Early pregnancy diagnosis by transrectal ultrasonography in ewes

J.E. Romano⁠,⁎, C.J. Christians b

⁎ Corresponding author. Tel.: +1 612 624 9694; fax: +1 612 625 6241.
E-mail address: roma0033@umn.edu (J.E. Romano).

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Technical note

Abstract

Two studies were performed to evaluate early pregnancy diagnosis by transrectal ultrasonography (TRUS), using a 7.5-MHz transducer in ewes. In the first study, the objectives were to determine the earliest date that a reliable pregnancy diagnosis could be made (percentage of ewes detected pregnant between days 15 and 20 after mating). In the second study, the objective was to confirm the findings of the first study using a randomized controlled trial. In both studies, the ewes were restrained in dorsal recumbency, using a special chute that maintained the pelvis at an angle of 30–35° lower than the head. In the first study, 30 Suffolk ewes were synchronized and maintained with 2 rams for 5 days. Each ewe was subjected to the first TRUS on day 15 after mating and daily thereafter until day 20 (estrus = day 0). Pregnancy was defined as the presence of an embryo or extra-embryonic membranes. The percentage of ewes detected pregnant at days 15, 16, 17, 18, 19 and 20 were 0% (0/30), 26.7% (8/30), 86% (24/30), 90% (27/30), 96.7% (29/30) and 100% (30/30), respectively (P < 0.001). In the second study, 390 TRUS examinations (TRUS-1) were performed on ewes from 10 to 50 days after mating in a breeding program (group mating, hand mating, cervical and intrauterine AI; breeding group; n = 270) or with vasectomized rams (vasectomized group; n = 120). The breeding date and the status of breeding were unknown to the operator. Thirty of these ewes were mated with vasectomized rams and used repetitively four times as the non-pregnant control group. All females had a subsequent TRUS (TRUS-2) between 7 and 30 days after the TRUS-1 examination. The second TRUS was used as the standard test against which the performance of the TRUS-1 was compared. The percentage of ewes correctly diagnosed at day 15 or less, days 16, 17, 18, and 19 in the breeding group were 0% (0/29), 31.3% (5/16), 40% (8/20), 70% (7/10), and 100% (14/14), respectively (P < 0.001). All the diagnoses of ewes more than 20 days following mating in the breeding group, were correctly predicted (n = 181), as well as all ewes from the vasectomized group (n = 120). It could thus be concluded that the earliest pregnancy diagnosis using a 7.5-MHz transducer by transrectal route based on the presence of positive signs of pregnancy is at day 16 and the maximum sensitivity and negative predictive value was reached at day 20 following breeding. © 2008 Elsevier B.V. All rights reserved.

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1. Introduction

An early, reliable, non-invasive, fast, repeatable and reproducible method of early pregnancy diagnosis for immediate diagnosis of pregnancy or non-pregnancy is essential for the efficient reproductive management of a flock (Bretzlaff et al., 1993; Romano et al., 1998a,b). Different methods have been used for pregnancy diagnosis in sheep (Watt et al., 1984; West, 1986; Richardson, 1972; Bazer et al., 2007). In recent years, real-time ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants. Here, the two approaches...
for pregnancy diagnosis have been the transabdominal (Fowler and Wilkins, 1984; White et al., 1984; Taverne et al., 1985) and transrectal procedures (Buckrell et al., 1986; Gearhart et al., 1988; Garcia et al., 1993; Kähn et al., 1993). Transrectal ultrasonography offers several advantages over other methods used for pregnancy diagnosis in sheep. These include an earlier time of pregnancy diagnosis, earlier determination of conceptus number, embryo/fetus viability, the estimation of embryo/fetus age, reduction of misdiagnosis, sexing and the diagnosis of abnormal pregnancies (Bretzlaff et al., 1993; Coubrough and Castell, 1998; Romano et al., 1998a,c).

At present, the main problem in evaluating the accuracy of transrectal ultrasonography during early pregnancy in sheep has been the lack of a simultaneous non-invasive accurate standard method of comparison or a criterion standard test. Different approaches such as progesterone in milk or blood can be used (Robertson and Sarda, 1971; Shemesh et al., 1979; Thimonier et al., 1979) or the determination of pregnancy-specific proteins (PSP’s) in blood are performed. However, many of these methods yield high false positives and negatives, especially during the early stages of pregnancy (Ranilla et al., 1994; Karen et al., 2003; Vandaele et al., 2005).

Previous studies have indicated the possibility of diagnosing pregnancy as early as 12 days after breeding by transrectal ultrasonography (Garcia et al., 1993). However, these findings were based primarily on the ultrasonographic appearance of the uterine lumen and location of the uterus rather than on the presence of positive signs of pregnancy (Buckrell et al., 1986; Gearhart et al., 1988; Garcia et al., 1993). The presence of fluid in the uterine cavity cannot be considered to be a positive sign of pregnancy as other clinical conditions may often cause such accumulations (Kähn, 1992; Bretzlaff, 1993; Bretzlaff et al., 1993; Romano et al., 1998a; Bretzlaff and Romano, 2001). So for example in cattle, ultrasonicographic images around estrus have shown small fluid collections within the uterus (Kastelic et al., 1989). Similar studies have not been described in the ewe. Nevertheless, if intruterine fluid pockets exist during the follicular phase of a subsequent estrous cycle, they could be confused with a conceptus during the first 3 weeks of pregnancy (Bretzlaff et al., 1993). Moreover, pathological conditions that accumulate uterine fluid such as mucometra and early stages of pyometra could be also mistaken for pregnancy (Bretzlaff et al., 1993; Romano et al., 1998a). Unfortunately, the prevalence of these pathological conditions in ewes is not fully established. An ewe cannot be definitively considered pregnant until exclusive signs of pregnancy (positive or confirmatory signs) are visualized. Positive signs of pregnancy described both in ewes and cows are, e.g. the presence of allantochorionic membranes, the amniotic sac, embryo/fetus and the presence of placentomes (Green and Winters, 1945; Romano et al., 1998a).

In previous studies, transrectal ultrasonography has been performed by, e.g. restraining the ewes in a standing position (Buckrell et al., 1986; Garcia et al., 1993; Kähn et al., 1993), a combination of standing position and abdominal lift (Karen et al., 2004), in right lateral recumbency (Gearhart et al., 1988), dorsal recumbency (Schrick and Inskeep, 1993) or dorsal recumbency with the pelvis in a position lower than the head (Romano et al., 1998a). The position of the female will affect the distance between the uterus and the transducer, therefore, affecting the quality and accuracy of the scanned images. A transducer with high frequency generates a better image resolution, but has a shallower depth of penetration. Therefore, close proximity of the uterus to the rectal wall during transrectal ultrasonography will improve the visualization and scanning efficiency (Griffin and Ginther, 1992).

The objectives of the first study were to determine the earliest date for a reliable pregnancy diagnosis (based on positive signs of pregnancy), based on the percentage of ewes detected pregnant between days 15 and 20 after mating, using a 7.5-MHz transrectal probe in ewes restrained in the dorsal recumbent position. The objective of the second study was again to confirm the findings of the first study by using a randomized controlled pregnancy diagnosis experiment in sheep.

2. Materials and methods

The trials were performed during three consecutive fall seasons at the University of Minnesota, St Paul Campus, Sheep Teaching and Research Unit. In the first study, 30 Suffolk ewes (aged 1–3 years) were synchronized for estrus with intravaginal sponges impregnated with 60 mg MAP (Depo-provera, Sigma Chemical Co., St Louis, MO 63178) for a period of 12 days. Two fertile rams with marking crayon harnesses were used in the pen breeding period for 5 days, after sponge removal and reintroduced again at day 14. During the mating period, sexual behavior was observed for 45 min, twice a day (07:00 and 18:00; estrus = day 0). Each ewe was considered in estrus when observed to have a crayon mark on her rump or when she was directly observed to accept a service from a ram. Each ewe was subjected to the first transrectal ultrasonography (TRUS) on day 15 and daily thereafter until day 20 after estrus and mating. The females were restrained in dorsal recumbence, in a modified Commodore Tilting Cradle, that maintained the pelvis at an angle 30–35° lower than the head (Plate 1). Females were not deprived of feed or water before examination. All TRUS procedures were performed between 07:00 and 10:00 by the same operator using a B-mode scanner (Aloka SSD 500; Coromet-
ing, cervical and intrauterine artificial insemination; from different breeding programs (group mating, hand mat- 
beat. No attempts were made to count the number of embryos. A viable embryo was considered to be an embryo that showed a detectable heart 
confirmed on the following day of scanning. A viable embryo presence of extra-embryonic membranes—which were both 
primarily in the presence of an embryo and secondarily by the 
reproductive tract. The transducer was cleaned with an anti-
septic solution between examinations. Pregnancy was based 
mainly in the presence of an embryo and secondarily by the 
presence of extra-embryonic membranes—which were both 
confirmed on the following day of scanning. A viable embryo was considered to be an embryo that showed a detectable heart 
beat. No attempts were made to count the number of embryos. 

The second study included 390 TRUS examinations. Ewes from different breeding programs (group mating, hand mat-
ing, cervical and intrauterine artificial insemination; n = 270) as well as from breeding with vasectomized rams were used. Polypay, Hampshire, Suffolk, Dorset, Montadale and cross-
bred ewes (aged 1–4 years) were used. In this trial, the 30 ewes bred with vasectomized rams were used repetitively four times (n = 120) as the non-pregnant, control group. All ewes were presented to the operator at different days after breeding (from day 10 to 50) for pregnancy diagnosis. The breeding date and the status of each ewe were unknown to the operator at the time of TRUS diagnoses. Pregnancy diagnosis by TRUS was performed as described in the first study. 

All females were subjected to an initial TRUS (TRUS-1) examination and then to a subsequent transrectal ultrasonography (TRUS-2) 7–30 days after the first TRUS. The earliest day after breeding that each female had a TRUS-2 procedure performed, was day 25. Day 25 was chosen based on previous data which showed TRUS had 100% accuracy to detect pregnancy starting at this day post-breeding. During this second examination, the operator was also not aware of the results obtained in TRUS-1. Some of the ewes diagnosed non-pregnant by TRUS-2 in the breeding program were rebred and included again in the study, but each pair of TRUS examinations for a given ewe was considered to be an independent observation. The results of TRUS-2 were considered the criterion standard test against which the performance of the TRUS-1 was measured and compared. 

The sensitivity of diagnosis was defined as the ability of the first ultrasonography to correctly detect pregnancy in animals pregnant at the second examination. The specificity was defined as the capacity of TRUS-1 to correctly identify the non-pregnant females based on non-pregnancy at TRUS-2. Further the positive predictive value (PPV) was determined as the probability of a female diagnosed pregnant at TRUS-1 to be diagnosed pregnant at TRUS-2. A negative predictive value (NPV) on the other hand, was the probability of a female diagnosed non-pregnant at TRUS-1 to be further diagnosed non-pregnant at TRUS-2. A correct pregnancy diagnosis was thus stated as an ewe diagnosed to be pregnant with TRUS and subsequently confirmed pregnant during the next TRUS and an ewe diagnosed non-pregnant with TRUS and subsequently confirmed non-pregnant in the next TRUS. An incorrect diagnosis was seen as an ewe diagnosed non-pregnant with TRUS and subsequently confirmed pregnant at the next TRUS (false negative) and an ewe diagnosed pregnant with TRUS and subsequently confirmed non-pregnant in the next TRUS (false positive). The sensitivity, specificity and predictive values of pregnancy diagnosis were then calculated (Browner et al., 1988).

2.1. Statistical analysis

In the first study, the proportion of animals detected pregnant was compared with the results of the following day, as well as all comparisons between days of TRUS using a Chi-

square statistic analysis. In the second study, the proportion for each day (using TRUS-1) was compared with the result of TRUS-2 using the Chi-square test. A difference was considered statistically significant at a level of \( P < 0.05 \) (Devore and Peck, 1993).

3. Results

In the first study, the percentage of ewes detected pregnant on days 15, 16, 17, 18, 19 and 20 were 0% (0/30), 26.7% (8/30), 86.0% (24/30), 90.0% (27/30), 96.7% (29/30) and 100% (30/30), respectively. Ewes pregnant on day 15 was significantly different from day 16 (\( P < 0.01 \)) and days 17, 18, 19 and 20 (\( P < 0.001 \)), while those pregnant on day 16 were significantly different from days 17, 18, 19 and 20 (\( P < 0.001 \)). Day 17 was similar to day 18, but was significantly different from days 19 (\( P < 0.05 \)) and 20 (\( P < 0.01 \)). No significa-
test differences in ewes pregnant were detected between days 18, 19 and 20 following mating. The earliest day of
pregnancy detection was day 16 and the maximum sensitivity and negative predictive value was obtained on day 20 following mating.

In the second study, the percentage of ewes correctly diagnosed from days 10 to 15, 16, 17, 18, and 19 in the breeding group were 0% (0/29), 31.3% (5/16), 40% (8/20), 70% (7/10), and 100% (14/14), respectively ($P < 0.001$). Ewes pregnant on days 10–15 were significantly different to day 16 ($P < 0.01$) and days 17, 18 and 19 ($P < 0.001$), while day 16 was similar to days 17 and 18, but significantly different to day 19 ($P < 0.001$). Ewe pregnant at day 17 was not different from day 18, but significantly different from day 19 ($P < 0.001$). Day 18 was significantly different to day 19 of gestation. The sensitivity of the technique gradually improved to reach 100% at day 19. The negative predictive values improved from 50.8% from days 10–15 to 100% at day 19 in the breeding group. In the vasectomized group all the ewes exposed were correctly diagnosed in all periods ($n = 120$; Table 1). In the breeding group, all ewes further than 20 days after breeding, were correctly diagnosed ($n = 181$).

4. Discussion

The present study shows that pregnancy diagnosis in ewes based on the presence of positive signs of pregnancy using transrectal ultrasonography with a 7.5-MHz transducer was possible starting at day 16 of pregnancy. This method reached maximum sensitivity and negative predictive values at day 20 following mating. Previous studies reported the diagnosis of early pregnancy to be possible following the second week of mating. However, no mention of the percentage of ewes detected pregnant during successive days or the criteria used to determine pregnancy was made (ultrasonographic appearance of the uterine lumen and location of the uterus vs. presence of positive signs of pregnancy) (Gearhart et al., 1988; Garcia et al., 1993).

Currently, only one study using a 7.5-MHz transrectal probe for ultrasonography in the early pregnancy diagnosis of ewes has been reported. In this study, all the females were detected pregnant on day 15—based on the presence of extra-embryonic membranes and the presence of fluid (Schrick and Inskeep, 1993). Unfortunately successive days of diagnoses have not been reported. Buckrell et al. (1986) suggested the 87% sensitivity obtained from days 25 to 50 days of pregnancy to be due to the abdominal placement of the genital tract and the beyond transducer-reach scanning when ewes were in a standing position. Gearhart et al. (1988) detected the earliest pregnancy at day 20, using a 5-MHz TRUS and the sensitivity improved from 12.3% during the first 25 days of pregnancy to 65% from day 26 to 50 of pregnancy (scanning the ewes in right lateral recumbency). Garcia et al. (1993) showed the sensitivity for pregnancy diagnosis at days 17–19 of gestation to be only 58% and to improve to 85% by days 32–34 of pregnancy, when using a 5-MHz transrectal transducer for ewes in the standing position. Kaulfuss et al. (1996a) was also able to detect pregnancy at days 17–18 and see the embryo on days 20–21 using a 5-MHz transrectal transducer in ewes. In a further study, the same authors (Kaulfuss et al., 1996b) confirmed the accuracy of transrectal ultrasonography diagnosis to be over 95% at the beginning of the third week and 100% on day 35 after mating.

In a recent study, fasting prior to TRUS and the lifting of the abdomen in front of the udder during transrectal scanning using a 5 MHz in standing position ewes was shown to improve the sensitivity of pregnancy diagnosis. However, the level of sensitivity was 46% for days 18–24, 92.5% for days 25–30, 92.3% for days 31–40 and 96.8% for days 41–50 of pregnancy (Karen et al., 2004).

In the present study, 100% accuracy was obtained from day 20 after breeding. Two factors may however contribute to the higher accuracy of the present study. Firstly, the use of a good system to restrain the ewe—an inclined position that reduces the movement of the ewe and facilitates the transrectal scanning by retaining the genital tract within the pelvis in a comfortable position for the operator. Secondly, the use of a 7.5-MHz lin-

| Time after breeding(days) | Diagnosis | | | | | |
|----------------------------|-----------|---|---|---|---|
|                            | Breeding group | | | | | |
|                            | Number | Correct | Incorrect | Number | Correct | Incorrect |
| 10–19                      | 89     | 34    | 55     | 30     | 30     | 0         |
| 20–30                      | 71     | 71    | 0      | 30     | 30     | 0         |
| 31–40                      | 51     | 51    | 0      | 30     | 30     | 0         |
| 41–50                      | 59     | 59    | 0      | 30     | 30     | 0         |

Table 1
Accuracy of first transrectal ultrasonography (TRUS-1; from days 10 to 50) when considering the second transrectal ultrasonography (TRUS-2) as the criterion standard test.
eral transducer gave a more precise and detailed image definition than the 5 MHz used in previous studies.

Withholding of feed prior to TRUS examinations was not required as recommended in previous studies (Buckrell et al., 1986; Gearhart et al., 1988; Karen et al., 2004). The only side effect of the procedure, observed in some ewes, was a partial rectal prolapse. The combined action of compression on the abdominal organs against the pelvis by gravity and a relaxed anal sphincter could be factors involved in this clinical condition. The rectal prolapse corrected spontaneously, without intervention, when the ewes were returned to normal standing position.

The false negative levels (mis-diagnoses of non-pregnancy), in both trials, were high only for the period of less than 19 days after breeding. A correct diagnosis of non-pregnancy is important, as a management decision based on an incorrect diagnosis could be extremely costly. The proportion of false negatives gradually reduced on a per-day basis reaching 0% on day 20 after breeding. This was due probably to the positive signs of pregnancy becoming clearer as pregnancy progressed. Previous studies showed a higher percentage of false negative results during early pregnancy by transrectal ultrasonography. The reported percentages of false negative were 13% for days 25–50 of pregnancy (Buckrell et al., 1986), 35% for days 26–50 of pregnancy (Gearhart et al., 1988), 50% for days 21–23 of pregnancy (Garcia et al., 1993) and 54% for days 18–24 of pregnancy (Karen et al., 2004).

The absence of false positives (due to pregnancy loss) as described in previous studies requires clarification (Kaulfuss et al., 1996a, 1997). Different factors can account for the absence of false positives such as, e.g. the embryos not counted, examination of multiparturant ewes, low pregnancy loss and short-time intervals between TRUS procedures. Determination of the number of embryos would allow for detection of partial pregnancy loss at the second TRUS. False positive diagnosis would only result if all embryo/fetuses are lost at the second TRUS. In the case of single pregnancies, the presence of embryo/fetal death would be easily detected as an open female following the second TRUS. The interval between TRUS (first and second) was, in most of the ewes, less than 10 days. Shorter intervals reduce the possibility of detecting pregnancy loss, compared to longer intervals (Schrick and Inskeep, 1993). In the present study, this last possibility was confirmed, as some ewes detected pregnant following both TRUS or at TRUS-2 did not lamb.

TRUS, in the present study, was shown to be an earlier and more accurate method of pregnancy diagnosis at day 20, compared to studies employing the determination of pregnancy specific proteins (PSP). Concentrations of ovine pregnancy associated glycoprotein 1 (PAG-1) using a heterologous radioimmunoassay (RIA) were undetectable during the first 2 weeks of pregnancy and began to be only detectable at week 3 of pregnancy (in 67%) and 100% detectable in pregnant females by week 4 (Ranilla et al., 1994). In a further independent study, using the same PAG-1 test, a sensitivity of 93.5% was obtained on day 22 and maximum sensitivity on day 29 of pregnancy (Karen et al., 2003). The use of a homologous RIA for PAG-1 provided 98–99% sensitivity at day 25% and 100% at day 45 of pregnancy (Vandaele et al., 2005). Testing for other PSP in ewes, e.g. PSP-B, has been shown to be detectable for the first time using a homologous RIA test at days 19–20 of pregnancy (Willard et al., 1995) and from day 26 of pregnancy when the heterologous RIA was used (Ruder et al., 1988). TRUS, in addition, gives an immediate diagnosis and permitted evaluation of the viability of the embryo/fetus, compared to PSP determinations.

Sensitivity and negative predictive values for pregnancy diagnosis of TRUS in this study are different to those reported in previous studies. It is difficult to compare studies, where conditions and animals may vary. In addition technician skills, restraining positions, ultrasound equipment and resolution were not the same. When a technique such as TRUS is used, two concepts need to be defined—the threshold and confidence level. The threshold level is the earliest day that a pregnancy can be detected and the confidence level is the day on which all animals pregnant as well as all non-pregnant are diagnosed correctly. These two concepts have important practical implications. In the present study, the earliest pregnancy detection was possible at day 16 (threshold level), but not all the pregnant females were detected correctly at that time. The maximum level of accuracy was obtained only from day 20 of mating (confidence level). Therefore, it is recommended that each person determines his/her own threshold and confidence level based on experience, animal bred and category, restraining system, equipment and examination conditions and audit these results as has previously been recommended for cattle (Romano et al., 2006).

5. Conclusion

It is concluded from both the studies, that the earliest pregnancy diagnosis using transrectal ultrasonography using a 7.5-MHz transducer based on the presence of positive signs of pregnancy could be made on day 16 and
the maximum sensitivity and negative predictive value reached at day 20 following mating.

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