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ULTRASOUND DIAGNOSIS OF EARLY PREGNANCY IN SWINE*

IVAN STANČIĆ, MILOŠ BEUKOVIĆ, IVAN RADOVIĆ,
BLAGOJE STANČIĆ, SAŠA DRAGIN, MIHAJLO ERDELJAN[1]

SUMMARY:Early pregnancy diagnosis significantly affects the efficiency of the intensive swine production. Furthermore, early pregnancy diagnosis of non-pregnant animals reduces the number of non-productive feeding days per sow, considerably increases the utilisation efficiency of housing space, and enables timely interventions during medical examinations of each animal. Traditional methods of pregnancy diagnosis, such as the detection of the lack of oestrous cycle in inseminated animals, the determination of progesterone and oestrone sulphate in blood and/or urine, the examination of vaginal and cervical discharge, and the rectal palpation of reproductive system, are either imprecise or impractical regarding production conditions. Ultrasound diagnosis is a sophisticated method of highly precise early pregnancy diagnosis deprived of negative effects on examined animals. Moreover, this method provides a real-time visualisation of conceptus, uterus, and ovaries. Ultrasound diagnosis enables pregnancy diagnosis as soon as 17 days after insemination. Well over 95% of accurate diagnoses are obtained during examinations conducted between 25 and 35 days after insemination. The only disadvantage of this method, which notably impedes mass application in production, is a fairly steep price of ultrasound equipment.

***Keywords**: ultrasound, early pregnancy, diagnosis, swine.*

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[1]Ivan B. Stančić, DVM, PhD, Assistant Professor; Miloš Beuković, PhD Associate Professor; Ivan Radović, PhD, Assistant Professor; Blagoje Stančić, PhD, Full Professor; Saša Dragin, PhD, Assistent Professor; Mihajlo Erdeljan, DVM, Teaching Assistant. University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia.
Corresponding author: Ivan B. Stančić, DVM, PhD, University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg Dositeja Obradovića 8, 21000 Novi Sad; E-mail: dr.ivan.stancic@gmail.com; Phone: +381 21 485 3496.
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INTRODUCTION

The reproductive efficiency of a sow herd is the result of the number of farrowed piglets per sow annually. One of the main parameters, which significantly influence the reproductive efficiency of a sow herd, is the farrowing index, i.e. the average number of farrowings per sow per year. This index is directly conditioned by the number (%) of sows and gilts, which conceive successfully after the first insemination. Thus, the number of non-productive feeding days per sow per year is reduced, and consequently the cost price of the total piglet production (Koketsu, 2005). Therefore, it is exceptionally important to use efficient methods of early pregnancy diagnosis in production within the period of 30 days after insemination. This procedure enables detection of non-pregnant sows and timely interventions. Moreover, the number of non-productive feeding days per sow per year is greatly reduced, and the reproductive efficiency of a sow herd is considerably increased from the technological, veterinary, and economic viewpoint (Stančić et al., 2003; Radović et al., 2006; Stančić et al., 2008; Stančić et al., 2008; Gagrčin et al., 2009; Stančić et al., 2009; Stančić et al., 2010).

The detection of pregnancy in sows and gilts on farms in our country is still conducted by the traditional method of detecting the lack of oestrus with a teaser boar 18 to 24 days after insemination. This method is not highly efficient due to numerous reasons which hinder the external exhibition of oestrus signs apart from pregnancy (Stančić et al., 2004). Pregnancy diagnosis methods such as the determination of progesterone and oestrone sulphate in blood and/or urine, the rectal palpation of reproductive tract, the histological and/or bio-electronic testing of vaginal discharge are not suitable for practical application in the intensive swine production (Flowers et al., 1999; Stefanakis et al., 2000; Stančić et al., 2001; Chadio et al., 2002; Stančić, 2002; Stančić et al., 2003; Stančić, 2004; Boma and Bilkei, 2008). The application of the ultrasound early pregnancy diagnosis in swine started in the late 1970s (Bosc et al., 1975). However, the practical application of ultrasonography is still very limited by a fairly steep price of necessary equipment. Nevertheless, the method of ultrasound pregnancy diagnosis is highly efficient because it enables the accurate diagnosis of early pregnancy as soon as 20 days after insemination. The method itself is very practical because it does not require any special preparations of animals, it does not cause great stress in animals, it does not have negative effects on animals, and the results are obtained in real time (Flowers et al., 1999).

Table 1. Efficiency of various techniques for pregnancy diagnosis in swine (Flowers and Knox, 2000)

Technique	Physiological Basis	Period of Efficiency	Accuracy
Detection of oestrus	Non-pregnant females exhibit oestrus	Any time during gestation	>98*
A-mode ultrasound	Identification of fluid in pregnant uterus via speed at which emitted sounds return to probe	Days 28 to 80 of gestation	>95
Doppler ultrasound	Identification of sound patterns of increased blood flow in uterine and umbilical arteries during pregnancy	After day 29 of gestation	>95

Real-time ultrasound (B-mode)	Visualization of fluid and fetal tissue in pregnant uterus	After day 21 of gestation	>95
Progesterone concentrations	Increased blood progesterone concentrations (>5.0ng/ml) in pregnant females	Days 17 to 20 of gestation	>85
Prostaglandin-$F_{2\alpha}$ concentrations	Increased blood prostaglandin concentrations in non-pregnant females (>200pg/ml)	Days 13 to 15 of gestation	>80
Oestrone sulphate concentrations	Increased oestrone sulphate concentrations in pregnant females (> 0.5 ng/ml)	Days 25 to 30 or after day 80 of gestation	>93

*Disadvantages: (1) pseudopregnant sows do not exhibit oestrus; (2) irregular exhibitions of oestrus prolong the period from insemination to the exhibition of oestrus; (3) the visualisation of reproductive tract is not possible; (4) repeated tests of pregnant sows are necessary to detect oestrus exhibition; (5) potential errors in detection of oestrus.

The aim of this paper is to show the basic technology and efficiency of the ultrasound early pregnancy diagnosis in swine, as well as the application of this diagnostic method in contemporary swine production.

DIAGNOSTIC ULTRASOUND TECHNOLOGY

The basic operation mode of an ultrasound apparatus consists of the following: (1) the apparatus transmits electric current to the wave-generating transducer (probe), (2) electric current stimulates piezoelectric crystals in the transducer to generate ultrasound waves (USW) of certain frequency, (3) USW are emitted towards organs and tissues, (4) some tissues reflect these waves (echogenic tissues), and some tissues or fluids (such as the allantoic fluid) absorb the waves (non-echogenic tissues) , (5) the obtained ultrasound echo of certain frequency is received by the transducer and transmitted to the apparatus where it is transformed in a black and white image displayed on the screen (Doppler real-time B-mode) or shown as a chart in a coordinate system (A-mode ultrasonograpgy).

Basically, the tissues and matters of higher density (e.g. fetal bones) generate stronger echo (so-called echogenic structures) so their ultrasound imaging is brighter, whereas the ultrasound imaging of tissues and matters of lower density is darker (e.g. the allantoic fluid, i.e. non-echogenic structures). The image is displayed in shades ranging from the absolute black to the absolute white colour. Fluids (liquids) are non-echogenic so they are shown black, whereas tissues, depending on their density, are shown in shades ranging from the absolute white to the bright grey colour (e.g. bones are very echogenic). Probes or transducers can generate USW ranging from 3.5 to 7.5 MHz. Probes with the total power of 3.5 MHz generate shortwave frequencies which can penetrate deeper into tissues, but the resolution of the obtained echo image is lower, whereas probes with the total power of 7.5 MHz generate high frequencies with weaker tissue penetration and higher resolution of the obtained image (Mannion, 2006).

Diagnoses can be obtained by means of transabdominal and transrectal probe. Transabdominal sonography is more common because it is less complicated and traumatic, but the visualisation of the organs and tissues is of lower quality in comparison with the transrectal method. Transabdominal ultrasonography is performed by placing a probe on the borderline area between the ventral part of the abdomen and the final 3 mammary complexes, usually on the right side (Moeller, 2002). Easily detectable echo frequencies 15-20 cm deep imply pregnancy, whereas in non-pregnant animals this echo is 5 cm deep. Convex 3.5-5.0 MHz probes are widely used in practice for early pregnancy diagnosis in sows and gilts. Transrectal probes are inserted into the rectum of animals and this procedure shortens the distance between the probe and the examined organ, which enables images of higher resolution. Nevertheless, the procedure is complicated, it requires the sedation of animals, and creates substantial negative impact on the organs and tissues (Kyriazakis i Whittemore, 2006).

Nowadays, there are two types of ultrasound apparatus in use for early pregnancy diagnosis in sows and gilts: the Amplitude-depth sonography (A-mode or Pulse-Echo) and the Doppler ultrasonography (B-mode, real-time).

The A-mode is the simplest method. The reflected ultrasound echo is registered via graphic signals. The Y-axis of the chart shows the echo-amplitude, whereas the X-axis shows the depth, which is the distance from the organ. There is no visualisation of the organs or tissues. The physiological basis of this method is the determination of the fluid in the pregnant uterus, which reflects the ultrasound echo. The Doppler (B-mode, real-time, a black-and-white image) shows two-dimensional black-and-white anatomical image of organs, tissues, and various fluids in the pregnant uterus. It can detect fetal movements, heartbeats, and blood vessel pulsation in real time. This type of ultrasonography provides the information on the anatomy and function of the examined organs (Flowers and Knox, 2000).

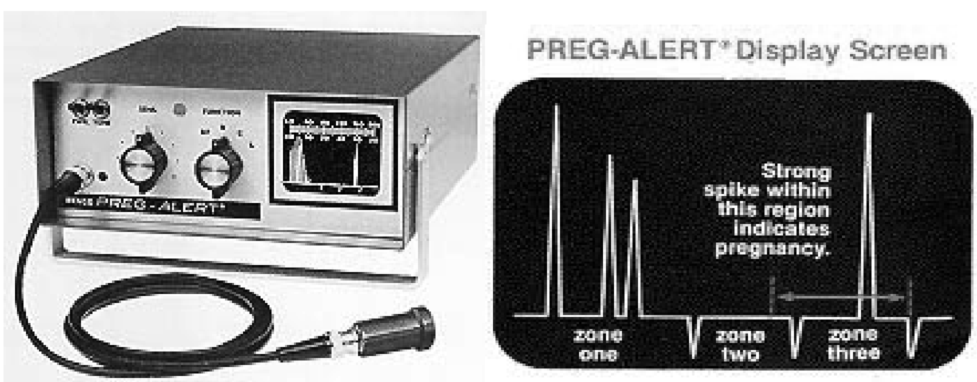

Image 1. A-mode, Pulse-Echo (the apparatus and the graphic image on the screen)

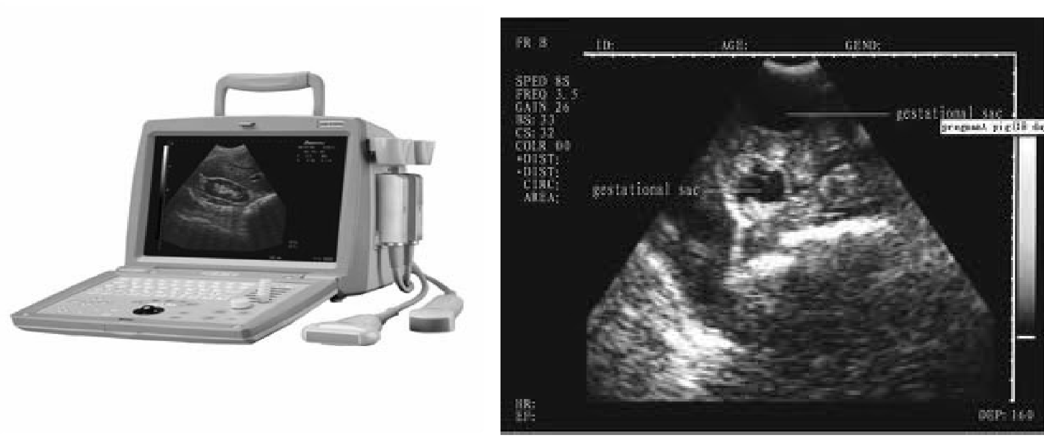

Image 2. Dopler B-mode real-time (the apparatus and the image on the screen)

THE APPLICATION OF ULTRASOUND DIAGNOSIS

The efficiency of the ultrasound pregnancy diagnosis entails the following: (1) it is possible to diagnose pregnancy very early (as soon as 17 days after insemination), (2) the results are obtained immediately and in real time, (3) the accuracy of the diagnosis is very high (over 90%), (4) the diagnostic procedure is simple and short, and (5) the procedure does not require any special preparations of animals, and it displays no negative effects on the health of animals. Therefore, it is very suitable for practical application in the intensive swine production. On farms in the USA, it is common practice to test the inseminated sows and gilts for the exhibition of oestrus with a teaser boar within the period of 17-24 days after insemination. Moreover, this traditional procedure is combined with the ultrasound pregnancy diagnosis obtained within the period of 28-45 days after insemination (Almond and Dial, 1987).

The accuracy of the diagnosis is increased with the length of pregnancy. Thus, on the 18th day after insemination positive pregnancy diagnoses were confirmed in 60% of sows, whereas on the 24th day after insemination they were confirmed in 96% of sows using the Doppler ultrasonography (Flowers et al, 1999). The studies conducted by Williams et al. (2008) show positive pregnancy diagnoses in over 90% of the observed sows within the period of 17-24 days after insemination using the Doppler ultrasound method. These authors came to a conclusion that there is no significant difference in diagnostic accuracy between the Doppler real-time and A-mode ultrasonography performed within the period of 17-24 days of gestation. In practice, it is very important to detect non-pregnant animals as soon and as accurate as possible. The results of the study indicate that the accuracy of the negative diagnosis is somewhat lower than the accuracy of the positive diagnosis. Furthermore, it is also demonstrated that the negative diagnosis is obtained more accurately using the Doppler real-time ultrasonography in comparison with the A-mode ultrasonography. Namely, the A-mode ultrasonography can hardly distinguish the bladder fluid from the allantoic fluid; whereas the Doppler real-time ultrasonography enables clear visualisation of the bladder. This is the main reason of the more accurate diagnosis by means of the Doppler real-time ultrasonography (Flowers et al., 1999). Therefore, it is highly recommended to repeat the diagnostic tests in sows with the negative diagnoses in 10 to 15 days (Maes et al., 2006). Repeated tests are recommended even in the case of suspected early embryonic mortality because it is not possible to anticipate whether the pregnancy will be continued (Kauffold et sal., 1997; Milller et al., 2003; Knox and Flowers, 2004).

Basically, ultrasonography enables early pregnancy diagnosis via the sonogram image of a larger or a smaller black circle (the non-echogenic area of the allantoic fluid). The diameter of this area is increased with the length of pregnancy. The volume of the allantoic fluid per embryo is known to be increased from approximately 3ml on the 17th day of gestation to approximately 200ml on the 30th day of gestation. However, a substantial increase of the amniotic fluid is not detected prior to the 30th day of gestation. Nevertheless, it has been confirmed that the volumes of amniotic and allantoic fluids per embryo vary greatly within the first 25 days of gestation (Wildt et al., 1975; Knight et al., 1977). After the 21st day of gestation, there is a sufficient volume of the fluid in the allantoic sac to be clearly visualized by means of the Doppler real-time ultrasonography (Flowers et al., 1999).

According to the researches we have conducted (Stančić et al., 2011., unpublished data) by means of the Doppler real-time ultrasonography, it is possible to visualize the allantoic sac on the 20th day of gestation with the diameter of 9.54mm, and the embryo with the length of 0.8-1.6 cm at the depth of 24.8 cm (Image 3).

Image 3. The ultrasonogram on the 20th day of gestation *(Stančič I., 2011)*

The amniotic sacs are shown in the uterine horn (arrows).

Within the period of 23-26 days of gestation, the increase of the allantoic sac diameter is clearly visible ranging from 2.2 cm to 2.5 cm, as well as the embryo with the length ranging from 1.8 cm to 2.5 cm. The depth is decreasing from 10.80 cm to 7.55 cm due to the allantoic sac enlargement (Image 4 and 5).

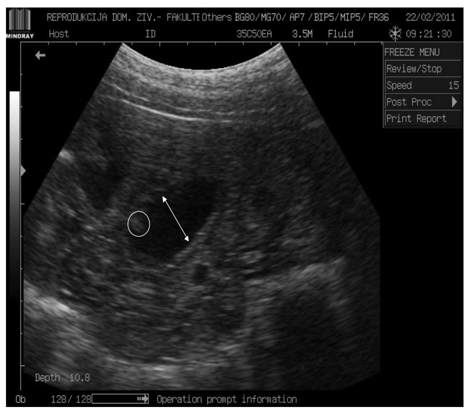

Image 4. The ultrasonogram on the 23rd day of gestation *(Stančič I., 2011)*
The amniotic sac (the arrow) and the embryo (in the circle) are shown.

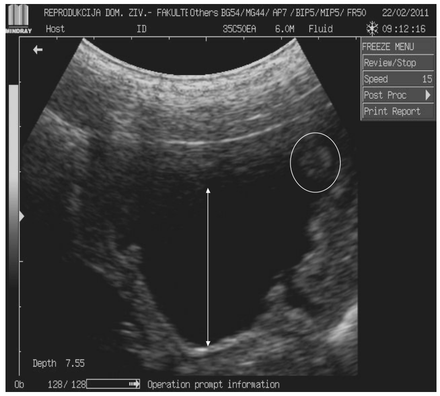

Image 5. The ultrasonogram on the 26th day of gestation *(Stančič I., 2011)*
The enlarged amniotic sac (the arrow) and the larger embryo (in the circle) are shown.

During the second half of the gestation period, the method of ultrasound pregnancy diagnosis is totally accurate. The fetal visualisation is completely clear, as well as the skeletal ossification. The foetuses are 5.7-6.0 cm long on the 56th day of gestation, and 8.0-9.0 cm on the 84th day of gestation (Image 6 and 7).

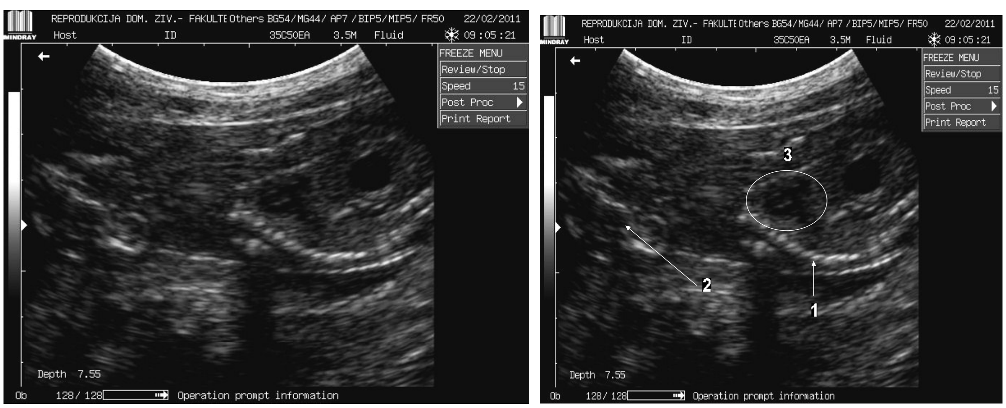

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Image 6. The ultrasonogram on the 56th day of gestation *(Stančič I., 2011)*
The embryonic vertebral column (1), head (2) and heart (3, in the circle) are shown.

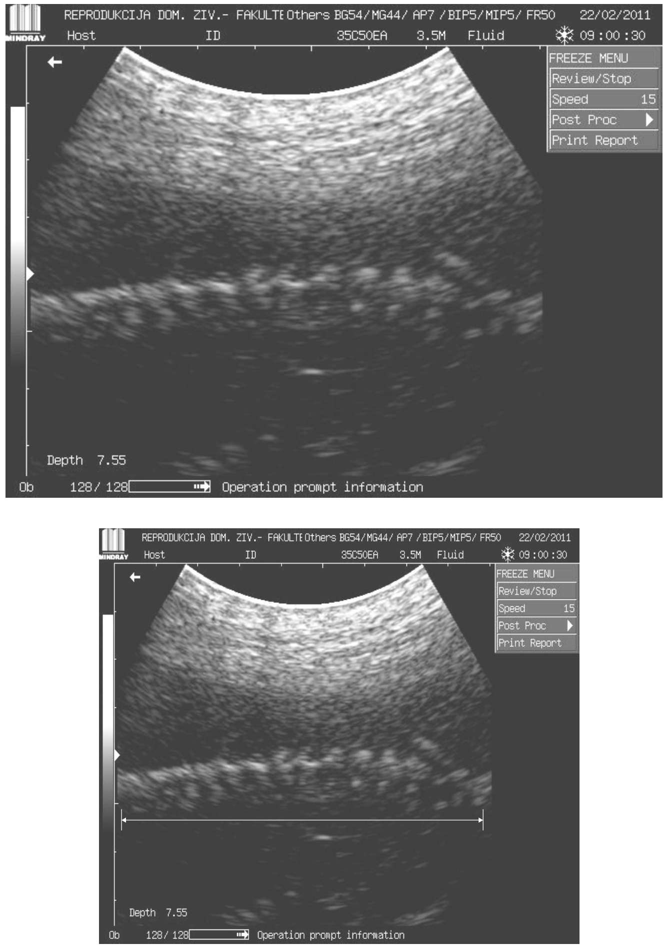

Image 7. The ultrasonogram on the 84th day of gestation (Stančič I., 2011)
Clearly defined vertebrae of the fetal vertebral column are shown (arrow)

The application of the real-time ultrasound methods (e.g. the Doppler or B-mode) enables pregnancy diagnosis approximately two weeks sooner than the A-mode ultrasonography. By means of the Doppler real-time visualisation it is possible to detect fetal mortality in sows previously diagnosed as pregnant. It is also possible to diagnose pseudopregnant sows (Flowers et al., 1999) due to the retention of the uterine luminal fluid after fetal mortality. Moreover, the fetal skeletal calcification does not commence prior to the 60th day of gestation and hence pseudopregnancy is extremely hard to detect prior to the 65th day of gestation. However, fetal heartbeats are not detected in pseudopregnant animals (Knight et al., 1977).

The Doppler ultrasound is used for the detection of the blood flow in the fetal heart and large umbilical cord blood vessels. The procedure enables the detection of the blood flow in these blood vessels, especially in the uterine artery, as soon as on the 21st day of gestation, while a significantly more accurate diagnosis can be obtained following the 30th day of gestation (Kyriazakis and Whittemore, 2006).

By means of the ultrasound technology it is possible to diagnose various pathological lesions of reproductive organs. Moreover, ultrasonographic examinations of the function of ovarian structures (antral follicles and corpora lutea) enable the determination of the oestrous cycle phase in examined animals (Waberski and Weitze, 1998; Kauffold et al., 2004; Kauffold et al., 2007). This is very important for the practical production. It has been confirmed that in approximately 30% of breeding gilts on farms in Serbia, which are usually 8 months of age or more, the detection of oestrus exhibition is not possible with a teaser boar (Stančić et al., 2008; Stančić et al., 2008; Stančić et al., 2001). These gilts are not inseminated and they are excluded from the further reproduction as 'anoestrous'. However, the post-mortem examination of reproductive organs indicates that over 60% of these gilts exhibit the cyclic ovarian activity and their reproductive organs show no pathological lesions, which could cause them to be anoestrous. Therefore, it has been concluded that such gilts are not anoestrous and that they are unnecessarily excluded from reproduction due to the inefficient detection of oestrus exhibition with a teaser boar (Stančić et al., 2009; Stančić et al., 2011). Timely ultrasound examinations would detect the cyclic ovarian activity in these gilts and prevent the exclusion from reproduction based on the misdiagnosis 'prolonged pre-insemination anoestrus'. Consequently, the ultrasound procedure would be indubitably beneficial from the zoological, technological, and economic viewpoint.

CONCLUSION

The application of ultrasound technology enables highly accurate positive and/or negative early pregnancy diagnosis in sows as soon as 20 days after insemination. By means of the Doppler real-time ultrasonography it is possible to detect pseudopregnant sows after the 60^{th} day of gestation. The method also ensures the detection of cyclic ovarian structures and pathological lesions in female reproductive system. This is greatly important for the intensive swine production due to the reduction of non-productive feeding days per sow per year.

The traditional method of oestrus detection with a teaser boar 18-24 days after insemination does not facilitate the detection of non-pregnant animals. Furthermore, according to numerous researches of various authors, it is not possible to detect oestrus within this period in 20-30% of sows which are not pregnant. On the other hand, a considerable number of sows exhibit oestrus following the 24^{th} day after insemination (so-called irregular oestrus exhibition), as well as a considerable number of pseudopregnant sows (which are non-pregnant after insemination and do not exhibit oestrus almost until farrowing). Consequently, the average period from the first to the successful insemination is increased, as well as the average period from the first insemination to the exclusion from further reproduction (so-called non-reproductive period). The prolonging of this non-productive period causes significant reduction in efficiency of piglet production on farms: (a) the average annual farrowing index is decreased and thus the total number of produced piglets on a farm, (b) the average number of non-productive feeding days per sow per year is increased, and (c) the number of unnecessarily excluded sows from reproduction is increased. These factors substantially increase the cost price of piglet production and reduce the cost-effectiveness of such production on farms.

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ULTRAZVUČNA DIJAGNOSTIKA RANE GRAVIDNOSTI KOD SVINJA

IVAN STANČIĆ, MILOŠ BEUKOVIĆ, IVAN RADOVIĆ, BLAGOJE STANČIĆ, SAŠA DRAGIN, MIHAJLO ERDELJAN

Izvod

Rana dijagnostika graviditeta značajno utiče na efiksanost intenzivne proizvodnje svinja. Naime, što ranijom dijagnozom negravidnih životinja, smanjuje se broj neproduktivnih hranidbenih dana po krmači, značajno se povećava efikasnost iskorištavanja smeštajnog prostora, a mouguća je i pravovremena adekvatna intervencija kod svake pregledane životinje. Klasične metode dijagnoze gravidnosti, kao što su evidencija izostanka estrusne cikličnosti, 18 i više dana od osemenjavanja, detekcija progesterona i estronsulfata u krvi i/ili urinu, ispitivanja vaginalne i cervikalne sluzi i rektalne palpacije reproduktivnih organa, ili nisu dovoljno precizne ili nisu praktične za primenu u proizvodnim uslovima. Ultrazvučna dijagnostika je sofisticiran metod vrlo preciznog dijagnostikovanja rane gravidinosti, bez negativnih posledica po ispitivanu životinju. Osim toga, ovom metodom se dobija dobra vizuelizacija konceptusa, materice i jajnika u realnom vremenu. Ultrazvučnom metodom se, kod svinja, može dijagnostikovati gravidnost već od 17. dana posle osemenjavanja. Preko 95% tačnih dijagnoza se dobija pregledom izvršenim između 25. i 35. dana od osemenjavanja. Značajniji nedostatak, koji znatno limitira masovniju primenu ove metode u prakstičnoj poizvodnje, je dosta visoka cena ultrazvučne opreme.

Ključne reči: *Ultrazvuk, rani graviditet, dijagnoza, svinje.*

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