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Diagnostic Performance of Transrectal, Transvaginal, and Transabdominal Ultrasonography in Pregnancy Diagnosis in Ewes

Abstract

The aim of this study was to evaluate and compare the diagnostic efficiency of transvaginal (tvUSG) and transabdominal (taUSG) ultrasonography on the 21st, 35th, and 50th days of pregnancy in ewes, using transrectal ultrasonography (trUSG) as the reference method. A total of 100 Awassi ewes in anoestrus were used in the study. Ewes were mated after estrus synchronisation. Pregnancy diagnosis was performed using only tvUSG and trUSG on Day 21 post-mating, whereas on Days 35 and 50, taUSG was additionally applied, allowing the use of all three imaging modalities.

In tvUSG, the sensitivity on Days 21, 35, and 50 was 85.71%, 95.34%, and 87.80%, respectively, while specificity remained 100.0% at all time points. The positive predictive value was 100.0%, and the negative predictive value was 87.93%, 96.61%, and 91.18%, respectively. Overall accuracy was calculated as 93.00%, 98.00%, and 95.00% on Days 21, 35, and 50 (Kappa=0.860; 0.959; 0.895, P<0.001). Conversely, taUSG demonstrated a sensitivity of 88.37% on Day 35 and 95.12% on Day 50, with accuracy values of 95.00% and 98.00%, respectively (Kappa 0.897; 0958, P<0.001). When the mean examination times were evaluated, tvUSG had the shortest diagnostic time with 110.03±3.09 seconds and 57.06±1.19 seconds on days 21 and 35, respectively, while taUSG was by far the fastest method with 50.12±1.40 seconds on day 50 (P<0.001).

In conclusion, tvUSG emerges as an alternative to trUSG for early pregnancy diagnosis, whereas taUSG is clinically more practical in later stages of gestation due to its ease of application and shorter examination time.

Keywords: Ewes, pregnancy diagnosis, transabdominal, transrectal, transvaginal, ultrasonography

Koyunlarda Gebelik Teşhisinde Transrektal, Transvajinal ve Transabdominal Ultrasonografinin Tanısal Performansı

Öz

Bu çalışmanın amacı, koyunlarda gebeliğin 21., 35. ve 50. günlerinde transrektal ultrasonografiyi (trUSG) referans alarak transvajinal (tvUSG) ve transabdominal (taUSG) ultrasonografinin tanısal etkinliğini değerlendirmek ve karşılaştırmaktır. Çalışmada toplam 100 baş anöstrüsteki İvesi koyunu kullanıldı. Koyunlar östrüs senkronizasyonu sonrası çiftleştirildi. Gebelik tanısı, çiftleşmeden sonraki 21. günde yalnızca tvUSG ve trUSG kullanılarak yapılırken, 35. ve 50. günlerde ek olarak taUSG uygulanarak her üç görüntüleme yönteminin de kullanılmasına olanak sağlandı.

tvUSG'de duyarlılık 21., 35. ve 50. günlerde sırasıyla %85.71, %95.34 ve %87.80 olarak bulunurken, özgüllük tüm zaman noktalarında %100.0 olarak kalmıştır. Pozitif öngörü değeri %100.0, negatif öngörü değeri ise sırasıyla

%87.93, %96.61 ve %91.18 olarak bulundu. Genel doğruluk 21, 35 ve 50. günlerde sırasıyla %93.00, %98.00 ve %95.00 olarak hesaplandı (Kappa=0.860; 0.959; 0.895, P<0.001). Buna karşılık, taUSG 35. günde %88.37 ve 50. günde %95.12 duyarlılık gösterirken, doğruluk değerleri sırasıyla %95.00 ve %98.00 idi (Kappa 0.897; 0.958, P<0.001). Ortalama muayene süreleri değerlendirildiğinde, tvUSG'nin 21. ve 35. günlerde sırasıyla 110.03±3.09 saniye ve 57.06±1.19 saniye ile en kısa tanı süresine sahip olduğu, taUSG'nin ise 50. günde 50.12±1.40 saniye ile açık ara en hızlı yöntem olduğu görüldü (P<0.001).

Sonuç olarak, tvUSG erken gebelik tanısında trUSG'ye alternatif olarak ortaya çıkarken, taUSG uygulama kolaylığı ve daha kısa muayene süresi nedeniyle gebeliğin ileri evrelerinde klinik olarak daha pratiktir.

Anahtar kelimeler: Gebelik teşhisi, koyun, transabdominal, transrektal, transvajinal, ultrasonografi

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Introduction

Early pregnancy diagnosis plays a crucial role in improving flock management efficiency and reproductive performance in ewes (Karen et al., 2001; Jones and Reed, 2017). Identifying non-pregnant ewes as early as possible enables their timely inclusion in re-synchronisation programs or culling to prevent economic losses (Akköse et al., 2021; Çebi and Akköse, 2024). Conversely, confirming pregnancy and estimating gestational age or litter size facilitates appropriate nutritional and management strategies to reduce abortion and neonatal losses (Ulusoy and Kaymaz, 2009; Allabban and Erdem, 2020; Satıcı and Özyurtlu, 2024).

Various methods such as abdominal palpation, laparotomy, cervical mucus testing, hormone assays, and ultrasonography have been used for early pregnancy diagnosis in small ruminants (Karen et al., 2001; Allabban and Erdem, 2020; Akköse et al., 2021; Uyanık et al., 2026). Among these, real-time B-mode ultrasonography is currently the most practical, reliable, and animal welfare-friendly technique (Jones and Reed, 2017; Ali et al., 2024). Advances such as portable and wireless probes have further expanded its use under field conditions (Allabban and Erdem, 2020).

Transrectal ultrasonography (trUSG) is considered the reference method for pregnancy diagnosis in ewes due to its high accuracy between days 25 and 35 of gestation (Dinç et al., 2001; Petrujkić et al., 2016; Allabban and Erdem, 2020). However, fasting, rectal emptying, and dorsal recumbency required for this technique limit its practicality and animal welfare (Romano and Christians, 2008; Petrujkić et al., 2016). Transabdominal ultrasonography (taUSG), although easier to perform, provides reliable results only after days 45-55 and is therefore unsuitable for early diagnosis (Aziz and Lazim, 2012; Barbagianni et al., 2017).

Transvaginal ultrasonography (tvUSG) has recently gained attention as a rapid, easily applicable method that allows accurate diagnosis from day 25 onward without the need for fasting or rectal preparation (Köker et al., 2012; Moraes et al., 2009; Fasulkov et al., 2021). Thanks to special vaginal probes, the uterine contents can be visualised directly, offering shorter examination time and improved animal welfare (Philip et al., 2017).

Based on the literature, tvUSG appears promising for early pregnancy diagnosis; however, its comparative effectiveness with trUSG and taUSG in practical field conditions has not been fully assessed. Therefore, this study aimed to evaluate and compare the diagnostic efficiency of tvUSG and taUSG at 21, 35, and 50 days of pregnancy in ewes, using trUSG as the reference method.

Material and Methods

Animals and Management

The study was conducted in the Reyhanlı District of Hatay Province, located in the Southeastern Anatolia Region, outside the breeding season (April-June 2025). This area is located at 36° 15' 55.6308 "N and 36° 32' 46.4424 "E and has an altitude148 of metres. A total of 100 clinically healthy, 2-5 years

old, 40-45 kg live weight multiparous Awassi ewes were used in the study. All ewes included in the study were grazed in natural pastures. The ewes were not given extra roughage except for pasture, but they had access to water *ad-libitum* for 24 hours.

Estrus Synchronisation and Mating Procedure

All ewes in the study (n=100) were administered intravaginally 60 mg medroxyprogesterone acetate (MAP) containing sponge (Esponjavet®, Hipra, Spain) for seven days. At the time of sponge removal, 500 IU eCG (5 ml) was administered intramuscularly (i.m.) (Oviser®, Hipra, Spain), and 75 µg D-cloprostenol sodium ($PGF_2\alpha$) (Senkrodin®, Vetaş, Turkey) (1 ml) was administered i.m. (Uyanık and Gözer, 2024). From the 24th hour after removal of the sponges until the 72nd hour, the rams were added to the flock twice a day (06.00-08.00, 18.00-20.00) for two hours, and the matings were monitored. At 72 hours, mating follow-up was terminated, and rams were removed from the flock. The moment of mating was considered as day 0 (Figure 1).

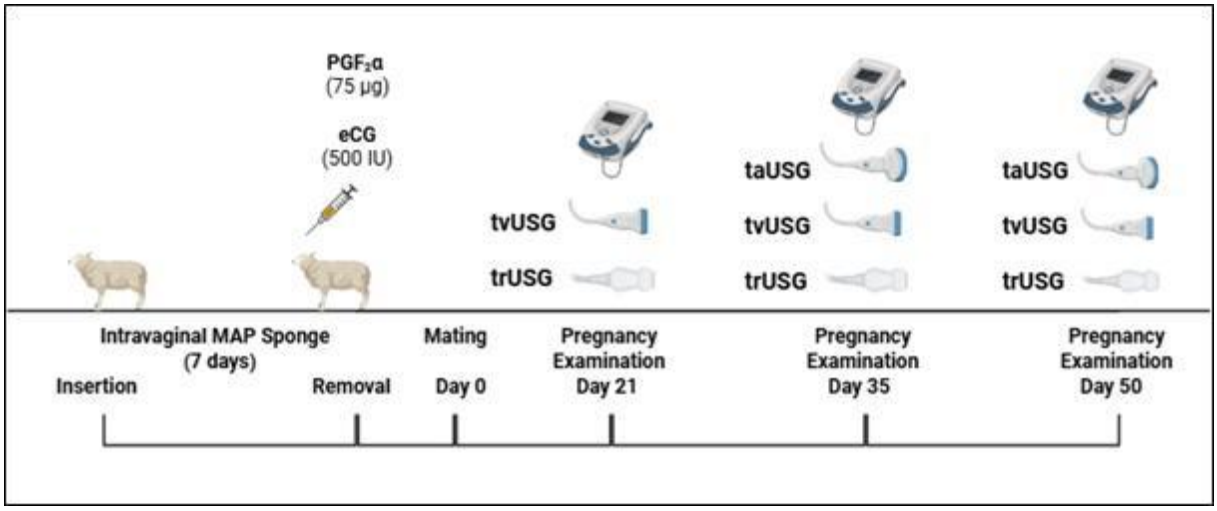

Figure 1. Time schedule of estrus synchronisation protocol, mating, and pregnancy examination applied in the study. Mating (Day 0) and pregnancy examinations (Days 21, 35, and 50) were performed after intravaginal MAP sponge insertion for 7 days and $PGF_2\alpha$ (75 µg) and eCG (500 IU) injections at sponge removal. Transvaginal (tvUSG), transrectal (trUSG), and transabdominal (taUSG) ultrasonography techniques were used during pregnancy examinations.

Ultrasonographic Pregnancy Examination

Pregnancy examinations were performed on days 21, 35 and 50 post-mating. Due to the limitations of taUSG in the early period (Jones et al, 2016), only tvUSG and trUSG were used on day 21, while on days 35 and 50, taUSG was included and evaluated with three methods (Figure 1). trUSG was performed with a real-time ultrasound device (SonoSite M-Turbo, Fujifilm, USA) equipped with a 5-10 MHz linear probe, tvUSG with a 3.5-6.5 MHz endocavitary transducer probe (C25, Landwind Medical, China), and taUSG was performed with a 5-7.5 MHz linear probe equipped device (CTS-800, Hasvet, Turkey). In tvUSG examination, the vulva area was disinfected with 2% benzalkonium chloride solution (Dermosept Zefiran, Aktaş, Turkey), and the probe, prepared with antiseptic solution and ultrasound gel was advanced vaginally until it contacted the cervix. In taUSG applications, ewes were kept standing, and the uterus was visualised over the hairless area between the right paralumbar fossa and udder (Ali et al., 2024). If the gestational sac was detected in the uterus and embryo or foetus, fetal membranes, amniotic fluid, heartbeats, and placentomes were visualised (Figure 2), pregnancy was considered positive and recorded (Uyanık et al., 2024). All examinations were performed independently and blindly by three different operators. In addition, video recordings were taken

during the procedure, and average examination times were calculated for each animal.

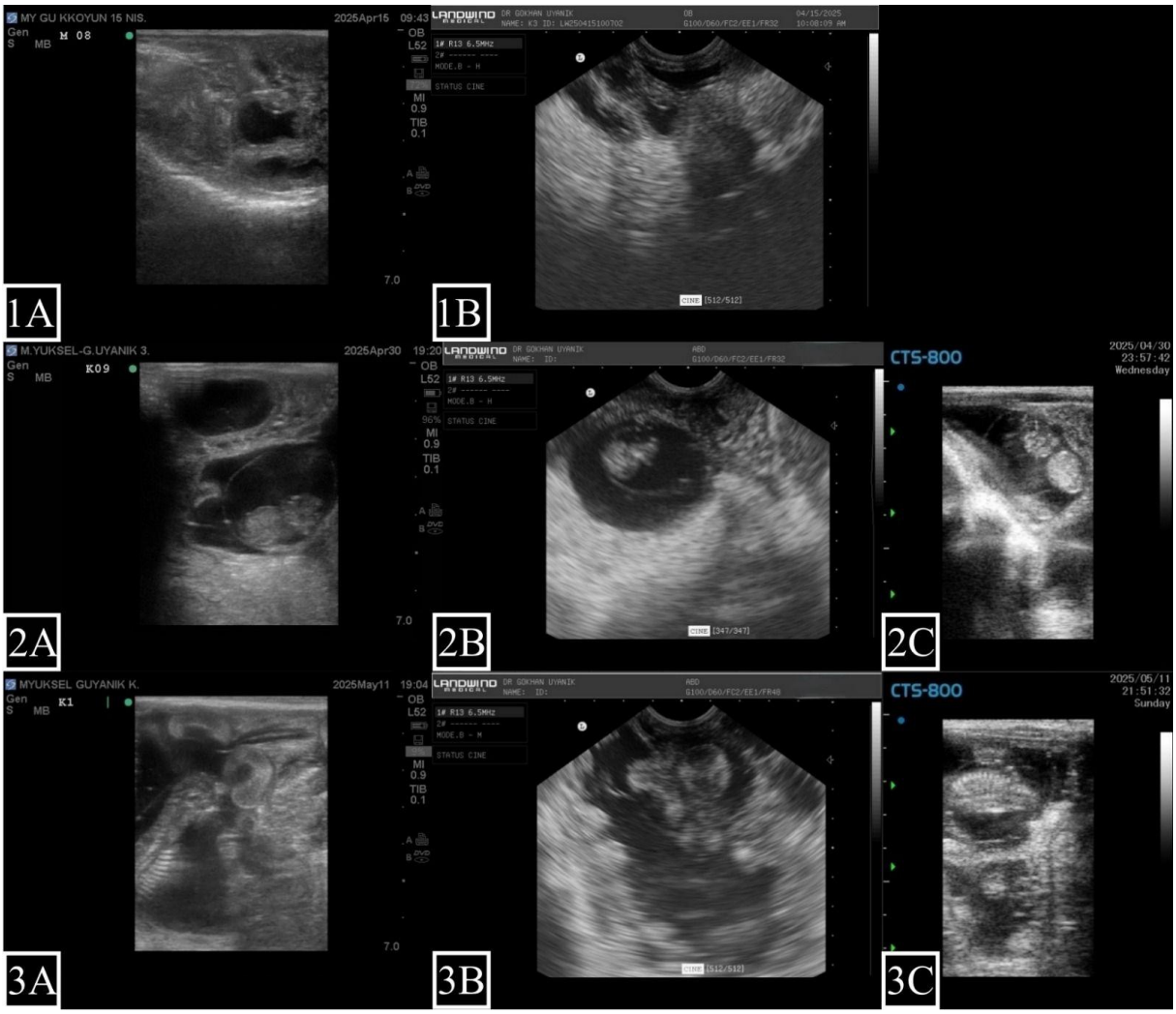

Figure 2. Ultrasonographic images obtained on days 21 (1), 35 (2), and 50 (3) of pregnancy using transrectal (A), transvaginal (B), and transabdominal (C) approaches.

Calculation of Parameters Determining the Effectiveness of Ultrasonography Method

The effectiveness of three ultrasonography methods applied at three different gestational periods in the diagnosis of pregnancy was based on the calculation of parameters determined according to method and time combinations. Diagnostic results were compared with the reference trUSG findings, and accuracy, sensitivity, specificity, positive predictive value, and negative predictive value parameters were calculated using the method described by Martin et al. (1987). Kappa analysis was performed to evaluate the level of agreement between the results of pregnancy examinations performed by tvUSG and taUSG on the 21st, 35th, and 50th days after ovulation and trUSG findings. Kappa coefficients were classified as minimal (≤0.20), weak (0.21-0.40), moderate (0.41-0.60), strong (0.61-0.80), and excellent (0.81-1.00) according to the level of agreement (McHugh, 2012). Mean pregnancy examination times for different ultrasonography methods on day 21 were evaluated using a paired t test, while those on days 35 and 50 were determined using repeated-measures analysis of variance. Bonferroni correction was used for multiple comparisons. Statistical analyses were performed using SPSS 23.0 software, and $P<0.05$ was considered statistically significant in all tests.

Results

During the study, six late embryonic losses were detected between days 21 and 35, and two early fetal losses were detected between days 35 and 50. These animals were considered pregnancy-negative at the relevant examination period. In addition, it was determined that the embryonic and fetal loss rates determined in the study were within acceptable limits (Uyanık et al., 2024), and the examination protocols applied did not adversely affect the pregnancy rates.

The diagnostic performance parameters for tvUSG at 21, 35 and 50 days of gestation were determined as follows: sensitivity 85.71%; 95.34%; 87.80%; specificity 100.0%; positive predictive value (PPV) 100.0%; negative predictive value (NPV) 87.93%; 96.61%; 91.18%; and accuracy 93.00%; 98.00%; and 95.00%, respectively (Table 1). In the light of these data, there was almost perfect agreement between trUSG and tvUSG for pregnancy diagnosis in ewes at 21, 35, and 50 days after mating (Kappa=0.860; 0.959; 0.895, P<0.001).

Table 1. The comparison of transvaginal ultrasonography (tvUSG) pregnancy examination results at 21, 35, and 50 days with transrectal ultrasonography (trUSG) results, and the validity and reliability of tvUSG in the diagnosis of pregnancy

Parameter	Day of pregnancy		
	21st	**35th**	**50th**
Correct positive (cp)	42	41	36
False positive (fp)	0	0	0
Correct negative (cn)	51	57	59
False negative (fn)	7	2	5
Sensitivity %, (CI)	85.71 (72.8 – 94.1)	95.34 (84.2 – 99.4)	87.80 (73.8 – 95.9)
Specificity %, (CI)	100.0 (93.0 – 100.0)	100.0 (93.7 – 100.0)	100.0 (93.9 – 100.0)
Positive predictive value %, (CI)	100.0 (91.6 – 100.0)	100.0 (91.4 – 100.0)	100.0 (90.3 – 100.0)
Negative predictive value %, (CI)	87.93 (76.7 – 95.0)	96.61 (88.3 – 99.6)	91.18 (82.7 – 97.4)
Accuracy %, (CI)	93.00 (85.4 – 97.4)	98.00 (93.0 – 99.8)	95.00 (88.7 – 98.4)

Sensitivity *[cp/(cp + fn)].* ***Specificity*** *[cn/(cn + fp)].* ***PPV*** *[cp/(cp + fp)].* ***NPV*** *[cn/(cn + fn)].*

Accuracy *[(cp + cn)/(cp + cn + fp + fn)].* ***CI****: 95% confidence interval.*

For taUSG, sensitivity was 88.37%, specificity was 100%, PPV was 100%, NPV was 91.93%, and accuracy was 95% on day 35. On day 50, sensitivity was 95.12%, specificity was 100%, PPV was 100%, NPV was 96.72%, and accuracy was 98% (Table 2). In conclusion, taUSG showed excellent agreement with trUSG in the diagnosis of pregnancy on days 35 and 50 (Kappa=0.897; 0.958, P<0.001).

Table 2. The comparison of transabdominal ultrasonography (taUSG) pregnancy examination results at 35 and 50 days with transrectal ultrasonography (trUSG) results, and the validity and reliability of taUSG in the diagnosis of pregnancy

Parameter	Day of pregnancy	
	35th	**50th**
Correct positive (cp)	38	39
False positive (fp)	0	0
Correct negative (cn)	57	59
False negative (fn)	5	2
Sensitivity %, (CI)	88.37 (74.9 – 96.1)	95.12 (83.5 – 99.4)
Specificity %, (CI)	100.0 (93.7 – 100.0)	100.0 (93.9 – 100.0)
Positive predictive value %, (CI)	100.0 (90.7 – 100.0)	100.0 (91.0 – 100.0)
Negative predictive value %, (CI)	91.93 (82.2 – 97.3)	96.72 (88.5 – 99.6)
Accuracy %, (CI)	95.00 (88.7 – 98.4)	98.00 (93.0 – 99.8)

Sensitivity *[cp/(cp + fn)].* ***Specificity*** *[cn/(cn + fp)].* ***PPV*** *[cp/(cp + fp)].* ***NPV*** *[cn/(cn + fn)].*
Accuracy *[(cp + cn)/(cp + cn + fp + fn)].* ***CI****: 95% confidence interval.*

When the mean examination times of different ultrasonography methods were examined, tvUSG had the shortest diagnostic time on day 21, with an average of 110.03±3.09 seconds, while trUSG took longer, with 146.92±3.76 seconds (P<0.001). On day 35, tvUSG (57.06±1.19 seconds) was the fastest, while on day 50, taUSG (50.12±1.40 seconds) achieved the shortest diagnostic time (P<0.001) (Table 3).

Table 3. The average time (in seconds) taken to diagnose pregnancy by transrectal (trUSG), transvaginal (tvUSG), and transabdominal (taUSG) ultrasonography according to different gestational days (Mean ± standard error of the mean [SEM])

Days	Average Time (Second) For Pregnancy Examinations			P-value
	trUSG	tvUSG	taUSG	
21st	146.92±3.76	110.03±3.09	-	<0.001
35th	79.11±1.52 ^{b}	57.06±1.19 ^{c}	160.09±1.97 ^{a}	<0.001
50th	93.38±2.19 ^{b}	172.05±2.34 ^{a}	50.12±1.40 ^{c}	<0.001

**Different superscripts (a, b, c) in the same row indicate significant differences among the groups (P<0.001).*

Discussion and Conclusion

The diagnostic performance of trUSG, tvUSG, and taUSG used in the diagnosis of pregnancy in ewes varies depending on the pregnancy period and application practices. This study aimed to determine the most clinically appropriate methods by comparing the sensitivity, specificity, and overall accuracy of tvUSG and taUSG at different time points (days 21, 35, and 50) compared to trUSG.

In the presented study, despite seven false-negative results in the pregnancy examination performed with tvUSG on the 21st day after mating, a significant majority of pregnancies were correctly detected with a sensitivity of 85.71% and an accuracy rate of 93%, demonstrating excellent agreement with trUSG (Kappa=0.860; P<0.001) (Table 1). In contrast to the present results, Rassu et al. (1997) reported a lower diagnostic performance in ewes, with an accuracy rate of 75.4% for tvUSG 25 days after mating. Similarly, Köker et al. (2012) reported an accuracy rate of approximately 70% for tvUSG in the first four weeks of gestation in goats. Possible reasons for these differences include the small size of embryonic structures in the early stages of pregnancy and the limited imaging area due to the location of the uterus in the pelvic cavity (Aria et al., 2004). In addition, failure to monitor

embryonic losses in previous studies and use of only lambing records as a reference led to the possible losses during pregnancy (Yadav et al., 2019; Uyanık et al., 2024) being overlooked; this may have led to the low diagnostic accuracy of tvUSG in the early period. Finally, technical factors such as operator experience, differences in the animal material used in the studies (age, body weight) and the frequency of the probe used may also have affected the diagnostic accuracy.

When the 35th day of pregnancy was reached, the uterine contents became more clearly distinguishable on ultrasonography and the diagnostic accuracy of all methods increased. tvUSG showed high success with a sensitivity of 95.34% and an accuracy of 98% (Kappa=0.959; P<0.001) (Table 1). Consistent with our findings, Fasulkov et al. (2021) reported that the sensitivity of tvUSG in goats increased significantly to 96.3% at day 35 compared to day 25. In contrast, taUSG performed relatively lower than tvUSG with a sensitivity of 88.37% (Kappa=0.897; P<0.001) (Table 2). This difference may be attributed to the inability of taUSG to detect some pregnancies in the early stages of pregnancy due to its limited imaging capacity of the uterus. Indeed, many sources have reported that taUSG should generally be preferred from the 45th day of pregnancy for reliable pregnancy diagnosis (Jones et al., 2016; Barbagianni et al., 2017). In parallel with our results, Tekin and Köse (2022) reported that taUSG correctly detected 93.93% of pregnancies on day 35 of pregnancy in ewes. Similarly, Parmar et al. (2024) found that the sensitivity of taUSG was 92.86% on day 35 in goats.

On the 50th day of pregnancy, a sensitivity of 95.12%, a specificity of 100%, and an overall accuracy rate of 98% were obtained with taUSG (Kappa=0.958; P<0.001) (Table 2). This finding is consistent with other studies reporting that the diagnostic accuracy of taUSG increases with the progression of pregnancy (Erdem et al., 2008; Jones et al., 2016; Barbagianni et al., 2017; Tekin and Köse, 2022). It has been reported that as the pregnancy progresses and the uterus moves towards the abdominal wall and reaches the limits of taUSG, the transabdominal approach becomes more suitable for the diagnosis of pregnancy (Kandiel et al., 2015; Jones et al., 2016; Tekin and Köse, 2022). On the other hand, tvUSG experienced some loss of sensitivity at this stage (87.80%), which was attributed to the fact that the uterus moved away from the pelvic region with the progression of pregnancy and became difficult to visualise with a vaginal probe (Kappa=0.895; P<0.001) (Table 1). Therefore, taUSG at day 50 and beyond stands out as the most appropriate method in terms of diagnostic accuracy and practicality.

In the present study, when the durations of different ultrasonography methods in pregnancy examination were compared, it was determined that tvUSG had the shortest diagnostic time on days 21 and 35, while taUSG was by far the fastest method on day 50 (Table 3). tvUSG's mean duration of 110.03±3.09 seconds on day 21 provides a significant advantage compared to trUSG. These findings are consistent with studies supporting tvUSG as a rapid and practical diagnostic method, especially in early pregnancy (Moraes et al., 2009; Köker et al., 2012; Jones et al., 2016; Fasulkov et al., 2021). In contrast, taUSG had the lowest mean examination time of 50.12±1.40 seconds at 50 days of gestation compared to other techniques. The present finding is in line with the values reported in previous reports (Meinecke-Tilmann, 2007; Jones et al., 2016) and increases the preferability of this method under field conditions in the late stages of pregnancy. The short duration of the examination both reduces stress in terms of animal welfare and contributes to practical applications by accelerating flock management.

When the applicability of the methods in clinical practice is evaluated, tvUSG has the advantages of short examination time, direct access to uterine contents, and safe application under aseptic conditions. On the other hand, although trUSG is the most sensitive method, especially in the diagnosis of early pregnancy, it has disadvantages such as the need for rectal cleaning, the length of the application time and the risk of mucosal damage. taUSG is the most advantageous method in terms of

non-invasiveness, rapid applicability and animal comfort in later pregnancy periods. However, since the accuracy rate may decrease in very early periods (first 3-4 weeks), it should be used only as a supportive method in this time period. In addition, late embryonic losses between days 21-35 and fetal losses between days 35-50 during the study period show the importance of repeated ultrasonographic examinations in monitoring pregnancy viability. Multiple ultrasonographic evaluations at critical gestational periods may be useful for monitoring embryonic viability, determining the number of offspring, early detection of possible pregnancy losses and prediction of dystocia.

In conclusion, the choice of ultrasonography method for pregnancy diagnosis in ewes should take into consideration the stage of pregnancy, equipment and farm objectives. While trUSG is the most suitable option for individual diagnoses in the early period, tvUSG can be preferred for practical screening at flock level. Especially after the 6th week, pregnancy examination in ewes becomes more practical with taUSG. Sequential and complementary use of these methods can provide high diagnostic accuracy and effective reproductive management.

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Declarations:

1. Statement of Originality:
This work is original.

2. Author Contributions:
Concept: GU,MY,MA; ***Conceptualization:*** GU,MY,MA; ***Literature Search:*** GU, AG; ***Data Collection:*** GU,MY,AG,FK,EC; ***Data Processing:*** UK; ***Analysis:*** GU,UK; ***Writing – original draft:*** GU; ***Writing – review & editing:*** GU,MY,AG,MA,FK,UK,EC.

3. Ethics approval:
This study was performed with the approval of the Local Ethics Committee for Animal Experiments of Mustafa Kemal University, Hatay, Turkey (2025/03-13).

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5. Competing Interests:
The authors declare no competing interests.

6. GenAI Usage Statement:
No GenAI tools were used at any stage of the study.

7. Sustainable Development Goals:

☐ 1 NO POVERTY ☐ 2 ZERO HUNGER ☐ 3 GOOD HEALTH AND WELL-BEING ☐ 4 QUALITY EDUCATION ☐ 5 GENDER EQUALITY ☐ 6 CLEAN WATER AND SANITATION ☐ 7 AFFORDABLE AND CLEAN ENERGY
☐ 8 DECENT WORK AND ECONOMIC GROWTH ☐ 9 INDUSTRY, INNOVATION AND INFRASTRUCTURE ☐ 10 REDUCED INEQUALITIES ☐ 11 SUSTAINABLE CITIES AND COMMUNITIES ☐ 12 RESPONSIBLE CONSUMPTION AND PRODUCTION ☐ 13 CLIMATE ACTION ☐ 14 LIFE BELOW WATER
☒ 15 LIFE ON LAND ☐ 16 PEACE AND JUSTICE STRONG INSTITUTIONS ☐ 17 PARTNERSHIPS FOR THE GOALS THE GLOBAL GOALS For Sustainable Development

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