Characterization of image and labor requirements for positive pregnancy diagnosis in swine using two methods of real-time ultrasound

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Summary

In Experiment 1, in order to compare and characterize labor and image requirements for early pregnancy diagnosis by transrectal and transabdominal real-time ultrasound (RTU), 100 sows were examined at 16 to 24 days of gestation using both methods. On day 20, over 71% of sows were diagnosed using transrectal RTU compared to 2% with transabdominal RTU. By day 22, 98% were diagnosed using transrectal RTU compared to 53% for transabdominal RTU, and by day 24, there was little difference between methods. Accuracy was greater for transrectal RTU prior to day 22, but also required more time for diagnosis. In Experiment 2, 183 sows were examined using transrectal RTU at gestation days 15 to 21 (uterine fluid diameter measured), and by transabdominal RTU between days 22 and 72 (fluid diameter and time to make a diagnosis measured). Fluid diameter increased to day 30, decreased to day 39, and increased thereafter. Diagnosis required more time prior to day 24. These results indicate that pregnancy can be diagnosed accurately in most sows by day 22 using transrectal RTU and by day 24 using transabdominal RTU. The largest fluid vesicles and least amount of time required for diagnosis occurred on day 30.

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The average number of nonproductive sow days for US swine herds was recently estimated at 87.[1] With the cost of nonproductive days reaching as much as $1.61 per day (US$), profit margins are reduced in sow herds with high nonproductive days.[2] Early identification of nonpregnant animals for rebreeding or for removal from the herd reduces nonproductive days. Beginning as early as the third week after breeding, real-time ultrasound (RTU) provides more accurate pregnancy detection than A-mode ultrasound.[3–5] With the development of portable and less expensive equipment, use of RTU technology for pregnancy diagnosis has increased rapidly over the last several years.

In many studies involving the use of RTU, animals were examined externally using a 3.5-MHz transducer.[2,3–5] This noninvasive method allows the deep, wide-angle tissue penetration that is necessary to quickly visualize the uterus. In contrast, transrectal RTU places a 7.5-MHz transducer much closer to the uterus. The 7.5-MHz transducer provides greater resolution over a shorter distance compared to the 3.5-MHz transducer. The higher transducer frequency also allows for visualization of the ovaries and internal contents of the uterus.[6] Visualization of ovarian structures that are uncharacteristic of pregnancy (eg, large follicles or ovarian cysts), as well as expected structures such as corpora lutea (CLs) of the appropriate size (1.2 cm), may reduce the uncertainty of making a false pregnancy diagnosis that is based on the appearance of single or multiple fluid vesicles with an uncharacteristic echogenic image profile.

Swine herds in the United States average approximately 1000 sows,[1] and with limitations on the availability of labor to manage these large groups, there is a greater need for the method of pregnancy diagnosis to be both quick and accurate. When RTU is used, the amount of fluid accumulation in the uterus may affect both time required for diagnosis and accuracy. Real-time B-mode (brightness mode) ultrasonography displays bright dots in a gray scale, two-dimensional image. Dense tissues such as the fetus and uterus reflect sound waves and appear hyperechoic, while fluid within the allantoic and amniotic membranes do not reflect sound waves and appear anechoic. When performing pregnancy detection, technicians look for black vesicles within the surrounding gray endometrial tissue. At some stages of gestation, the amount of visible fluid decreases, and the fetus, which appears white, may blend in with the surrounding tissues on the screen, making pregnancy detection more difficult and prone to error. A rapid increase in the volume of allantoic fluid has been observed between days 20 and 30 of gestation, followed by a decrease to day 40.[7] In contrast, fetal crown-rump length increases steadily throughout gestation. Because of the reduced volume of fluid and increasing size of the fetus, pregnancy diagnosis based on visualization of fluid may be less accurate near day 40 of gestation. It is important to know whether this is true, so that routine pregnancy checks may be

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adjusted to occur on days when diagnosis is most efficient and reliable.

The objectives of this study were to compare transrectal and transabdominal RTU for early pregnancy diagnosis and for reproductive tract evaluation, and also to characterize the time requirement and diagnostic image used to make a positive pregnancy diagnosis when RTU is performed on days 15 through 72 of gestation.

Animals and housing

Experiment 1 was conducted in confinement facilities at two of the University of Illinois swine research farms. The study animals, which were crossbred sows representing commercial maternal-line genotypes and crossbred and purebred maternal lines, were selected from groups of sows weaned and mated in the fall of 2000 and spring of 2001. The two research herds were similar in average parity (2.5), previous lactation length (farrowing-to-weaning interval, 22.0 days), farrowing rate (75% and 85% respectively) and total pigs born (10 and 11 respectively). Throughout the experiment, sows were housed in gestation stalls.

Experiment 2 was performed at a 6000-sow commercial swine confinement unit in Illinois (summer of 2001), and at the same two University of Illinois swine research farms described for Experiment 1 (fall of 2001). Sows in the commercial herd had an average lactation length of 16 days and an average herd parity of 4.9.

Real-time ultrasound examination

Sows were examined in gestation crates in a standing position using an Aloka 500V B-mode ultrasound machine (Aloka Inc, Tokyo, Japan). A 7.5-MHz linear-array transducer placed in a rigid, fixed-angle PVC adaptor was used for transrectal examinations,[6] and a 3.5-MHz convex linear-array transducer was used for transabdominal examinations.[3]

Experiment 1

To determine whether transrectal RTU was advantageous for diagnosing early pregnancy, compared to transabdominal RTU, 100 sows were examined by a single technician on days 16 to 24 after the first day of estrus and insemination. All sows within a gestation day were examined first by transabdominal RTU, then by transrectal RTU. The low resolution transabdominal method was performed before the higher resolution transrectal method to limit bias caused by the influence of the results of the first method on the outcome of the second method. In addition, 8 to 15 sows were examined consecutively each day, so that the technician was unable to remember the diagnosis of individual females. The time required to diagnose animals as Pregnant, Not pregnant, or No decision was recorded. Time (seconds) was determined from the moment the transabdominal transducer touched the animal to the moment when the technician made a diagnosis. The requirement for a positive pregnancy diagnosis (Pregnant) included the presence in the uterus of multiple, anechoic fluid pockets, with an average diameter >1 cm. The presence or absence of fluid in the uterus is not a reliable indicator of pregnancy before day 22 of gestation, as most sows do not have 1-cm fluid vesicles until day 20. In addition, between days 18 and 20, it may not be possible to definitively classify regressing CLs and large growing follicles (>0.65 cm) in nonpregnant sows that will return to estrus at 21 days. Therefore, a "No decision" category of response was necessary to avoid erroneous diagnoses before day 22. On day 35, a final examination was performed by transabdominal RTU alone for the purpose of confirming the earlier diagnosis.

Since ovaries and fetuses could not be visualized reliably using transabdominal RTU, the capability of only transrectal RTU to support and improve pregnancy diagnosis was evaluated by recording Time to visualize a CL, Time to visualize a fetus, and Diameter (cm) of the largest noncystic CL (defined as a CL approximately 1.2 cm in diameter that did not contain a fluid-filled cavity). Time (seconds) was determined from the moment the transrectal transducer touched the animal to the moment when the technician located the desired structure.

Experiment 2

To characterize the labor requirements and diagnostic image used to make a positive pregnancy diagnosis using RTU, a total of 183 pregnant sows that ultimately farrowed were observed. Sows were examined between days 15 and 72 of gestation. Observations for day of gestation were grouped in a 2-day interval for Day 15 (days 15 to 16) and in 3-day intervals for Days 18 (days 17 to 19), 21 (days 20 to 22), 24 (days 23 to 25), 27 (days 26 to 28), and continuing throughout gestation to day 72. The average number of animals examined in each group was 22 ± 3. As it was impossible to visualize and measure uterine fluid diameter before day 22 using transabdominal RTU, transrectal RTU was used to obtain diameter measurements for days 15 to 21, and transabdominal RTU was used to obtain both diameter and timing measurements for days 22 to 72. Three technicians (A, B, and C) performed these procedures. Measurements obtained from the pregnant sows included the time (seconds) to diagnose animals as Pregnant (Time to decision) and the largest cross-sectional diameter (cm) of the fluid-filled uterus (Uterine fluid diameter).

Calculations and analysis for Experiment 1

Farrowing data were used to confirm accuracy (correct diagnoses divided by total diagnoses), specificity (number of correct Not pregnant diagnoses divided by total number of sows that did not farrow), and sensitivity (number of correct Pregnant diagnoses divided by total number of sows that farrowed) of all diagnoses.

For Experiment 1, the effects of Day of gestation 16 (days 15 to 16), 18 (days 17 to 18), 20 (days 19 to 20), 22 (days 21 to 22) and 24 (days 23 to 24), Method (transrectal and transabdominal RTU), and their interaction were evaluated on the continuous variable Time to make a diagnosis using the General Linear Models (GLM) procedures of SAS (SAS Institute, Cary, North Carolina). Diagnosis (Pregnant, Not pregnant, or No decision) was included in the model for the response variable Time to make a diagnosis. The continuous measures (obtained using only transrectal RTU) Time to visualize a fetus, Time to visualize a CL, and Diameter of the largest CL, were evaluated using GLM for the main effect of Day. A categorical model (CATMOD) in SAS was used to detect the effect of Method on the percentage of total animals diagnosed (all sows observed on that day of gestation), accuracy, specificity, and sensitivity by Day of gestation. Data for Time to make a diagnosis, Time to visualize a CL, and Time to visualize a fetus were not normally distributed, and the means of these values were transformed to logarithms for statistical analysis. However, means were transformed back to the original scale for reporting results. Differences between pair means comparisons were performed using a Student *t* test, while multiple means comparisons were performed using the Scheffé test.

Analysis for Experiment 2

For Experiment 2, the continuous response variable Time to decision was analyzed using GLM procedures for the main effects of Day, Technician, and their interactions. The continuous response variable Uterine fluid diameter was analyzed using GLM procedures for the main effect of Day. Logarithmic transformation was used for Time to decision to normalize data, but means were transformed back to the original scale for reporting results.

Results of Experiment 1

Three animals were removed from the study because uterine infection was diagnosed by transrectal RTU. Fluid associated with infection contains considerable echogenic material, in contrast to the clear fluid evident in the pregnant uterus. Of the remaining 97 sows, 80 farrowed (farrowing rate 82%). Five sows failed to farrow after being diagnosed as pregnant both by transabdominal and transrectal RTU between days 16 and 24 of gestation and later on day 35. This rate of "RTU fallout" is similar to the 4 to 7% rate reported by others.[8,9]

More sows were diagnosed earlier in gestation by transrectal RTU compared to transabdominal RTU ($P <.01$; Table 1). No evidence of pregnancy was observed on Day 16 by either method. By Day 18, a few sows could be diagnosed using transrectal RTU, while no animals were diagnosed using transabdominal RTU. By Day 20, more sows were diagnosed by transrectal RTU than by transabdominal RTU ($P <.001$), and accuracy of transrectal RTU tended to be greater than that of transabdominal RTU ($P <.10$). By Day 22, almost all sows were diagnosed using transrectal RTU and approximately half were diagnosed using transabdominal RTU ($P <.001$). By Day 24, all sows could be diagnosed using transrectal RTU, while 9% of sows still could not be diagnosed using transabdominal RTU: this difference was not statistically significant. Accuracy did not differ for the two methods at Day 22 or Day 24. Animals that were not pregnant could be correctly diagnosed with high specificity by transrectal RTU on Day 18, and with 55 to 71% specificity between Days 20 and 24. Fewer than 50% of nonpregnant sows could be correctly diagnosed using transabdominal RTU even on Day 24. However, there was no significant difference in specificity or sensitivity between methods on any gestation day.

For reporting all time measures, the data recorded and analyzed in seconds was converted to minutes to aid interpretation. There was a Method by Day interaction for Time to make a diagnosis ($P <.001$; Table 2). Time to make a diagnosis did not differ by Day for Transabdominal RTU, even though the percentage of sows diagnosed was lower or diagnosis was not possible on some days. Diagnosis by transrectal RTU took longer on day 16, and Time to make a diagnosis decreased until day 24.

The time required to use transrectal RTU to support pregnancy diagnosis by locating and evaluating ovarian structures and fetuses is shown in Table 3. Using transrectal RTU, it was determined that CL diameter changed by day ($P <.001$), with size similar between days 16 and 20, and smaller on day 22. There was an effect of Day on Time to visualize a fetus ($P <.05$), but not on Time to visualize a CL ($P >.05$). It took an average of 1.8 minutes to visualize a CL and 1.3 minutes to visualize a fetus between days 16 and 24.

Results of Experiment 2

There was an effect of Day ($P <.001$) on Uterine fluid diameter (Figure 1). Fluid

Table 1: Accuracy, sensitivity, and specificity of transabdominal and transrectal real-time ultrasound pregnancy diagnosis in 97 crossbred sows on days 16 to 24 of gestation (Experiment 1)

Parameter	Day of gestation							
	18		20		22		24	
	Abdominal	**Rectal**	**Abdominal**	**Rectal**	**Abdominal**	**Rectal**	**Abdominal**	**Rectal**
Sows diagnosed[1] (%)	0	5	2[a]	71[b]	53[a]	98[b]	91	100
Accuracy[2] (%)	NP[5]	100	50[c]	93[d]	90	94	95	95
Sensitivity[3] (%)	NP	100	100	100	100	100	99	100
Specificity[4] (%)	NP	100	NP	55	NP	60	45	71

1 Percentage of total sows examined that were diagnosed as pregnant or not pregnant. No sows could be diagnosed on day 16.
2 Correct diagnoses/total diagnoses
3 Number correctly diagnosed pregnant/total sows that farrowed
4 Number correctly diagnosed not pregnant/total sows that did not farrow
5 NP = Not possible to make a diagnosis.
ab Within a row, values with different superscripts by day of gestation are different ($P <.01$; CATMOD logistical model).
cd Within a row, values with different superscripts by day of gestation tend to differ ($P >.05$ but $<.10$).

Table 2. Least squares means for Time (minutes) to make a diagnosis (Pregnant, Not pregnant, or No decision) by transrectal and transabdominal real-time ultrasound in 97 crossbred sows on days 16 to 24 of gestation (Experiment 1)

	Day of gestation[1]				
	18	20	22	24	SE
n	45	46	45	40	NA[2]
Rectal	2.5[a]	1.9[a]	1.7[a]	1.4[a]	0.2
Abdominal	0.6[b]	0.7[b]	0.8[b]	0.3[b]	0.2

1 No sows could be diagnosed on day 16

2 NA: not applicable.

a,b Within a column, values with different superscripts are different (*P* <.01; Student *t* test)

was detectable on gestation Days 15 to 18 using transrectal RTU, and by Day 22 using transabdominal RTU. Uterine fluid diameter increased between Days 18 and 30 (*P* <.05), reaching a peak between Days 27 and 33 and declining thereafter to Day 39, with a difference of 24% between the maximum and minimum uterine fluid diameter measurements. Fluid diameter began to increase again after Day 42. Figure 2 illustrates images obtained using transabdominal RTU on days 29 and 39 of gestation. On Day 29, there were distinct, large fluid vesicles (6.5 cm), but by Day 39, vesicle fluid diameter had decreased (3.5 cm) and the fetus had grown to fill much of the visible fluid pocket.

There was an effect of Day on Time to decision (*P* <.001). Time to decision was not recorded for transrectal RTU on Days 15 to 18 to avoid confounding Day with Method. Time to decision using transabdominal RTU between Days 22 and 72 ranged from 1 second to 2.2 minutes. Diagnosis required significantly more time prior to Day 24, compared to all other days (*P* <.001). Time to decision using transabdominal RTU was greatest on Day 21, and required <15 seconds for the remainder of the evaluation days. Time to decision declined numerically but not significantly after Day 27, reached its lowest point between Days 30 and 36, increased to Day 39, remained at a plateau until Day 54, and then declined steadily until Day 72. There was no effect of technician on Time to decision (*P* >.05).

Discussion

The results of this study indicate that pregnancy can be detected earlier in gestation using transrectal RTU compared to transabdominal RTU. Using transrectal RTU, 70% of sows were diagnosed by day 20 of gestation with >90% accuracy, and 98% of sows were diagnosed by day 22 of gestation with 94% accuracy. If transrectal RTU is performed on gestation days 20 to 22, sows returning to estrus may be identified earlier, allowing them to be rebred in a timely manner or culled from the herd if they are repeat breeders. However the time required to perform transrectal RTU might limit its use for pregnancy diagnosis on a routine basis. In comparison, when transabdominal RTU was used, 91% of sows were diagnosed with 95% accuracy by day 24 of gestation. This procedure is faster and easier to perform than transrectal ultrasound, but pregnancy cannot be diagnosed in 9% of sows at day 24 of gestation.

The ability to visualize ovarian CLs and fetuses in 1 to 2 minutes might make transrectal RTU more useful for identifying causes of poor farrowing rates or high numbers of nonproductive days. It has been observed that 20 to 48% of all mated sows that fail to farrow return to estrus 18 to 25 days after breeding.[8–10] Therefore, most sows that fail to farrow do not return to estrus at 21 days and cannot be diagnosed as not pregnant when a boar is used for estrus detection. It is likely that many of these sows are pseudopregnant, maintaining CLs but failing to accumulate fluid identifiable by RTU. Data suggest that RTU might be used to effectively reduce the number of nonproductive days that occur as a result of pseudopregnancy.[11]

When transabdominal RTU and a 3.5-MHz transducer are used, pregnancy can be diagnosed accurately as early as day 24 of gestation, but the optimum day for routine pregnancy diagnosis, on the basis of uterine fluid diameter and speed of diagnosis, is near day 30. Errors in pregnancy diagnosis may occur when RTU is used later (ie, days 36 to 45 of gestation) when there is less fluid in the uterus, fetuses are growing, and fetal skeletons are calcifying. Initial pregnancy diagnosis usually is performed between days 22 and 35 of gestation, with follow-up RTU examinations usually per-

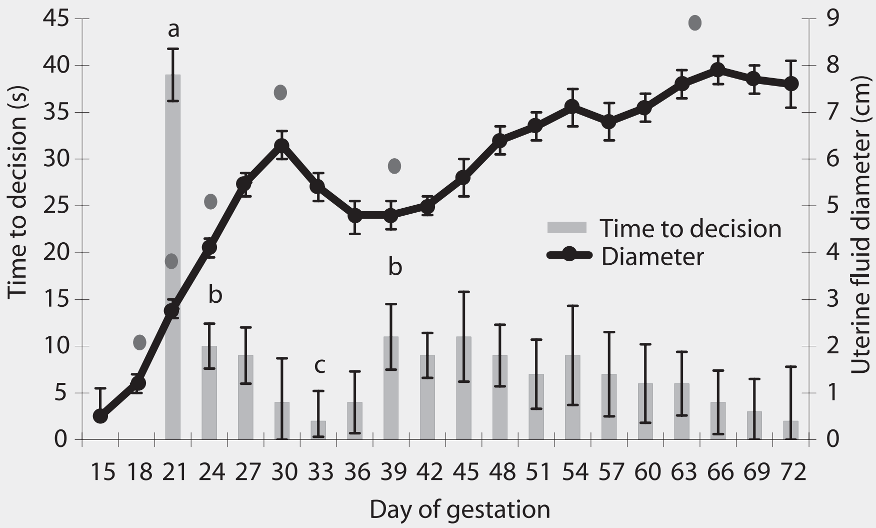
Table 3: Least squares means for Diameter of the largest corpus luteum (CL), Time to observe a CL, and Time to observe a fetus using transrectal real-time ultrasound in a total of 100 sows on days 16 to 24 of gestation (Experiment 1)

Measure	Day of gestation (n)					SE
	16	18	20	22	24	
CL diameter (cm)	0.94[ab] (21)	0.99[a] (16)	0.96[ab] (19)	0.89[b] (18)	0.87[b] (14)	0.04
Time to observe a CL (min)	2.2 (21)	2.3 (25)	1.4 (24)	1.4 (15)	1.9 (21)	0.3
Time to observe a fetus (min)	NA[1]	1.7[a] (3)	1.3[a] (24)	1.3[a] (25)	0.8[a] (21)	0.3

a,b Within a row, means with different superscripts are different (*P*<.05; Scheffé test).

1 NA: not applicable. It was not possible to observe a fetus at 16 days gestation.

Figure 1: Least squares means (± SE) for time to diagnose a sow as pregnant (Time to decision; seconds) and the largest cross-sectional diameter (Uterine fluid diameter; cm) of the fluid-filled uterine horn on days 15 to 72 of gestation (Experiment 2). Transrectal RTU was used to obtain Uterine fluid diameter measurements for days 15 to 21, and transabdominal RTU was used to obtain Uterine fluid diameter for days 22 to 72. Means for Time to decision with different letters are different (*P* <.05). Gray dots denote differences in Uterine fluid diameter (*P* <.05).



formed within the next 2 weeks in cases where a diagnosis could not be made or the sow was diagnosed not pregnant. Thus, culling decisions might be based on examinations performed between days 38 and 50 of gestation, when errors in diagnosis are likely to occur.

The accuracy of the RTU methods evaluated in this study was based on the percentage of females that farrowed. There was every indication that the methods used were highly accurate in diagnosing females that were pregnant, on the basis of our previously defined criteria for a positive pregnancy diagnosis and our observation of the fetus and reproductive status of the ovaries. In most cases when sows diagnosed pregnant failed to farrow, fluid had clearly accumulated, and in some cases, fetuses were observed, and it was not readily evident why pregnancy failed. However, because the sows failed to farrow, the method of diagnosis was determined to be inaccurate. In reality, this may not be the best way to evaluate RTU for pregnancy diagnosis, as there is evidence in this study and others[8,9,11] that early pregnancy failure does occur. Perhaps RTU is a reliable method for diagnosing early pregnancy failure.

In this study and others,[4,5] accuracy of transabdominal RTU was >90% by day 22 of gestation. However, in this study, only 53% of the animals examined could be diagnosed on day 22, possibly because the technician was given the option to remain undecided. If no decision could be made, RTU was repeated 2 days later. Under field conditions, if no diagnosis can be made on day 22, ultrasound should be repeated 6 to 8 days later to allow adequate time for the diameter of the fluid vesicles to reach maximum detectable levels in pregnant females. In this study, no decision was made in some cases in which uterine fluid was visible, because a diagnosis of Pregnant required observation of multiple, fluid-filled pockets >1 cm in diameter. Another possible reason that the technicians in this study failed to make a diagnosis on day 22 was that they were unable to positively identify nonpregnant animals before day 24 of gestation. There is a need for accurate pregnancy diagnosis before day 24 to identify nonpregnant females and either rebreed them or remove them from the herd, in order to reduce nonproductive days. The inability of transabdominal RTU to diagnose animals as not pregnant before day 24 of gestation limits its use for this purpose. In contrast, transrectal RTU was effective in identifying both pregnant and nonpregnant animals before day 24. Although this method requires more than 1.5 minutes per animal and is also somewhat invasive and uncomfortable for the female, the ability to accurately diagnose almost all pregnant females and more than 50% of nonpregnant females suggests that using transrectal RTU to detect pregnancy on days 20 to 22 could aid in determining reasons for pregnancy failure and reducing open days.

Transrectal RTU provided an advantage over the transabdominal method in that it allowed visualization of the ovaries and fetuses as additional confirmation of pregnancy. With the potential ability to detect fetuses by day 18 of pregnancy, transrectal RTU may reduce the possibility of error due to fluid accumulation in the uterus as a result of endometritis, pseudopregnancy, or some other factor. As the ovary of a pregnant sow contains CLs necessary for maintenance of pregnancy, the presence of CLs on the ovary may aid in pregnancy diagnosis, but should not be used as the sole determining factor. Luteal regression begins on day 15 of the estrous cycle in nonpregnant females.[12] It is possible for pseudopregnant sows to have fully formed CLs (1.2 cm) on the ovary after day 15. Pseudopregnant sows may be identified by transrectal RTU for selective administration of prostaglandins.

Changes detected in uterine fluid diameter in Experiment 2 were similar to results obtained by Martinat-Botte et al[13] on days 18 to 38 of gestation. Allantoic fluid volume increases rapidly between day 18 and day 30 of gestation.[7,14] Knight et al[7] suggested that this is due to expansion of the chorioallantoic membranes and attachment of the placenta to the endometrium. Allantoic fluid volume decreases between days 30 and 40, increases again to day 60, then decreases. Changes in the uterine fluid vesicles observed in Experiment 2 reflect this pattern. In contrast, amniotic fluid increases between days 30 and 85, then decreases to day 112. Since the diameter of uterine fluid vesicles remains high after day 60, when allantoic fluid has decreased, the observed fluid measurements may reflect the increasing volume of amniotic fluid.

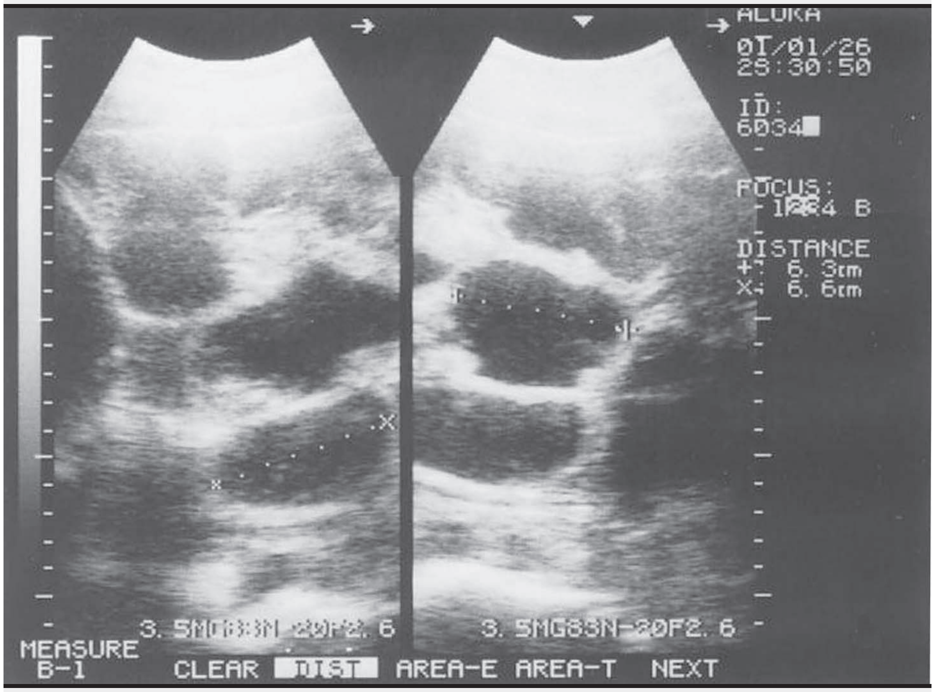
Fetal weight increases throughout gestation, with the most rapid increase occurring after day 50. Ossification of fetal skeletons begins at approximately day 35 of gestation.[15] Intuitively, it is likely that pregnancy diagnosis performed between days 38 to 46 would be more difficult and prone to error, since the fluid-to-fetus ratio declines, resulting in more time required to make a diagnosis.

Implications

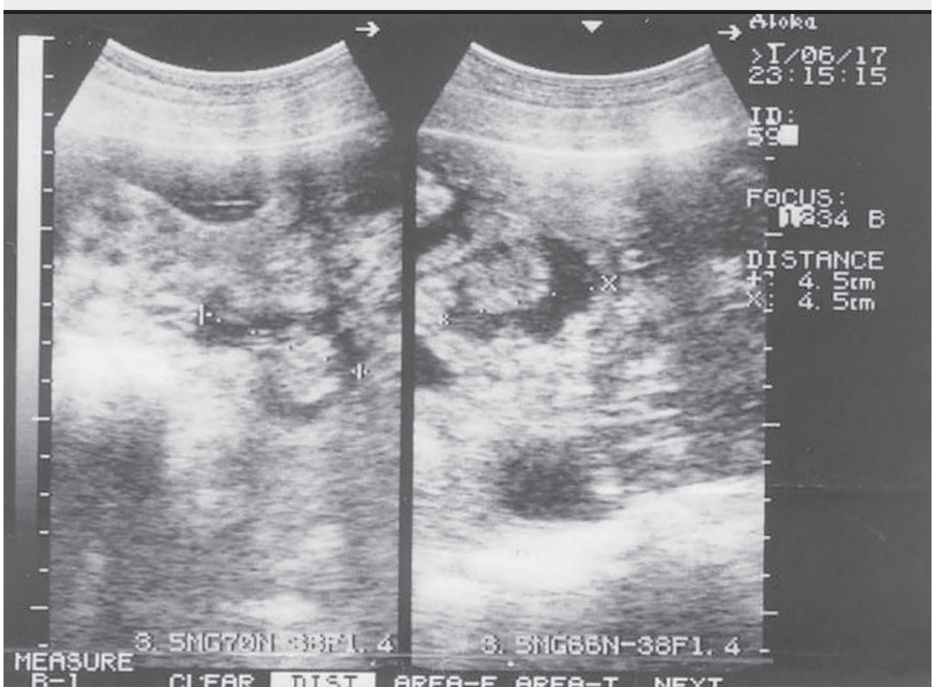
- Transrectal ultrasound accurately identified pregnant and nonpregnant sows in at least 98% of cases at days 22 to 24 of gestation.
- Transabdominal RTU accurately identified pregnant sows in 91% of cases at day 24, but was limited in its ability to identify most nonpregnant sows.
- More time was required for routine pregnancy diagnosis using transrectal RTU compared to transabdominal RTU.
- The ability of transrectal RTU to detect follicles, CLs, and fetuses might make it useful for supporting field diagnosis and determining causes of pregnancy failure.
- For routine pregnancy diagnosis using transabdominal RTU, early and accurate diagnosis was possible by day 24, but day 30 was optimum for accurate diagnosis.
- Accurate diagnosis at 30 days gestation allows identification of nonpregnant sows that may be rebred or culled, reducing nonproductive days in the herd.
- Use of RTU after day 35 may increase the risk for errors in pregnancy diagnosis.

Figure 2: Representative transabdominal RTU cross-sectional images of a sow uterus on day 29 of gestation (A) and on day 39 of gestation (B). Magnification of the image in Figure 2B is 2× that of the image in Figure 2A. The fetus is present within the uterus on day 39. The bar on Figure 2A is 6.6 cm and the bar on Figure 2B is 4.5 cm; in each case, the bar measures the width of the fluid vesicles.

2A



2B



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