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Introduction

Over the past 40 years the pig production landscape has changed dramatically, influenced by several factors including the massive application of technological tools coupled with advanced feeding strategies, improvements in herd health management, advances in housing systems and environments, as well as increased awareness for sustainable farming taking into consideration animal welfare principles. All these features have contributed to the industrialization of pig farming, bringing about many benefits for the major pork-producing countries. Notwithstanding the advances seen in many areas of pig production, precise methods for troubleshooting reproductive dysfunction in pig operations are still lacking, which results in economic losses.

In this context, real-time ultrasonography (RTU) is an invaluable tool to optimize reproductive efficiency in pig herds. Indeed, trans-abdominal RTU can be used to characterize reproductive dysfunction (Castangna et al., 2004; Kauffold et al., 2005), to assess ovulation (Waberski et al., 2000), the attainment of puberty (Kauffold et al., 2004) and for early diagnosis of pregnancy (Flowers et al., 1999; Maes et al., 2006).

The ability to assess the sows' reproductive tract in real-time and make a precise diagnosis assumes great importance when the current culling rates are observed. Indeed, it is estimated that as many as 40% to 50% of breeding sows are culled each year; of these, 30% are culled by third parity. Reproductive failure is one of the major reasons for involuntary culling (Tani et al., 2018), with disappointing litter size, anestrus and return to estrus following insemination as major underlying causes for removal of young sows. Nevertheless, aside from been caused by intrinsic imbalances, reproductive failures can be influenced by external factors such as deficient estrus detection, incorrect timing of insemination, poor semen quality, and disease. Hence, the information obtained through RTU examination would substantiate the decision for removal, enabling producers to re-inseminate, treat or remove females from the breeding herd (Flowers et al., 2000; De Rensis et al., 2000; Maes et al., 2006), reducing the number of non -productive days (Willians et al., 2008) and maximizing reproductive efficiency.

Use of RTU in pig reproduction

Real-time ultrasonography utilizes sound waves of high frequency emitted from transducers that travel in different patterns. It is a non-invasive and biologically safe technique for both operator and sow. Upon contact with tissues, the ultrasound waves are reflected back to the transducer, where they are converted into electrical signals and displayed on a monitor as a two-dimensional image in greyscale (Flowers et al., 2000). The image displayed on the screen varies from white to light grey, representing dense tissues such as bone and muscle, and from dark grey to black, corresponding to fluid-filled structures such as bladder and uterus (Knox; Flowers, 2006).

Pregnancy diagnosis

Transcutaneous RTU is preferentially used for pregnancy diagnosis in pigs because it is readily accessible and quicker to perform (Kauffold et al., 2019). To visualize the gravid uterus, the transducer should be placed on the right abdominal wall, just above the most caudal mammary glands. The transducer should be pointed towards the spine in a 45-degree angle and directed dorsocaudally and dorsocranially. Ultrasound gel must be applied to the transducer surface in order to maximize wave propagation and contact between the skin and the transducer. Both the 3.5 MHz and 5.0 MHz transducers can be used for pregnancy diagnosis (Knox; Flowers, 2006); however, according to Kauffold et al. (2019) if a single transducer frequency should be selected, 5 MHz is preferred as lower frequencies typically provide for lower resolution.

The non-gravid uterus is characterized by circles (cross-section of uterine horns) of moderate echogenicity. It is worth mentioning that the non-gravid uterus is more difficult to visualize, which poses a challenge for the inexperienced operator to rule out pregnancy. The visualization of multiple fluid-filled pockets within the uterus, representing the embryonic vesicles, can be considered as the first sign of pregnancy. Embryonic vesicles, measuring from 10 mm to 20 mm, can easily be visualized on day 20 of pregnancy (Figure 1A). After day 21 of pregnancy the embryos can be observed; they are represented by echogenic structures within the vesicles. Around day 30 of pregnancy it is possible to distinguish the head, the abdomen, and the limbs of the embryos (Figure 1B). From day 60 it is possible to visualize the ocular orbits, the spine, the stomach and the beating foetal heart (Figure 1C).

Using RTU for pregnancy diagnoses is advantageous when compared to other methods, as sows' pregnancy status can be determined early and reliably, allowing pig producers to make quick decisions, limiting the number of non-productive days (Maes et al., 2006). Table 1 summarizes the outcomes of testing for pregnancy at various days post-insemination.

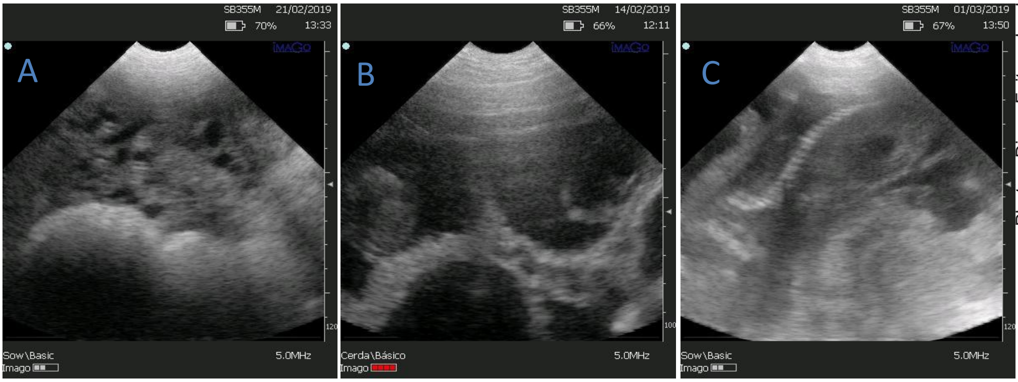

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Figure 1. Transabdominal ultrasonographic images of the gravid uterus. Day 20 of pregnancy (A); around day 35 of pregnancy (B); > 90 days of pregnancy (the stomach and the spine of the embryos can be seen).

Table 1. Sensitivity (%), specificity (%), overall accuracy (%) and predictive values (%) of pregnancy diagnoses from day 17-21 post-insemination.

	17	18	19	20	21
Sensitivity	51.43	73.68	93.02	100.00	100.00
Specificity	42.86	12.50	62.50	100.00	100.00
Accuracy	48.98	63.04	88.24	100.00	100.00
Positive predictive value	69.23	80.00	93.02	100.00	100.00
Negative predictive value	26.09	9.09	62.50	100.00	100.00

Ultrasonographic examination of the ovaries: follicular dynamics and ovulation

The employment of RTU to monitor follicular dynamics and the time of ovulation, as part of the reproductive management in pig herds, could constitute a valuable strategy to increase reproductive efficiency as it make possible to determine the best moment for insemination, maximizing farrowing rate and litter size, and also identify non-cyclic females (anestrus).

Both transrectal and transabdominal routes can be applied for ovary scanning. Transabdominal scanning is performed by placing the transducer on the lower flank above the mammary glands in the inguinal area in a similar way as in pregnancy diagnosis (Figure 2). The ovaries are localized dorsocranially in relation to the hind leg; the transducer should be pointed upwards toward the spine and angled slightly back and forth to visualize the bladder, which serves as a reference to localize the ovaries; in the ultrasound image, the ovaries will appear cranially to the bladder. When performed transrectally, the transducer is hand-held and manually guided through the rectum. The ovaries are localized ventrally in relation to the rectum and approximately 30 cm - 40 cm inside. It is important to mention that the transrectal examination requires the prior removal of faeces in order to have an adequate tissue/probe interface to obtain a high-quality imaging (Kauffold et al., 2019).

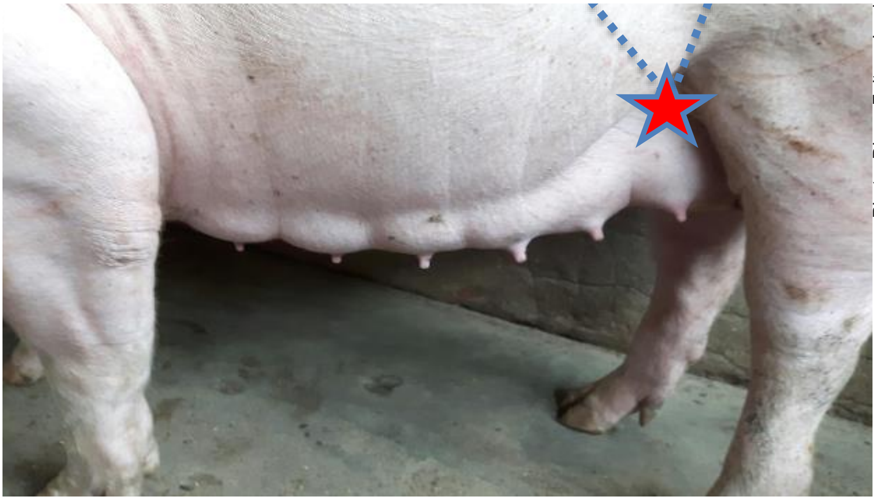

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Figure 2. Transducer positioning for ovary scanning and pregnancy diagnosis through the transabdominal route.

The transrectal approach is often preferred because the transabdominal scanning requires more practice to acquire a good image. However, from the authors' experience, good results are obtained using transabdominal scanning after 50 examinations (Viana, 1998).

The main limitations of transabdominal scanning are related to obliteration of the ovaries by surrounding tissues (i.e. colon), especially on the left abdominal wall. Moreover, a detailed image of the ovarian structures is often difficult to obtain due to constant movement of the sow. On the other hand, transrectal scanning allows for a better and detailed scanning of the ovaries; it is even possible to count ovarian structures.

In both techniques the fluid-filled follicles appear as non-echogenic ovarian structures of 3 mm - 11 mm in late proestrus and estrus. Attention should be paid to differentiate the follicles from surrounding blood vessels, cysts and corpora hemorrhagica. According to Waberski et al. (2000) the echogenicity of corpora lutea is similar to that of the ovarian stroma, being only visible for well-trained investigators in approximately 50% of sows.

To identify the time of ovulation, repeated sonographic investigations (Figure 3) are required and the time of ovulation is considered to have occurred halfway between the two investigation intervals in which follicles were last detected and subsequently disappeared (Waberski et al., 2000).

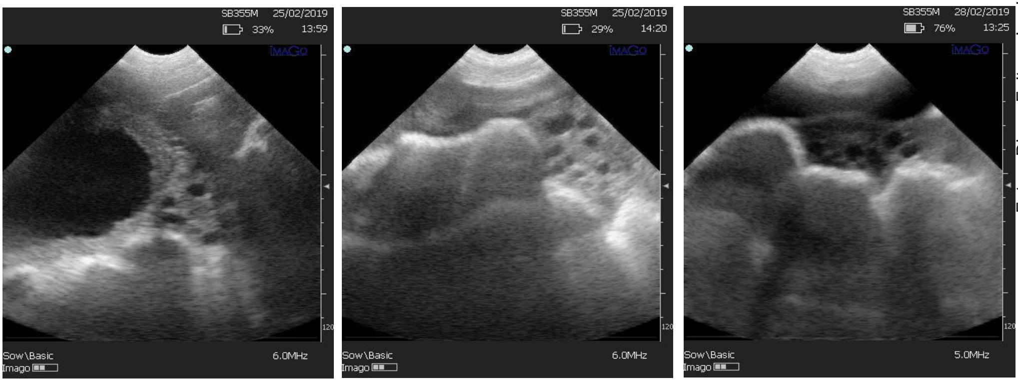

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Figure 3. Transabdominal ultrasonographic images of the ovaries showing pre ovulatory follicles

Using rtu for diagnosis ovarian and uterine pathologies

Using the transabdominal route, ovarian cysts can be easily identified during the examination with both 3.5 MHz and 5 MHz transducers. They appear as fluid-filled ovarian structures measuring more than 12 mm and can be classified as follicular or luteal cysts. It is noteworthy that the ovarian cystic degeneration can affect 10% of sows in a herd, being an important cause of reproductive failure (Castagna et al., 2004). With the aid of RTU ovarian cysts can be readily identified, avoiding economic losses and the unnecessary culling of breeding sows.

Pyometra is another uterine condition that can be diagnosed using RTU. The echographic image of affected sows shows heterogeneous echogenic material inside an enlarged uterine lumen. Note that some sows may not have vulvar discharge as a sign of uterine infection; these cases can only be quickly identified through ultrasonographic examination.

Biosecurity considerations

Ultrasound machines can serve as fomites for transmission of diseases, putting farm biosecurity at risks. Indeed, ultrasound machines have been found to function as a carrier of pathogenic microorganism such as *Streptococcus* spp. and the PRRS viral RNA (Kauffold et al., 2010). Our recommendation is that ultrasound machines should be cleaned and disinfected after each use. Its use between farms should be limited to a minimum. In case of movement between pig farms, the equipment down time should be respected. In order to avoid damage, only the manufacturers recommended sanitizers should be applied onto the ultrasound machine (Kauffold et al., 2007).

Concluding remarks

The use of RTU is an invaluable technological tool to aid in the process of decision making on pig farms. Using this tool, it becomes possible to examine the reproductive tract of sows with reproductive disorders and an accurate decision can be made either to remove the sow or to implement correction measures. This is of particular importance for young sows which are removed from the breeding herd chiefly for reproductive failure, increasing sow retention. Finally, it should be emphasised that the technique of RTU does not increase reproductive efficiency and correct management deficiencies *per se*; it is how the information gathered is interpreted and implemented on the farm that will count.

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