# Investigation of the effectiveness of ultrasonography in determining pregnancy and the number of fetuses on the 35th day of pregnancy in Awassi sheep

Research Article

**Tufan Can Tekin**[1a]
**Ayşe Merve Köse**[2b]

[1]Altınözü District Directorate of Agriculture and Forestry, Hatay, Turkey
[2]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Turkey

ORCİD-
[a]0000-0001-9737-4233
[b]0000-0003-1863-5955

## ABSTRACT

In this study, it was aimed to determine the effectiveness of transabdominal ultrasonographic imaging on the 35th day of pregnancy in determining the pregnancy status and number of fetuses in sheep. 69 Awassi sheep were used in the study. Sheep were mated after oestrus synchronization during the breeding season. On the 35th and 50th days after mating, a pregnancy examination was performed twice transabdominally in each animal by ultrasonography. Sheep with single/multiple offspring were noted according to examination performing on day 35 and lambing record. According to ultrasonography results, early foetal death rate was determined as 6.1%. Sensitivity, specificity, positive predictive value, negative predictive value ratios for pregnancy examination findings and single/multiple offspring data by transabdominal ultrasonography on day 35 were determined as 91.30%, 100%, 100%, 83.3% and 38.46%, 81.25%, 76.92%, 44.82%, respectively. While the consistency of transabdominal ultrasonographic imaging for pregnancy on day 35 and 50 were high agreement (Kappa=0.864, p<0,001), consistency of single/multiple pregnancy findings on day 35 and at birth were low (Kappa=0,170, p>0.05). The rates of transabdominal ultrasonographic examination on day 35 for correct diagnosis of pregnancy status and the number of fetuses were found as 93.93% and 54.76%, respectively. Finally, it can be concluded that transabdominal ultrasonographic examination on day 35 for early pregnancy diagnose in sheep is highly effective in determination of pregnant sheep, and recurrent examination on days 35 and 50 may be useful for detecting of early foetal deaths. In order to determine the number of fetuses, repeated examinations should be performed in the following days of pregnancy.

**Keywords:** Early fetal death, early pregnancy diagnosis, fetal number, sheep, transabdominal ultrasound

## INTRODUCTION

Sheep show a seasonal reproductive activity, so losing the opportunity to breed could mean losing a whole productive year. Diagnosing pregnancy at an early stage is of great significance in sheep production (Ganaie et al., 2009). If pregnancy cannot be detected early, economic losses may occur in milk and lamb production due to longer lambing intervals (Ishwar, 1995). The widespread use of controlled breeding techniques, in-season/off-season synchronization, and artificial insemination increases the need for accurate and practical tests to diagnose pregnancy at an early stage (Ganaie et al., 2009). Reproductive and production losses in the form of abortion, stillbirth, or weak lambs at birth can be reduced by dividing the herd into pregnant and non-pregnant groups. Accurately knowing the time of pregnancy can be helpful for ceasing to milk lactating females at the appropriate time and for close follow-up of late-pregnant females.

***How to cite this article***
**Tekin, TC., Köse AM. (2022).** Investigation of the effectiveness of ultrasonography in determining pregnancy and the number of fetuses on the 35th day of pregnancy in Awassi sheep. *Journal of Advances in VetBio Science and Techniques, 7*(2), 143-152.
https://doi.org/10.31797/vetbio.1091526

***Correspondence***
**Ayşe Merve KÖSE**
aysemervekose@gmail.com

***Article info***
*Submission*: 22-03-2022
*Accepted*: 26-06-2022
*Online First:* 04-08-2022
*Publication:* 31-08-2022

***e-ISSN:*** 2548-1150
***doi prefix:*** 10.31797/vetbio
- http://dergipark.org.tr/vetbio

Another benefit to early pregnancy detecting is the determination of the number of fetuses in each sheep, an essential information for sheep herds. Because this allows nutritional management to target the different needs of each sheep group. This way, the herd's management can be planned, and reproductive losses can be minimized (Crilly et al., 2017; Mali et al., 2022).

Numerous methods have been used to diagnose pregnancy in sheep. These methods include some less practical ones like not returning to oestrus, abdominal palpation, palpation of the caudal uterine artery, laparotomy, peritonoscopy, vaginal biopsy, and vaginal smear, and some rather practical methods like radiography, hormonal tests, pregnancy protein tests, and ultrasonography (Karen et al., 2001; Singh et al., 2004). In small ruminants, ultrasonography is a safe, fast, accurate, cost-effective, and practical method that can be used to detect pregnancies at an early stage (Crilly et al., 2017). It also provides other advantages like determining the number of fetuses, fetal age and sex, fetal deaths, and monitoring fetal development (Gürler and Kaymaz, 2011, Alkan et al., 2020).

Early detection of pregnancy by ultrasonography gives breeders, clinicians, and researchers the necessary information to improve prenatal care, improve the condition of postnatal lambs, and increases production efficiency in small ruminants (Jyothi et al., 2020). Ultrasonography can be used transrectally or transabdominally with high sensitivity. Although, in practice, transabdominal scanning is less painful for animals, easier and faster for practitioners, and provides a very wide field of view, so it is preferred more (Crilly et al., 2017). For the transrectal approach, the ideal time is 25-30 days; this method also requires more technical equipment and may cause rectal damage and embryonic death (Erdem et al., 2008; Gürler and Kaymaz, 2011). Lone et al. (2016) report that real-time transabdominal ultrasound systems in sheep are reliable for determining pregnancy and numbers of fetuses 50 days after mating. The sensitivity of ultrasound increases as pregnancy progresses. Jones et al. (2016) found that the transabdominal method had a sensitivity of 40% on the 21st day of pregnancy and 100% on the 39th day. In most cases, sensitivity increases from the 40th day of pregnancy, when the uterus becomes intra-abdominal. Hence, the ideal time for diagnosing pregnancy by the transabdominal method is the 40-75th days (Fthenakis et al., 2012; Mali et al., 2022; Lone et al., 2016).

In the present study, we aimed to evaluate the level of agreement between transabdominal ultrasonographic examination on the 35th day of pregnancy and on the 50th day of pregnancy, the latter being more accurate, for diagnosing early pregnancy in sheep. We also tried to examine the level of agreement between single/multiple fetuses detected at the early stage (35th day) and lambing records.

## MATERIAL and METHOD

The animal used in the study consisted of 69 Awassi sheep (45-50 kg b.w.) and 10 Awassi ram (60-70 kg b.w.), healthy and fertile, between the ages of 3-5 and in an animal breeder in Altınozu district of Hatay province. While the animals were taken to herding in fields with dry grass and pasture in the morning, were supplemented with a ration prepared with a mixture of barley grain and straw in the evening. For the drinking water to the animals, clean and fresh water was supplied.

In the study oestrus synchronization was performed to the sheep during the breeding season. Cylindrical polyurethane sponge (Esponjavet, HIPRA) containing 60 mg of Medroxyprogesterone acetate (MPA) was applied intravaginally to all sheep. Sponges were kept in the vagina for 10 days. 125 µg d-

cloprostenol (Gestavet Prost, HIPRA) and 400 IU Pregnant Mare Serum Gonadotropin (PMSG; Oviser 500, HIPRA) were administered intramuscularly to all sheep on the day of sponge removal. At 24 hours following sponge removal, the rams joined the flock and kept with the sheep for 2 hours in the morning and evening, twice a day. The oestrus of the sheep was observed for three days and sheep in oestrus were naturally mated. Rams were left from herd following the mating.

The first examinations for pregnancy were performed 35 days after mating and the second examinations 50 days after mating. All pregnancy examinations were performed transabdominally (tUSG) using a 5 MHz convex probe real-time ultrasound device (Falco, Pie Medical, Netherlands). For the examination area, we chose the hairless region above the udder, ventral to the right fossa paralumbalis when the animals stand and on ventrodorsal recumbency (Dinç et al., 1994). Pregnancy results were accepted as positive when fluid-filled uterus, placentome, fetus with movement, and heartbeat were observed all together (Figure 1). And, sheep with lack of these appearances on tUSG were accepted as non-pregnant. Besides, the diagnosis of fetal losses were also made by determining pregnancy at the first diagnosis and then non-pregnancy at the next diagnosis by tUSG. (Ridler et al., 2015). We recorded animals with single/multiple offspring on examination on the 35th day. All ultrasonographic examinations were performed by the same experienced veterinarian. We also recorded the animals that gave birth to single/multiple lambs.


**Figure 1.** Ultrasonographic image of pregnancy viewed by using a 5 mHz transabdominal probe on day 35 in a sheep.

The fertility parameters were calculated according to the formulae below.

**Oestrus rate:** (Number of sheep in oestrus/Number of sheep undergoing oestrus synchronization) x 100

**Conception rate:** (Number of pregnant sheep/Number of mating sheep) x 100

**Pregnancy rate:** (Number of pregnant sheep/Number of sheep in the group) x 100

**Lambing Rate:** (Number of lambing sheep/Number of pregnant sheep) x 100

In the study, ultrasonographic examination findings on the 50th day were accepted as reference for the confirmation of the pregnancy determined on the 35th day transabdominal ultrasonographic imaging. And, lambing records were accepted as reference for confirmation of single/multiple pregnancy data determined on 35th day transabdominal

ultrasonographic imaging. To analyze the validity and reliability of pregnancy examination findings and single/multiple offspring data by transabdominal ultrasonography on the 35th day, we determined the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rate (Kastelic, 2006; Kaya et al., 2017).

We performed a Kappa analysis to determine the level of agreement between transabdominal ultrasonography findings on the 35th and 50th days after mating, and the level of agreement between single/multiple offspring data on the 35th day and the lambing records (excluding the 4 sheep with false negative results). The level of agreement for the Kappa coefficients were classified as minimal (≤ 0.20), weak (0.21-0.40), moderate (0.41-0.60), strong (0.61-0.80), or perfect (0.81-1.00) (Altman, 1991). We used the SPSS 23.0 software for statistical analysis. For all statistical analyses, $p<0.05$ was considered significant.

## RESULTS

We observed oestrus was in 58 sheep, with an oestrus rate of 84.05%. According to transabdominal ultrasonography examinations, the conception and pregnancy rates were 77.5% and 65.21% on the 35th day and 79.3% and 66.7% on the 50th day, respectively (Table 1). The lambing rate for the whole sample was 100%.

**Table 1.** Fertility parameters

| *Oestrus rate* | *Conception rate* | | *Pregnancy rate* | | *Lambing rate* | *Early fetal death rate* |
|---|---|---|---|---|---|---|
| | **tUSG on Day 35** | **tUSG on Day 50** | **tUSG on Day 35** | **tUSG on Day 50** | | |
| 84.05% (58/69) | 77.5% (45/58) | 79.3% (46/58) | 65.2% (45/69) | 66.7% (46/69) | 100% (46/46) | 6.1% (3/49) |

Repeated ultrasonographic examinations (on the 35th and 50th days after mating) revealed early fetal death in 3 animals. The early fetal death rate was 6.1% (3/49). These 3 animals were excluded from the Kappa analysis for the transabdominal pregnancy examination findings on the 35th and 50th days.

The transabdominal pregnancy examination findings on the 35th and 50th days were found to be perfectly fit (Kappa=0.864, $p<0.001$) (Table 2). Table 2 shows the validity and reliability of transabdominal ultrasonography findings on the 35th day.

**Table 2.** Compatibility of tUSG results on days 35 and 50 and validity and reliability of tUSG results on day 35

| *Pregnancy Status* | *tUSG on Day 35 and 50* | | *Total* |
|---|---|---|---|
| | **available** | **unavailable** | |
| **Positive** | 42 (TP) | 0 (FP) | 42 |
| **Negative** | 4 (FN) | 20 (TN) | 24 |
| **Total** | 46 | 20 | 66 |
| **Sensitivity** | TP/(TP+FN)x100 | | 91.30% |
| **Specificity** | TN/(FP+TN)x100 | | 100% |
| **Positive Predictive Value** | TP/(TP+FP)x100 | | 100% |
| **Negative Predictive Value** | TN/(FN+TN)x100 | | 83.3% |
| **Accuracy ratio** | (TP+TN)/(TP+FP+FN+TN)x100 | | 93.3% |

Kappa=0.864; Compatibility between the two conditions = EXCELLENT True Positive (TP): Pregnant according to ultrasound examination both on day 30 and on day 50, False Positive (FP): Pregnant according to ultrasound examination on day 35, but not on day 50, False Negative (FN): Pregnant according to ultrasound examination on day 50, but not on day 35, True Negative (TN): Not pregnant according to ultrasound examination both on day 35 and on day 50.

The single/multiple offspring findings on the 35th day had minimal agreement with the lambing records (Kappa=0.170, p>0.05) (Table 3). Table 3 shows the validity and reliability of day 35 single/multiple pregnancy findings.

**Table 3.** Compatibility of single/multiple pregnancy findings of tUSG on day 35 with lambing records and validity and reliability of day 35 single/multiple pregnancy findings

| *Offspring status* | *tUSG on Day 35 and lambing records* | | *Total* |
|---|---|---|---|
| | **single** | **multiple** | |
| **Single** | 10 (TS) | 3 (FS) | 13 |
| **Multiple** | 16 (FM) | 13 (TM) | 29 |
| **Total** | 26 | 16 | 42 |
| **Sensitivity** | TS/(TS+FM)x100 | | 38.46% |
| **Specificity** | TM/(FS+TM)x100 | | 81.25% |
| **Positive Predictive Value** | TS/(TS+FS)x100 | | 76.92% |
| **Negative Predictive Value** | TM/(FM+TM)x100 | | 44.82% |
| **Accuracy ratio** | (TS+TM)/(TS+FS+FM+TM)x100 | | 54.76% |

Kappa=0,170; Compatibility between the two conditions = POOR. True Single (TS): Single according to both ultrasound examination on day 35 and lambing records, False Single (FS): Single according to ultrasound examination on day 35, but multiple in lambing records, False Multiple (FM): Multiple according to ultrasound examination on day 35, but single in lambing records, True Multiple (TM): Multiple according to both ultrasound examination on day 35 and in lambing records.

## DISCUSSION

In sheep, progesterone or prostaglandins have been used most commonly for oestrus synchronization. Gonadotropins (eCG and GnRH) are often used to increase the efficiency of P4-based protocols (Hameed et al., 2021). In small ruminants, oestrus occurs 24-48 hours after removing the intravaginal sponges for oestrus synchronization (Uçar and Özyurtlu, 2015). In sheep, the conception rates for progesterone applications are reported to be around 70-80% (Gordon, 1997). Synchronization studies using different doses of MAP+eCG report pregnancy rates between 20% and 100% in sheep (Hameed et al., 2021). In the present study, consistent with the literature, the oestrus rate was 84.05%, the conception and pregnancy rates were 77.5% and 65.21% on the 35th day and 79.3% and 66.7% on the 50th day, respectively (Table 1). Awassi ewes bred during June and July reported 60.7% pregnancy rate, 90% lambing rate and 1.08 fecundity (Talafha and Ababneh, 2011).

The method for diagnosing pregnancy in small ruminants depends on equipment availability, costs, number of days since mating, desired accuracy, and the examiner's experience (Singh et al., 2004). B-mode real-time diagnostic ultrasonography is a useful management tool for reproduction and has been used successfully to detect pregnancy (Karadaev, 2015; Mali et al., 2022). In small ruminants, early diagnosis of pregnancy is possible by detecting fetal heartbeat and uterine characteristics, which indicate implantation and fetal viability (Garcia et al., 1993). Due to the initial position of the uterus and the proximity of the ultrasound probe to the uterine wall, the transrectal method is suitable for early screening before the 30th day (Amer, 2008). As the pregnancy progresses and the uterus moves towards the abdominal wall and into the limits of transabdominal ultrasound imaging, the transabdominal approach becomes more suitable for diagnosing pregnancy (Jones et al., 2016; Kandiel et al., 2015).

In sheep, placentomes develop from the 33rd day of pregnancy (Crilly et al., 2017) and placentome units become viewable transabdominally or transrectally from the

32nd-33rd days of pregnancy. This initially presents as irregular shapes on the uterine wall and then matures into hollow hemispheric structures after the 39th-40th days (da Silva et al., 2018; Jones and Reed, 2017). The transabdominal approach can be used to detect fetal heartbeat between the 27th-30th days in both goats and sheep (Amer, 2008; Amer, 2010; Karen et al., 2009). The limb buds extending from the fetal body become observable from the 35th day (Jones et al., 2016). In the current study, pregnancy findings were evaluated as positive when fluid-filled uterus, placentome, fetal movement, and heartbeat were observed. In our research, we detected pregnant sheep with an accuracy of 93.93% by the transabdominal approach on the 35th day (Table 2). According to the transabdominal ultrasonography findings on the 50th day, the number of false positives was 0 and the number of false negatives was 4. The transabdominal examination findings on the 50th had a perfect agreement (Kappa=0.864, Table 2). Goel and Agrawal (1992) stated that the ultrasound scanner (B-mode) has been used successfully in sheep and the abdominal (5 MHZ) or rectal (7 MHZ) probe is ideal for sheep and goats. Garcia et al. (1993) suggested that while early pregnancy status could be determined at any time after the 30th day using the transabdominal technique with linear-array 5 MHz transducer, these procedures are more accurate when performed between the 40th-80th days. Goel and Agrawal (1992) found that images of uterine fluids, placentomes, and fetuses were evidence of pregnancy with 90% accuracy on the 45th-50th days in sheep. Çelik et al. (2019) determined pregnant sheep with an accuracy of 84% on the 50th day by using ultrasonography device with 3.5 MHz linear transabdominal probe. Küplülü et al. (2002) detected pregnant sheep with an accuracy of 89.4% on the 32nd day after insemination by using 5 MHz transabdominal rectal probe.

Ganaie et al. (2009) reported that using a real-time ultrasound scanner equipped with a 3.5MHz sector array transducer had an accuracy of 68% on the 15th-30th days, which later increased to 100% on the 61st-75th days and remained constant until lambing. Aziz and Lazim (2012) used a real-time ultrasound scanner equipped with a 3.5 MHz convex probe to detect pregnancy in Awassi sheep and reported accuracy rates of 53%, 80%, and 100% on the 21st-24th, 28th-32nd, and 40th-43rd days after insemination, respectively. Navarrete et al. (2021) reported that with transabdominal ultrasonography at 3.5 MHz, pregnancy can be detected with 100% accuracy at day 31 and the embryonic vesicle depth can be used to predict fetal age. Anwar et al. (2008) stated that on the 42nd day of pregnancy in Balkhi sheep, 3.5 MHz transabdominal probe ultrasound with 100% accuracy. Georgel et al (2021) stated that they detected pregnancy with an accuracy of 45% on the 25th day and the accuracy increased to 100% on the 31st day at a transducer frequency of 3.5 MHz transabdominal ultrasound.

Roberts et al (2019) pregnant sheep on the 30th day using a 3.5-5 MHz probe transabdominal ultrasound detected that sensitivity, specificity, positive predictive value, negative predictive value and accuracy ratio determined as 98.6%, 100%, 100%, 96.36%, 98.98%, respectively. In our study sensitivity, specificity, positive predictive value, negative predictive value and accuracy ratios for pregnancy examination findings determined as 91.30%, 100%, 100%, 83.3%, 93.93%, respectively (Table 2). According to literature data, as the penetration depth of the probes increases, the accuracy of the pregnancy diagnosis increases in the early stages. We diagnosed pregnancies with rate of accuracy 93.93% by using 5 MHz probe transabdominal approach on the 35th day. This may be associated with using a convex probe, which improves scanning penetration and allows for

deeper scanning into the abdominal cavity. Goel and Agrawal (1992) highlighted that sector scanners provide a much wider field of view, allowing to visualize the entire uterus. Besides, the accuracy rates are quite variable among studies; these rates seemingly depend on many factors like the sheep's breed, age, type of probe, examination day and region, and operator's experience (Erdem and Sarıbay, 2015).

In sheep, the period until the 34th day of pregnancy is defined as the embryonal period, and the period from the end of the embryonal period to lambing is called the fetal period (Fthenakis et al., 2012). Embryonic or fetal deaths in sheep cause great economic loss. According to research, embryonic and fetal death rates are nearly 30% (Dixon et al., 2007; Fthenakis et al., 2012; Sarıbay and Erdem, 2007). There is limited information on early fetal deaths in sheep, with rates ranging between 3.5-12% (Jones et al., 2016). Researchers show that most embryonic losses occur before the 18th day, with 9.4% of losses from the 18th day to lambing, and 1-5% of late embryonic or fetal losses from the 30th day to lambing (Dixon et al., 2007).

The diagnosis of a fetal loss is made by detecting non-viable fetuses on transabdominal ultrasonography or by determining pregnancy at the first diagnosis and then non-pregnancy at the next diagnosis (Ridler et al., 2015). Studies on embryonic and fetal losses emphasize that plasma progesterone concentrations are similar among animals with fetal loss and continuing pregnancy, they are insufficient to determine losses, and therefore ultrasound examination is necessary (Ridler et al., 2017; Sarıbay and Erdem, 2007). Ridler et al. (2015) performed transabdominal ultrasonography examinations twice per animal and detected 6.8% fetal loss in the second examinations. In our research, repeated transabdominal ultrasonography on the 35th and 50th days revealed that 3 sheep that were pregnant at the first examination were non-pregnant at the second examination, indicating a fetal death rate of 6.1%. We believe that making two examinations per sheep can help determine fetal death rates and allows to make the necessary planning without losing time.

Erdem et al. (2008) reported that, despite finding all sheep to be pregnant after transrectal examination, they could detect single pregnancies at a rate of only 64% in transabdominal examinations on the 34th day. Alkan et al. (2020) reported that singleton pregnancy determination by using transabdominal ultrasonography with 5 MHz convex transducer was detected with percentage 62.06%, 84.21%, 81.81%, 73.80% and 70.27% on days 40, 45, 50, 55, 60, respectively. Karen et al. (2006) investigated 61 single offspring pregnancies and 39 multiple offspring pregnancies and correctly diagnosed 21 of the 39 multiple pregnancies by transabdominal ultrasonography on the 43rd-56th days. The authors found that the remaining 18 sheep were falsely diagnosed with single offspring. They reported that transabdominal ultrasonography had a specificity of 78.6% for diagnosing of single offspring pregnancies. Alkan et al. (2020) stated that the accuracy of the diagnosis of twin pregnancies by transabdominal ultrasonography was higher on day 60 and its specificity was 91.66%. Fridlund et al. (2013) assessed accuracy and affecting factors for pregnancy screening by transabdominal ultrasonography and found that the accuracy (percentage of fetuses scanned/number of lambs born) decreased as the number of fetuses increased. The authors demonstrated that for the number of fetuses diagnosed, the sheep's breed, age, gestational age, and operator's experience significantly impacted the accuracy. They reported accuracy rates of 71.8%, 91.6%, and 89.3% on days <40, 40-80, and 81-100, respectively. They found that the number of fetuses was often overestimated during screening.

Karen et al. (2006) reported that the rate of accurate detection of multiple pregnancy was 53.8% on days 43-56 and 60% on days 76-87. In the present study, the lambing records showed that 26 sheep had single offspring and 16 had multiple offspring. According to the transabdominal findings on the 35th day, there were 16 false diagnoses for multiple offspring (Table 3). As stated by Allabban and Erdem (2020), the reason for false diagnosis of multiple offspring may be that the existing fetus may have been counted twice, or premature fetal death or fetal death may have occurred after the examination. Because, we performed no further examination for the number of offspring until lambing.

Gonzalez-Bulnes et al. (2010) stated that embryos can be viewed from the 28th day with transabdominal ultrasonography, however they are recommended to postpone the examination to the 35th day. In addition, same authors highlighted that pregnancy diagnosis is recommended to be performed after days 40–55 as efficiency in counting the number of conceptuses in multiple pregnancies reaches 100%. Compared to the lambing records, the transabdominal examinations on the 35th day had an accuracy rate of 76.9% for single offspring and 44.8% for multiple offspring (Table 3). We observed that the results obtained for diagnosing single offspring were similar to the literature, and the rate of detecting single offspring by the transabdominal approach on the 35th day was sufficient. However, the accuracy rate for diagnosing multiple offspring by transabdominal ultrasonography may decrease in the earlier days of pregnancy. By knowing the intervals of pregnancy in sheep, more practical and rapid examinations can be done transabdominal in field conditions (Erdem et al., 2020). High accuracy rates can be obtained by performing transabdominal examinations on the 30th day for diagnosing pregnancy and after the 44th day for the number of fetuses (Erdem et al., 2008). We diagnosed pregnancies with a high rate of accuracy (93.93%) by the transabdominal approach on the 35th day under field conditions, but the accuracy rate for diagnosing single/multiple offspring was only 54.76%.

## CONCLUSION

In conclusion, we believe that performing transabdominal ultrasonographic examinations on the 35th day is sufficient for diagnosing early pregnancy in sheep. If the number of fetuses needs to be determined, transabdominal ultrasonograhy should be performed in the following days of pregnancy rather than day 35. Besides, it should be kept in mind that the accuracy rates for diagnosing pregnancy and determining the number of fetuses by transabdominal examination depend on many factors like the sheep's breed, age, probe type, day and region of examination, and the operator's experience. Moreover, performing two transabdominal examinations per sheep on the 35th and 50th days could help detect early fetal losses at a significant rate. This allows to investigate the causes and to take measures on time, contributing to higher efficiency in production.

## ACKNOWLEDGMENT

This study was funded by Hatay Mustafa Kemal University Coordinatorship of Scientific Research Projects, Project number of 21.D.008. This study was carried out by using some of the data from Tufan Can Tekin's PhD thesis project. The authors wish to thank Dr. Pınar Ambarcıoğlu from ‘Department of Biostatistics, Faculty of Veterinary Medicine University of Hatay Mustafa Kemal’ for helping in the statistical analysis in this study.

Ethical approval: Hatay Mustafa Kemal University Rectorate Animal Experiments Local Ethics Committee, 20/10/2021 No: 2021/06-12

Conflict of interest: The authors declared that there is no conflict of interest.

## REFERENCES


**Alkan, H., Kivrak, M. B., Satilmis, F., Tekindal, M. A., & Dinc, D. A. (2020**). Detection of twin pregnancies in ewes by pregnancy-associated glycoprotein assay and transabdominal ultrasonography.*Domestic Animal Endocrinology*, *72*, 106399. doi.org/10.1016/j.domaniend.2019.106399

**Allabban, M., & Erdem, H. (2020).** Koyunlarda real-time ultrasonografik muayene ile gebelik tanısı. *Bahri Dağdaş Hayvancılık Araştırma Dergisi*, *9*(1), 47-55.

**Altman, D.G. (1991).** *Practical statistics for medical research*. Chapman and Hall, London.

**Amer, H. A. (2008).** Determination of first pregnancy and foetal measurements in Egyptian Baladi goats (Capra hircus). *Veterinaria Italiana*, *44*(2), 429-37.

**Amer, H. A. (2010).** Ultrasonographic assessment of early pregnancy diagnosis, fetometry and sex determination in goats. *Animal Reproduction Science*, *117*(3-4), 226-31. doi.org/10.1016/j.anireprosci.2009.05.015

**Anwar, M., Riaz, A., Ullah, N., & Rafiq, M. (2008).** Use of ultrasonography for pregnancy diagnosis in Balkhi sheep. *Pakistan Veterinary Journal*, *28*(3).

**Aziz, D. M., & Lazim, E. H. (2012).** Transabdominal ultrasonography in standing position for pregnancy diagnosis in Awassi ewes. *Small Ruminant Research*, *107*(2-3), 131-135. doi.org/10.1016/j.smallrumres.2012.05.007

**Çelik, H. T., Aslan, F. A., Arıcı, Y. K., Kahveci, M. E., & Kiper, İ. (2019).** A study on the use of transabdominal ultrasonography and pregnancy condition score in pregnancy in Karayaka sheep. *Turkish Journal of Agriculture-Food Science and Technology*, *7*(11), 1789-1794. doi.org/10.24925/turjaf.v7i11.1789-1794.2616

**Crilly, J. P., Politis, A. P., & Hamer, K. (2017).** Use of ultrasonographic examination in sheep veterinary practice. *Small Ruminant Research*, *152*, 166-73. doi.org/10.1016/j.smallrumres.2016.12.021

**da Silva, P. D. A., Uscategui, R. A. R., Santos, V. J. C., Taira, A. R., Mariano, R. S. G., Rodrigues, M. G. K., Simoes, A. P. R., Maronezi, M. C., Avante, M. L., Vicente, W. R. R., & Feliciano, M. A. R. (2018).** Qualitative and quantitative ultrasound attributes of maternal-foetal structures in pregnant ewes. *Reproduction in Domestic Animals*, *53*(3), 725-32. doi.org/10.1111/rda.13163

**Dinç, D. A., Taşal, İ., Erdem, H., Semacan, A., & Aral, S. (1994).** Koyunlarda transabdominal ultrasonografi ile yavru sayımı. *Eurasian Journal of Veterinary Sciences 10*, 81-83.

**Dixon, A. B., Knights, M., Winkler, J. L., Marsh, D. J., Pate, J. L., Wilson, M. E., Dailey, R.A., Seidel, G., & Inskeep, E. K. (2007).** Patterns of late embryonic and fetal mortality and association with several factors in sheep. *Journal of Animal Science*, *85*(5), 1274-84. doi.org/10.2527/jas.2006-129

**Erdem, H. & Sarıbay, M. K. (2015).** Çiftlik Hayvanlarında Doğum ve Jinekoloji (2nd ed.). In A. Semacan, M. Kaymaz, M. Fındık, A. Rişvanlı, & A. Köker (Eds.), *Gebelik ve tanı yöntemleri* (pp 507-521). Medipres, Malatya.

**Erdem, H., Alkan, H., Kıvrak, M. B., Satılmış, F., & Alabban, M. (2020).** İvesi ırkı koyunlarda gebelik muayenesi amacıyla dorsal transrektal ve transabdominal yolla yapılan ultrasonografik muayenenin etkinliği. *Bahri Dağdaş Hayvancılık Araştırma Dergisi*, *9*(1), 7-12.

**Erdem, H., Sarıbay, M. K., & Tekeli, T. (2008).** Geç embriyonal ve erken fötal dönemde Konya Merinosu koyunlarda gebelik tanısı ve fötal sayım amacıyla transabdominal ultrasonografik muayene tekniğinin etkinliğinin değerlendirilmesi. *Eurasian Journal of Veterinary Sciences*, *21*(1), 15-20.

**Fridlund, C., Humblot, P., Bage, R., & Söderquist, L. (2013).** Factors affecting the accuracy of pregnancy scanning in ewes. *Veterinary Record*, *173*(24), 606-606. doi.org/10.1136/vr.101935

**Fthenakis, G. C., Arsenos, G., Brozos, C., Fragkou, I. A., Giadinis, N. D., Giannenas, I., Mavrogianni, V. S., Papadopoulos, E., & Valasi, I. (2012).** Health management of ewes during pregnancy. *Animal reproduction science*, *130*(3-4), 198-212. doi.org/10.1016/j.anireprosci.2012.01.016

**Ganaie, B. A., Khan, M. Z., Islam, R., Makhdoomi, D. M., Qureshi, S., & Wani, G. M. (2009)**. Evaluation of different techniques for pregnancy diagnosis in sheep. *Small Ruminant Research*, *85*, 135-41. doi:10.1016/j.smallrumres.2009.09.003

**Garcia, A., Neary, M. K., Kelly, G. R., & Pierson, R. A. (1993).** Accuracy of ultrasonography in early pregnancy diagnosis in the ewe. *Theriogenology*, *39*(4), 847-61. doi.org/10.1016/0093-691X(93)90423-3

**Georgel, V., Navarrete, J., Kibler, M., Rickard, J., Earing, J., & Lugar, D. (2021).** Embryonic vesicle depth using transabdominal ultrasound early in gestation as a predicter of gestational age in sheep. *Transactions of the Illinois State Academy of Science*, *114*, 19-22.

**Goel, A. K., & Agrawal, K. P. (1992).** A review of pregnancy diagnosis techniques in sheep and goats. *Small Ruminant Research*, *9*(3), 255-64. doi.org/10.1016/0921-4488(92)90155-W

**Gonzalez-Bulnes, A., Pallares, P., & Vazquez, M. I. (2010).** Ultrasonographic imaging in small ruminant reproduction. *Reproduction in Domestic Animals*, *45* (2), 9-20. doi.org/10.1111/j.1439-0531.2010.01640.x

**Gordon, I. (1997).** *Controlled Reproduction in sheep and goats*. Universty College Dublin Ireland.

**Gürler, H. & Kaymaz, M. (2011).** Akkaraman ırkı koyunlarda transrektal ve transabdominal muayene tekniği kullanılarak embriyonik ve fötal yaşın belirlenmesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *58*, 99-104.

**Hameed, N., Khan, M. I. U. R., Zubair, M., & Andrabi, S. M. H. (2021).** Approaches of estrous synchronization in sheep: developments during the last two decades: a review. *Tropical Animal Health and Production*, *53*(5), 1-10. doi.org/10.1007/s11250-021-02932-8

**Ishwar, A. K. (1995).** Pregnancy diagnosis in sheep and goats: a review. *Small Ruminant Research, 17*, 37-44. doi.org/10.1016/0921-4488(95)00644-Z

**Jones, A. K., & Reed, S. A. (2017).** Benefits of ultrasound scanning during gestation in the small ruminant. *Small ruminant research*, *149*, 163-71. doi.org/10.1016/j.smallrumres.2017.02.008

**Jones, A. K., Gately, R. E., McFadden, K. K., Zinn, S. A., Govoni, K. E., & Reed, S. A. (2016).** Transabdominal ultrasound for detection of pregnancy, fetal and placentallandmarks, and fetal age before day 45 of gestation in the sheep. *Theriogenology 85*, 939-45, e931. doi.org/10.1016/j.theriogenology.2015.11.002

**Jyothi, K., Sudha, G., Krishna, K. M., Sahadev, A., Swamy, M. N., Rao, S., & Kshama, M. A. (2020).** Transabdominal ultrasonographic measurement of placentome length to estimate and validate gestational age in Nellore brown ewes. *Journal of Entomology and Zoology Studies*, *8*(6), 1460-67.

**Kandiel, M. M., Watanabe, G., & Taya, K. (2015).** Ultrasonographic assessment of fetal growth in miniature Shiba goats (Capra hircus). *Animal Reproduction Science, 162*, 1-10. doi.org/10.1016/j.anireprosci.2015.08.007

**Karadaev, M. (2015).** Pregnancy diagnosis technıques in goats-a review. *Bulgarian Journal of Veterinary Medicine*, *18*(3). 183-193. doi.org/10.15547/bjvm.837

**Karen, A. M., Fattouh, E. S. M., & Abu-Zeid, S. S. (2009).** Estimation of gestational age in Egyptian native goats by ultrasonographic fetometry. *Animal Reproduction Science*, *114*(1-3), 167-174. doi.org/10.1016/j.anireprosci.2008.08.016

**Karen, A., El Amiri, B., Beckers, J. F., Sulon, J., Taverne, M. A., & Szenci, O. (2006).** Comparison of accuracy of transabdominal ultrasonography, progesterone and pregnancy-associated glycoproteins tests for discrimination between single and multiple pregnancy in sheep. *Theriogenology*, *66*(2), 314-322. doi.org/10.1016/j.theriogenology.2005.11.017

**Karen, A., Kovács, P., Beckers, J. F., & Szenci, O. (2001).** Pregnancy dagnosis in sheep: Review of the most practical methods. *Acta Veterinaria Brno*, *70*(2), 115-26.

**Kastelic, J. P. (2006)**. Critical evaluation of scientific articles and other sources of information: an introduction to evidence-based veterinary medicine. *Theriogenology, 66*, 534-42. doi.org/10.1016/j.theriogenology.2006.04.017

**Kaya, M. S., Bademkıran, S., Köse, M., Uçar, E. H., Mutlu, H., & Atlı, M. O. (2017).** Sığırlarda gebelikle ilişkili glikoproteinlerin tespitinde kullanılan iki test kitinin karşılaştırılması. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 12,* 45-53. doi.org/10.17094/ataunivbd.309772

**Küplülü, Ş., Çetin, Y., Macun, H. C., & Taşdemir, U. (2002).** Akkaraman ırkı koyunlarda transrektal ve transabdominal ultrasonografi yöntemi ile erken gebelik tanı sınırlarının belirlenmesi. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, *42*(1), 25-33.

**Lone, S. A., Gupta, S. K., Kumar, N., Prakash, K., Ganaie, B. A., Rather, H. A., & Kumar, S. (2016).** Recent technologies for pregnancy diagnosis in sheep and goat: an overview. *International Journal of Environmental Science and Technology*, *3*, 1208-16.

**Mali, A. B., Mehar, R. M., Kapane, S. H., & Hadimani, M. R. (2022).** Scanning the future: veterinary reproductive ultrasonography. *Animal Reproduction Update*, *1*(2), 82-89. doi.org/ 10.48165/aru.2022.1201

**Navarrete, J., Kibler, M., Earing, J., & Lugar, D. W. (2021).** Estimating fetal age using transabdominal ultrasonic measurements in sheep early in gestation. *Journal of Animal Science*, *99*(1), 25-25. doi.org/10.1093/jas/skab054.044

**Ridler, A. L., Corner-Thomas, R. A., Kenyon, P. R., & Griffiths, K. J. (2017).** Investigation of fetal loss in ewe lambs in relation to liveweight changes and progesterone concentrations in early to mid gestation. *New Zealand Veterinary Journal*, *65*(1), 34-38. doi.org/10.1080/00480169.2016.1241726

**Ridler, A. L., Vallee, E., Corner, R. A., Kenyon, P. R., & Heuer, C. (2015).** Factors associated with fetal losses in ewe lambs on a New Zealand sheep farm. *New Zealand Veterinary Journal*, *63*(6), 330-334. doi.org/10.1080/00480169.2015.1037813

**Roberts, J., May, K., Ajani, O., & Kaneene, J. (2019).** A comparison of pregnancy diagnosis methods in commercial sheep using lambing as a gold standard. *Clinical Theriogenology*, *11*(2), 107-113.

**Sarıbay, M. K., & Erdem, H. (2007).** Kovunlarda real-time ultrasonografi İle embrivonik ölümlerin insidansının belirlenmesi. *Eurasian Journal of Veterinary Sciences*, *23*(3-4), 19-25.

**Singh, N. S., Gawande, P. G., Mishra, O. P., Nema, R. K., Mishra, U. K., & Singh, M. (2004).** Accuracy of ultrasonography in early pregnancy diagnosis in doe. *Asian-australasian journal of animal sciences*, *17*(6), 760-68. doi.org/10.5713/ajas.2004.760

**Talafha, A. Q., & Ababneh, M. M. (2011)**. Awassi sheep reproduction and milk production. *Tropical animal health and production*, *43*(7), 1319-1326. doi.org/ 10.1007/s11250-011-9858-5

**Uçar, M., & Özyurtlu, N. (2015).** Çiftlik Hayvanlarında Doğum ve Jinekoloji (2nd ed.). In A. Semacan, M. Kaymaz, M. Fındık, A. Rişvanlı, & A. Köker (Eds.), *Üremenin denetlenmesi* (pp 491-505). Medipres, Malatya.