highly accurate and reliable (Taverne 1989). A reliable and early pregnancy diagnosis enables pig farmers to either rebreed or cull non-pregnant animals in the breeding herd as soon as possible, and accordingly, it is of great significance to maintain a low average post-service interval and good productivity in the herd.

Pregnancy diagnosis in pigs can be performed using different methods. Physical methods, vaginal biopsy, and methods based on hormone concentrations are described, but these are unsuitable for systematic application under practical conditions (Almond and Dial 1986, 1987; Vos et al. 1999). The technique of ultrasound imaging has important applications in veterinary practice both in research field and for diagnostic purposes (Yeager et al. 1992). A-mode and Doppler imaging technique used in the past were considered non-imaging system (Lindhal, 1969). The advent of real-time (B-mode) ultrasound (RTU) scanning has made it possible to establish relatively more reliable and early pregnancy determination and its integration into modern swine production systems can significantly facilitate the optimal use of reproductive imaging in swine production sector (Kauffold et al. 2019).

Real-time ultrasonography provides two-dimensional image of the scanned part on a real-time screen. Transabdominal real-time ultrasound imaging with multifrequency sector probe (ranging from 3.5 to 5 MHz) can be useful in diagnosis of pregnancy and status of estrous cyclicity in breeding sows (Almond et al. 1998; Gokuldas, 2015). For pregnancy determination in sows using ultrasonography, decisions can be made based on the appearance of fluid-filled gestational sacs within the surrounding white-gray echogenic endometrial tissue (Miller et al. 2003). With the development of portable and advanced equipment, the use of RTU imaging for pregnancy diagnosis in pigs has increased rapidly over the past few decades and has now been used even with advanced machine-learning techniques (Kousenidis et al. 2022). In this backdrop, the present study was carried out to evaluate the diagnostic accuracy and effectiveness of a portable trans-abdominal real-time ultrasound imaging system visà-vis pregnancy status in breeding sows under intensive management system.

Materials and methods

Location and experimental animals

The animals represented in this study were apparently healthy crossbred sows with mean age of 18.5 ± 0.32 months and in their first and second parity maintained in the Institute Farm Complex, ICAR-National Research Centre on pig, Guwahati, Assam (26.01° Lat. N., 91.34° Long. E, 56 m above MSL). A total of forty-three crossbred (cross between Hampshire and Ghungroo, a registered indigenous pig breed of India) were selected and estrus detection in these animals were performed by twice-daily checks and primarily through back pressure test (standing reflex) and by observing other important signs of estrus. Sows observed in estrus were artificially inseminated twice during estrus using good quality liquid-preserved boar semen (Gokuldas et al. 2018). Selected animals were housed in individual pens $(1.25 \times 3.5 \text{ m})$ under intensive system of rearing. Sheds were adequately ventilated with asbestos roofing, water proof, and rough finish concrete floors and oriented in north-south direction. Animals were fed with 2.5 kg/day of nutritionally balanced and standard diets and were given ad libitum access to water during the experimental period from July 2015 to April 2016.

Procedure of ultrasound imaging

Trans-abdominal real-time B-mode ultrasonographic examinations were performed in the selected animals using a mechanical sector array transducer (3.5-MHz frequency) and portable ultrasound scanner. Every sow was individually monitored for pregnancy daily during consecutive days, starting 20 ± 2 days after the insemination for up to next 40 days. The day of first artificial insemination was designated as day 0 for each experimental animal. The imaging was performed on the sows while they were kept in standing position in individual pens using a real-time B-mode ultrasound machine (MINISCAN®, Meditech Equipment Co. Ltd., Qingdao) and all the experimental sows were tested by the same person on the right abdominal wall of the sow, below the region of the fold of the flank, near the caudal three mammary glands. Before scanning, surface of the transducer or probe was lubricated with coupling gel (AquaSolid[®], Sushobh Meditech, Pune) to maximize wave propagation and contact between skin and the probe. The transducer was applied to contact the lower abdomen just lateral to nipple line and ahead of the rear leg and was directed dorso-caudally and dorso-cranially with sufficient pressure to visualize the organs in the pelvic cavity (Maes et al. 2006). Appearance of non-echogenic and hyper-echoic areas in the uterus, fluid-filled anechoic vesicles and presence of embryo(s) or fetal mass (Fig. 1) were observed as the evaluated parameters (Miller et al. 2003). All procedures involving the use of animals were conducted in accordance with the extant guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPC-SEA), Govt. of India, and were duly approved by the Institute Animal Ethics Committee (IAEC).

Measurement of diagnostic accuracy

Animals were followed up for subsequent reproductive performance with farrowing data as definitive test or gold standard for deriving accuracy measures and predictive values. Confirmation of non-pregnancy was based on return to estrus, rebreeding as well as recorded non-farrowing. Accuracy for diagnosis was determined by diagnostic accuracy measures like sensitivity (proportion of sows diagnosed as pregnant that farrow), specificity (proportion of sows diagnosed as not pregnant that fail to farrow), likelihood ratio (alternative measure of accuracy which combines both specificity and sensitivity), and predictive values (measures of the usefulness of a test once the test results are known) (Campbell et al. 2007; Williams et al. 2008) as provided in the Table 1.

Statistical analysis

Data forms were manually entered into an electronic database (Microsoft[®] Excel[®] 2019 MSO (Version 2208 Build 16.0.15601.20148), Microsoft Corp., WA, USA), which was also used to perform part of the analysis. Additional data summaries and statistical analysis were performed using SAS[®] software (License site number 11601386, version 9.3 for Windows, SAS Institute Inc., NC, USA) and *p*-values \leq 0.05 were considered a statistically significant difference. The chi-square test was used to analyze the proportionate data and differences in the characteristics of diagnostic methods were tested by using Fisher's exact test (SAS Proc FREQ, SAS, version 9.3).

Results

In the present study, trans-abdominal ultrasonographic examinations were performed in forty-three crossbred sows from 20 days post-insemination for up to next 40 days. Using transabdominal RTU imaging technique, sows were positively diagnosed for pregnancy with > 81% accuracy by day 30 of gestation and with 94% accuracy by day 45 of gestation. Combined accuracy of RTU imaging, defined as the proportion of correct number of diagnoses for pregnant and non-pregnant

Fig. 1 Representative ultrasonograms (real-time B-mode) of artificially inseminated crossbred sows screened for the study. A Distinct hyper-echoic fetal mass (arrow) enclosed in anechoic (fetal sac) region (35 days post-insemination). B Uterine cross-section containing fetal fluid and the fetus (arrow) on day 45 post-AI. C Image of fetal vertebral column-arrow (65 days post-insemination). D Hyper-echoic fetal mass inside fluid-filled anechoic region (fetal sac)



	Pregnant animals	Non-pregnant animals	Total animals
	Expressed as	Expressed as	Expressed as
Positive Diagnosis	А	b	Total positive tests (a + b)
Negative Diagnosis	С	d	Total negative tests (c + d)
Total animals	Pregnant animals $(a + c)$	Non-pregnant animals (b + d)	Animals scanned $(a + b + c + d)$

 Table 1 Defining sensitivity, specificity and predictive values in swine pregnancy diagnosis

*Sensitivity: proportion of pregnant animals with a positive diagnosis result $\{a/(a + c)\}$

**Specificity*: the proportion of non-pregnant animals with a negative diagnosis result $\{d/(b + d)\}$ **Positive predictive value*: the proportion of animals with a positive diagnosis in total pregnant animals $\{a/(a + b)\}$

*Negative predictive value: the proportion of animals with a negative diagnosis in total non-pregnant animals $\{d/(c+d)\}$

**Positive likelihood ratio* (LR+) = sensitivity/(1-specificity)

**Negative likelihood ratio* (*LR*–) = (1-sensitivity)/(specificity)

 Table 2 Different diagnostic accuracy measures of trans-abdominal RTU (B-mode) imaging with 3.0-MHz transducer in artificially inseminated sows

Sl no	Diagnostic accuracy measures	Before day 30 of service	After day 30 of service
1	Sensitivity	84.21%	94.74%
2	Specificity	75.00%	70.83%
3	Positive predictive value	72.73%	72.00%
4	Negative predictive value	85.71%	94.44%
5	Combined accuracy	79.07%	81.40%
6	Positive likelihood ratio	3.37	3.25
7	Negative likelihood ratio	0.21	0.07

combined, was 79.07% before 30 days of service and 81.4% after 30 days of service (Table 2). Trans-abdominal real-time B-mode ultrasound imaging technique employing 3.5-MHz frequency probe had 85% sensitivity and 65% specificity in breeding sows after 30 days of artificial insemination. Significantly higher (p < 0.05) false diagnosis rates (false negatives and false positives) were recorded in animals scanned at or before 55 days post-insemination than in animals scanned after 55 days (21.73% versus 9.09%). Negative pregnancy rate was low with 29.16% false positives. Sensitivity and specificity of ultrasound imaging, using farrowing history as the gold standard, were 84.21% and 75.0% before day 30 of service while, after day 30 of service, the measures were 94.74% and 70.83%, respectively (Table 2). The sensitivity of testing tended to be slightly lower in sows with litter size of less than 8 total piglets, compared to sows with 8 or more piglets.

Likelihood ratio is another measure of diagnostic accuracy where it combines the values of sensitivity and specificity. In the current study, overall positive likelihood ratio (LR+) was 3.25 while negative likelihood ratio (LR-) was 0.07 (Table 2). Predictive values are measures of the usefulness of the diagnostic test once the test results were known. Predictive values of positive diagnosis were relatively lower with an overall value of 72%, whereas those of negative test results were high (Table 2). Significantly higher (p < 0.05) number of pregnant animals were positively diagnosed as compared to non-pregnant animals (Fig. 2). Similarly, a significantly higher (p < 0.05) number of non-pregnant animals were negatively diagnosed as compared to pregnant animals. Overall, out of 19 pregnant sows, 94.74% sows were diagnosed positive while 70.83% of total non-pregnant animals were diagnosed negative. In contrast to the low and constant predictive values of positive diagnosis, values of negative diagnosis were relatively higher with an overall value of 94.4% and showed increasing trend as gestation progressed towards term.

Discussion

In the present study, animals were positively diagnosed by day 30 of gestation with 81% accuracy. Miller et al. (2003) also reported an accuracy of 90% for identifying pregnant females during 30 to 35 days post-mating. In general, recognition of pregnancy is easier than non-pregnancy in sows using trans-abdominal RTU imaging (Kauffold et al. 1997). The allantoic vesicles undergoes apparently rapid growth in length around day 23 of pregnancy in pigs (Almond and Dial, 1987). Also, studies demonstrated that RTU is an accurate method of pregnancy diagnosis when used after 24 days of gestation (Flowers et al. 2000). When compared to A-mode, RTU imaging provides more accurate pregnancy diagnosis as early as third week after mating (Inaba et al. 1983; Taverne et al. 1985; Martinez et al. 1992). Williams et al. (2008) have reported relatively lower efficiency 70% for RTU imaging in pigs. It is worth noting that accuracy for positive pregnancy diagnosis during early gestation is based on the ability to detect multiple, clear, fluid-filled pockets within the uterus. A reduced accuracy of RTU imaging can occur in cases of misinterpretation of fluid vesicles within the abdomen and pelvic cavity (Knox and Flowers 2004).





In several studies involving the use of trans-abdominal real-time B-mode ultrasonography, animals were checked using a 3.5-MHz transducer (Taverne et al. 1985; Martinez et al. 1992; Armstrong et al. 1997). This non-invasive method allows the deep, wide-angle tissue penetration that is necessary to quickly visualize the uterus and uterine contents. Ultrasound imaging technique in the study employed 3.5-MHz frequency probe and had 85% sensitivity and 65% specificity in sows after 30 days of service. This is marginally lower compared to the earlier findings of Flowers et al. (2000), who reported 98.3% sensitivity and 74.3% specificity during 24 to 35 days of gestation. Gaggini et al. (2012) detected both embryonic vesicles and embryos in pregnant pigs on day 40 post-insemination using ultrasound imaging with sensitivity, specificity, positive, and negative predictive values as 93.75, 100, 100, and 90.91%, respectively. Stancic et al. (2012) evaluated pregnancy in gilts and sows using a 3.0-MHz ultrasonic probe on day 41 post-insemination and observed that the accuracy of positive diagnosis (99.17%) was higher than the negative diagnosis (89.7%). It is worth noting that the level of accuracy depended on factors like type of ultrasound probe used, transducer frequency (3.5, 5.0 or 7.5 MHz), stage of gestation, skill, and experience of the operator. In contrast to the findings in the present study, Flowers and Knox (2000) reported that 5-MHz or 7.5-MHz transducer tends to provide more accurate results than a 3.0-MHz transducer for early pregnancy determination.

In the present study, predictive values of positive diagnosis were relatively lower, whereas those of negative test results were high. This result is in contrast to the findings of Maes et al. (2006) who reported higher predictive values of positive test results and lower predictive values for negative test results both using trans-abdominal linear transducer with 5-MHz frequency and sector transducer with 3.5-MHz frequency. Significantly higher (p < 0.05) number of pregnant animals were positively diagnosed as compared to non-pregnant animals in the present study. Furthermore, predictive values of negative diagnosis were comparatively higher than positive diagnosis and showed increasing trend as gestation progressed towards term. This may be possibly due to the use of trans-abdominal approach with mechanical sector array transducer having low frequency which may not be sufficient to clearly visualize the deep uterine contents and fetal parts. Transducer with frequencies of 5 MHz or 7.5 MHz tends to provide more accurate results than a 3.0-MHz transducer for early pregnancy determination (Flowers and Knox 2000). Transrectal ultrasound scanning may facilitate a more detailed image compared to transabdominal approach in pigs (Kauffold et al. 2019). Also, incidences of early embryonic mortality and pseudopregnancy are comparatively high in swine species which can result in false positive diagnoses.

To sum up, the results of the present study revealed that pregnancy in pig breeding herds maintained under intensive system of management can be reliably detected as earlier by 30 days post-service using portable trans-abdominal real-time (B-mode) ultrasound imaging technique. Results also imply that animals with a positive diagnosis early in pregnancy should be re-examined later. In contrast, predictive values for negative results were comparatively high which indicates that the imaging method is more efficient in detection of non-pregnant animals. This safe, portable, and non-invasive technique can be used as an integral part of reproductive monitoring and sound management practices for profitable pig production systems and can be justified for use in intensive swine production systems.

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Data availability Further information on the data and methodologies will be made available by the corresponding author upon reasonable request.

Declarations

Statement of animal rights All procedures involving the use of animals were conducted in accordance with the extant guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were duly approved by the Institute Animal Ethics Committee (IAEC).

Consent to participate Not applicable.

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Conflict of interest The authors declare no competing interests.

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