The effectiveness of Ultrasonography in predicting Pregnancy, litter size, viability and embryo/fetal age during first three months post-breeding in Iraqi Shami does

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Abstract

This study aimed to evaluate the detection efficiency of early pregnancy by Ultrasonography (ULR), and also to estimate embryo/fetal age, embryo/fetal count and viability in Iraqi Shami Does. Thirty-five mature does were synchronized using progesterone intra-vaginal sponges for 12 days+ equine Chorionic Gonadotrophin (eCG) (500 IU) after sponge removal. Estrus was detected after two days in only 28 does, therefore, seven does were excluded. All does (n=28) were mated by four breeding bucks. Pregnancy monitoring, embryo/fetus number and developmental stages during the does gestation was carried out with B-mode Transrectal ultrasonography (TRU) (Transrectal probe) on days 17-29 Post mating (PM) using a frequency 6.5-7.5 MHz, and Transabdominal Ultrasonography (TAU) with in days 36-90 PM with frequency 3.5-4.5 MHz. The results revealed that the conception rate was 85.71% (24/28) and the Predicted +value (PV+), Predicted –value (PV-), Sensitivity (Se), Specificity (Sp) and Accuracy (Acc) were low on days 17-20 PM, which recorded 50%, 4%, 12%, 25% and 14% respectively. All detection values between 96-100% in the period 36-50 PM, and 100% in day 60 onwards. The efficiency in predicting embryo/fetal number was 77% and 75% for estimating twin (Se) and singleton (Sp) pregnancies in days 23-29 PM, and the detection parameters reached 100% by day 60, then declined after that. The embryo/fetal viability was 60% in the early gestational period and reached 96% between days 36–50 PM, and 100% after that. The correlation coefficient between embryo/fetal age and Embryonic Vesicle Diameter (EVD), Crown Rump Length (CRL) and Trunk Diameter (TD) was recorded as very high in different gestational ages; $R^2 = 0.99$ (23-42 days), $R^2 = 0.97$ (23-60 days) and $R^2 = 0.98$ (42-90 days). It was concluded that ULR (by using both TRU and TAU) was reliable, very efficient and safe to predict pregnancy, embryo/fetal count, viability and age between days 23-35 and 35-90 PM, respectively.

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I.   INTRODUCTION

Goats are recognized as a widely domesticated animal species worldwide due to their remarkable adaptability to changing environmental conditions and varying nutritional regimes (Haibel, 1990). Shami goats constitute an important livestock breed in Iraq, which is primarily raised for milk and meat production. They are distinguished by their high milk yield and a reasonably high rate of twinning (Mavrogenis *et al*., 2006).

Determining pregnancy in the early period is the most important factor in determining female reproductive efficiency. The early pregnancy diagnosis has economic benefits for sheep producers and play an important role in reproductive management. It enables farmers to plan for lambing and make necessary arrangements regarding feeding and healthcare. Early identification of non-pregnant ewes (a short period after mating) helps rapid intervention and opportunity to cull segregate and rebreed barren or non-pregnant ewes (Medan et al., 2004; Lianou 2022).Thus, many reproductive and production losses are minimized as a result of decrease abortions and stillbirths (Wani, 1998).

The use of ultrasonography for pregnancy diagnosis in small ruminants has several advantages over traditional methods such as rectal palpation and serum hormone assays. Conventional methods of pregnancy diagnosis are invasive, time-consuming, and have lower Acc compared to ULR (Bello *et al*., 2023).

Ultrasonography is a non-invasive, safe and practical diagnostic tool, which allows for highly accurate early detection of pregnancy, assessment of embryo/fetal number, estimation of embryo/fetal age, gender, viability and diagnosis of some pregnancy diseases and disorders (Amer, 2010; Ardakani *et al*., 2022; Brzozowska *et al*., 2022), prediction time of parturition and reduce the fetal loss due to dystocia (Barbagianni *et al*., 2017) and early fetal death (Osman, 2010; Hussein *et al*., 2020). Ultrasonography is highly accurate than other methods, with a Se and Sp of over 90% and 95%, respectively, and it allows for early detection of pregnancy as early as 24 days post-breeding (Dana *et al*., 2021).

Ultrasound systems provide several approaches to be used in small ruminants to cover the needs related to pregnancy; A-mode ultrasound device is used for detecting pregnancy status in the second and third trimester (Ammar and Baker, 2021). Real-time B-mode ultrasonography is used with 2D and 3D for early, mid and late pregnancy diagnosis with very high accuracy, It is rapid, safe and the practical means of pregnancy detection, estimation fetal numbers, and gestational age in sheep and ewes (Jones *et al*., 2020; Alkan *et al*., 2020). The Doppler system is also used to predict pregnancy and embryo/fetal viability, but the principle of this approach is based on the detection of fetal circulation, heart beating and/or fetal movements (Elmetwally *et al*., 2016).

According to Okorie-Kanu et al. (2018), 22-59% of the slaughtered doe were pregnant, and about 66% of them carried twins, that caused intensive economic loss in Nigeria. Moreover 7.6% and 23% of slaughtered ewes were found pregnant in Switzerland and Nigeria, respectively (Chama *et al*., 2019; Pagamici and Stephan, 2022). Therefore, early pregnancy diagnosis through ultrasonography is very crucial to minimize such losses.

According to our knowledge, there is little available information on this subject in Iraq. There is no published study on the efficiency of ULR to determine the pregnancy in early and mid-gestation in does, some studies were conducted to estimate pregnancy and fetal age in the mid and late stages of pregnancy in Iraqi goats (Rasheed, 2017; Muhammad and Aziz. 2022a and b). Therefore, the present study aimed to evaluate the detection efficiency of early and mid pregnancy, and also to estimate embryo/fetal age, embryo/fetal count and viability using B-mode ULR and Pulse wave Doppler ULR in local Shami does.

II.   MATERIALS AND METHODS

Experimental animals

This article was conducted in Al Saqlawia city, located in Al-Anbar governorate, Western Iraq. The expreiment extended from February to June, during this period; Thirty-five mature, pluriparous, cyclic Shami does with an average age of 2-3 years were utilized in this study. These does were reared with four breeding bucks in one farm under the same feeding systemnand conditions. The kidding data used a gold standard for determining pregnancy, gestational age and embryo/fetal count.

Estrus Induction and synchronization

Estrus was induced and synchronized using Intra-vaginal sponges containing 60 mg Medroxyprogesterone acetate (MPA) (Hipra/ Spain) for 12 days. At the time of sponge removal, each animal was intramuscularly injected with 500IU of eCG (Hipra, Spain). Two days after eCG injection, all female goats were subjected to heat detection, using four breeding bucks. The 28 does showed after two, which were allowed to mate at least one time with the fertile buck, then isolated from the flock. The day of initial breeding was regarded as day 0 of gestation. Seven females that failed to show estrus were excluded from the study.

Ultrasonographic examination

The pregnancy determination was carried out by utilizing a real-time B-mode ultrasound scanner (Chison/China), equipped with a dual-frequency (6.5 and 7.5 MHz), transrectal probe and a dual-frequency convex-probe (3.5 and 4.5 MHz) for TAU. Transrectal inspection was scanned four times at on days 17–20, and 23–29 post-breeding. While, TAU was used through interval 30-90 post-breeding.

The transcutaneous probe was applied to the inguinal region and scanned laterally above the udder, then 10 cm in front of the udder in advance of pregnancy.

Different embryo/fetal and maternal landmarks were identified to detect pregnancy, fetal number and stage of pregnancy such as; embryonic vesicle, embryo, amniotic and allantoic membranes and fluids, vertebral column, ribs, head, heart beats, extremities, trunk and placentomes. Additionally, it was used to diagnose the fetal number

The embryo/fetal measurements were done in terms of Embryonic Vesicle Diameter (EVD), Crown Rump Length (CRL), Trunk Diameter (TD) and Bi-Parietal Diameter (PBD). Moreover, the embryo/fetal viability was evaluated by using the Pulse wave Doppler approach. By placing the pointer on the selected area (heart or umbilical cord), the pulses appeared on the monitor and sound heard on the speaker according to Brzozowskaet al. (2022). Additionally, the fetal pulses and movements were also detected by B-mode.

Data analysis

The following diagnostic parameters were determined according to Yotov (2005) (50):

Predicted true positive (pregnant) = Diagnosis true positive (A)/ Diagnosis pregnant (True and false positive (A+B)) $\times$ 100.

Predicted True negative (non-pregnant) = Diagnosis true Negative (C)/ Diagnosis non-pregnant (true and false negative (C+D)) $\times$ 100.

Accuracy (Ac %) = Number of True diagnosis /Total Number of diagnoses

(A+(C/D)) $\times$100

Sensitivity (Se %) = Number of True Positive diagnoses (A) /Total Number of Positive diagnoses (A/(A+D)) $\times$100

Specificity (Sp %) = Number of True Negative diagnoses/ total number of negative diagnoses (C/(B+C)) $\times$100.

A. True positive (pregnant), B. False positive (non-pregnant), C. True negative (non-pregnant), D. False negative (pregnant).

III.   RESULTS AND DISCUSSION

 Out of 28 mated does, 24 were detected as pregnant by ULR and kidding data, while four does were not pregnant, indicating the conception rate of 85.71%. Additionally, the ULR and kidding data indicated that 13 does gave single births and 11 gave twin births.

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The reliability of ultrasonography in detecting pregnancy

A doe is considered pregnant when the gestational sac (embryonic vesicle) is first seen by TRU, it looked like a fluid-filled structure appeared at the earlier stage of pregnancy (day 23-29) accompanied by the presence of some embryonic mass, also CL seen in some cases as a circular shape at the top of view with central lcauna, with average size was 7.5 mm. Therefore, the reliable diagnosis of pregnancy via TRU was possible as early as 23 day PM. Before that, the pregnancy diagnosis depended only on the presence of some fetal fluid inside the uterine horn accompanied by the presence of CL (in some cases) (Fig. 1) (Fig. 2).
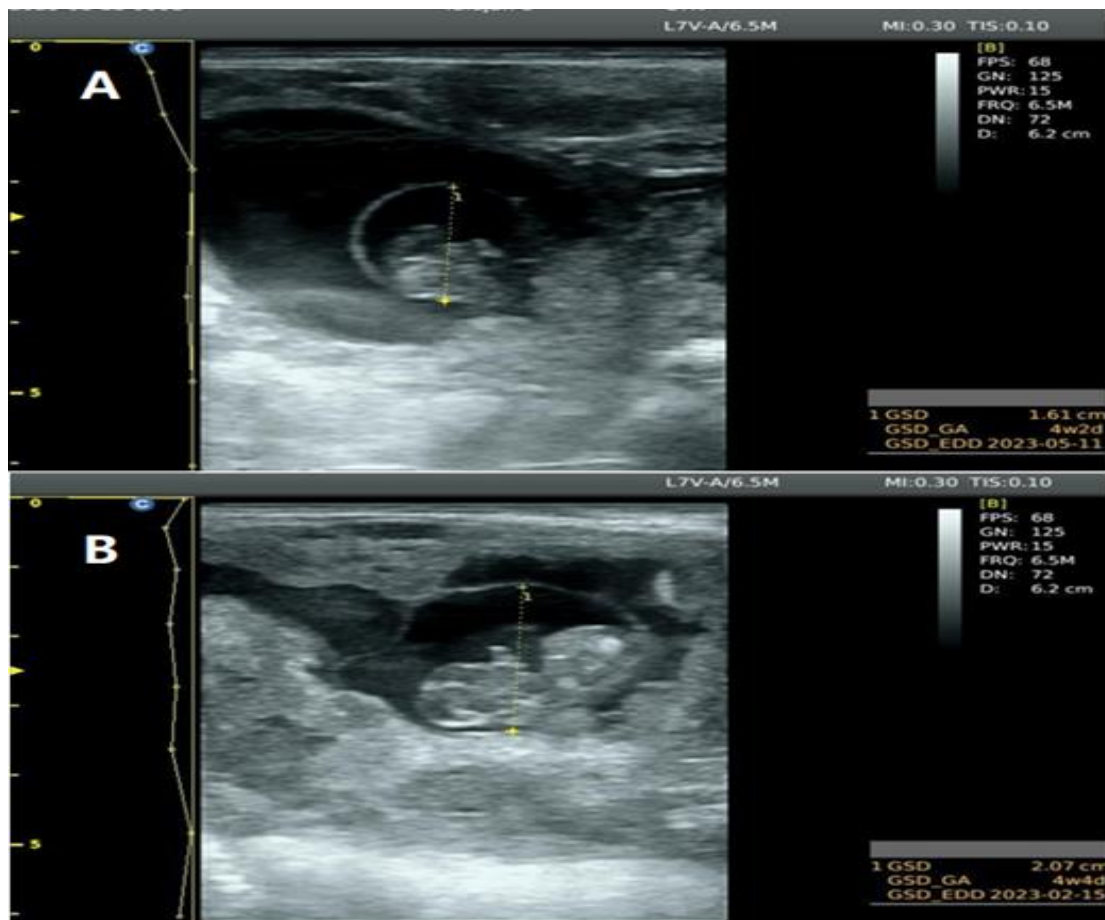

Figure 1: Transrectal Ultrasonography image showing embryonic vesicle and allantoic membrane with proper embryo on day 23 (A) and day 29 PM (B).

Figure 2: Transabdominal ultrasonography image showing embryonic vesicle with proper embryo on day 30 PM.

Abdelghafar et al. (2009) and Riza et al. (2019) reported that the presence of a fluid-filled gestational sac is an indicator that Doe was pregnant. Karen et al. (2009) mentioned that embryonic vesicle was observed in only one case before day 15 PM by TRU and it was easily detected on day 20 and beyond, ten embryonic vesicle out of 15 were detected on day 20 PM. Additionally, Suguna et al. (2008) recorded that embryonic vesicle was first scanned by day 21, which agrees with the present finding. The first embryonic vesicle was detected at 20-25 post-mating in does according to Omran and Hussein (2007). While, Hobi and Al-Jubouri (2016) confirmed the pregnancy by monitoring embryonic sac and fetus on day 35-37 PM. Before day 20, the detection of pregnancy only depends on the presence of fetal fluid in the uterine horn. A study conducted by Padilla-Rivas et al. (2005) revealed that the detection of allantoic fluid before day 20 (day 19.5) by TRU may be considered a sign of pregnancy. The same authors also mentioned that the corresponding time for the first reliable detection of pregnancy was from day 24 onwards depending on the presence of fetal fluid.

On period (17-20) PM, only three does appeared positive signs of pregnancy with a very high rate of false negatives (1-Se) (88%), the Se and Acc were very low during this period. On days (23-29) PM, the Se and Acc were improved because the positive signs of pregnancy became prominent. The Se was 70% (17 out of 24 does detected as pregnant) and Acc 67% with moderate Sp, the false negative was

approximately low (30%). All pregnancy parameters were improved after that, it reached near to optimal values (96-100%) in period (36-50) PM and 100% in periods 60–70 and 80-90 PM when examined by TAU (Table 1) (Fig 3-5).
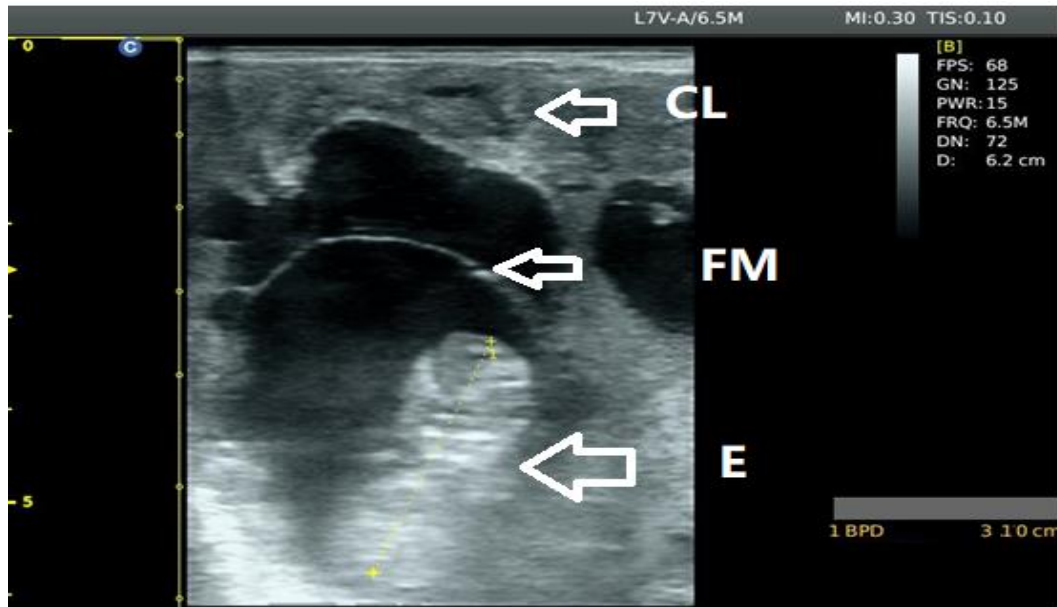

Figure 3: Transrectal Ultrasonography image showing gestational sac, fetal membrane (allanoic membrane) (FM) and corpus luteum (CL) on day 35 PM.
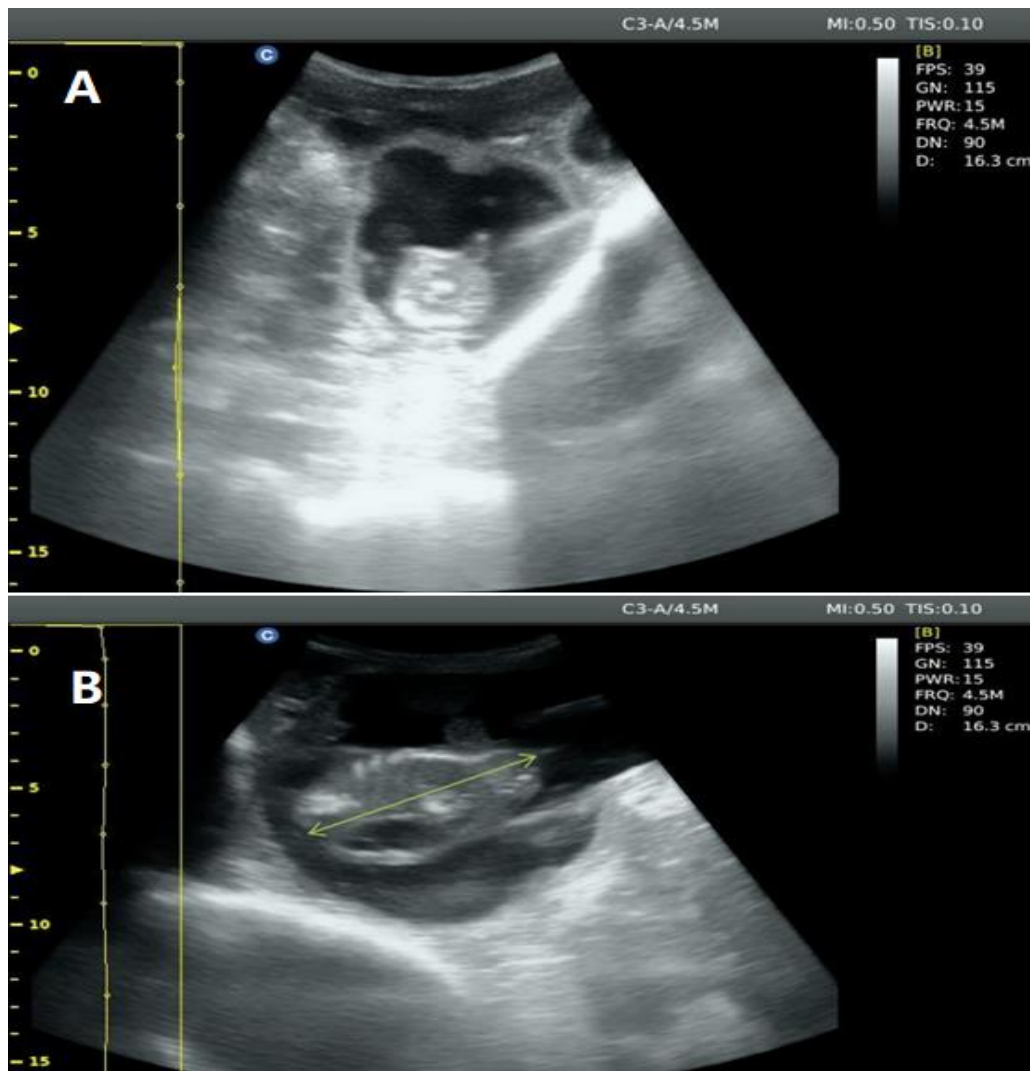
Figure 4: Transabdominal ultrasonography images showing: A. Gestational sac with embryos thoracic cavity bordered by ribs on day 36 PM; and B. Fetus Thoracic and body 50 PM.
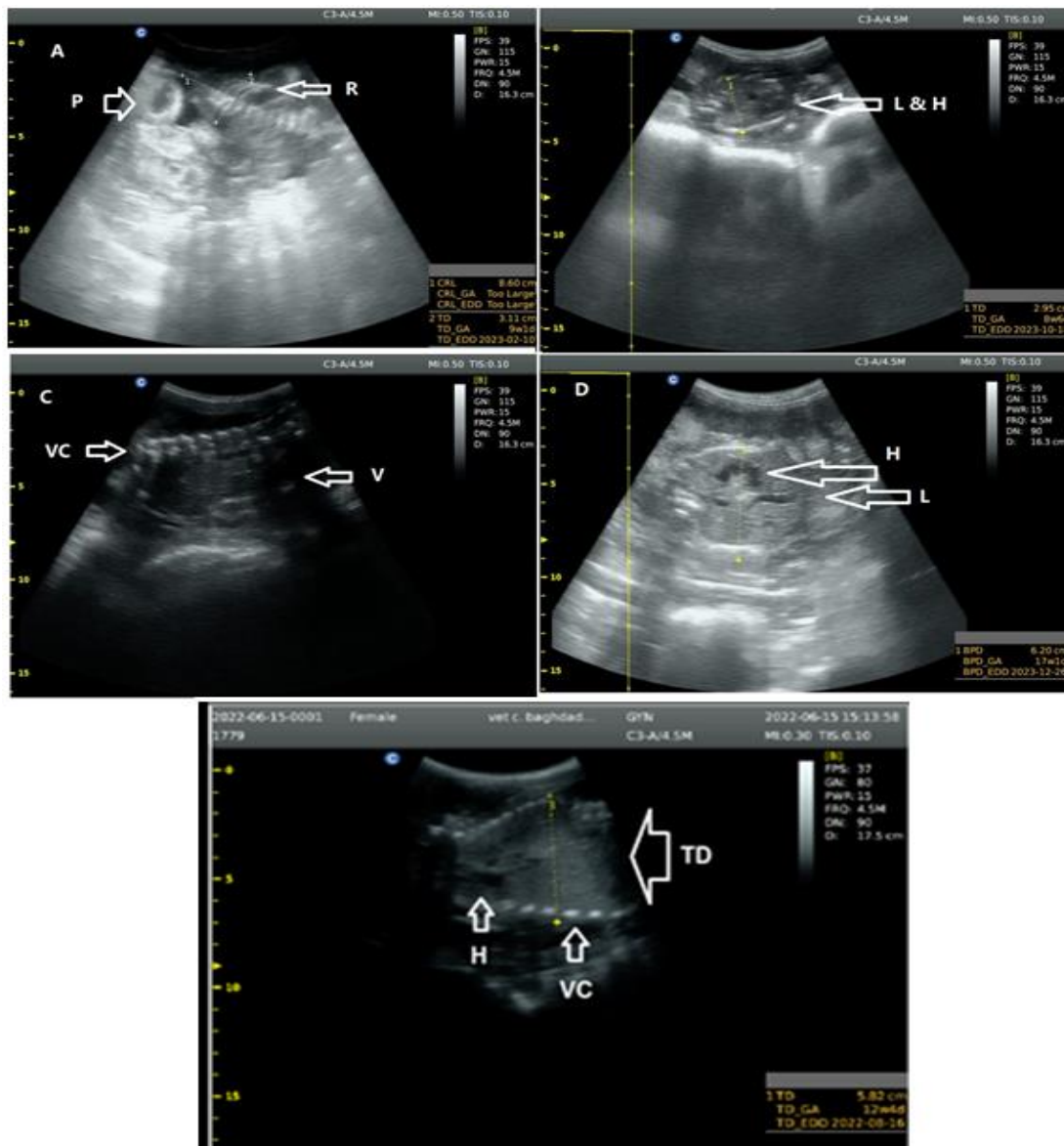
Figure 5: Transabdominal ultrasonography images showing fetal Ribs (R), lung (L), heart (H), Vertebral Column (VC) and thoracic cavity.

Table 1: The pregnancy parameters during different gestational intervals

Pregnancy status/Day	Pregnant/ 17–20 (n = 28)	Pregnant/ 23–29 (n = 28)	Pregnant/ 36–50 (n = 28)	Pregnant/ 60–70 (n = 28)	Pregnant/ 80-90 (n = 28)
Predicted + value	(3/6) 50%	(17/19) 89%	(24/25) 96%	(24/24) 100%	(24/24) 100%
Predicted - value	(1/22) 4%	(2/7) 28%	(3/3) 100%	(24/24) 100%	(24/24) 100%

Sensitivity	(3/24) 12%	(17/24) 70%	(23/24) 96%	(4/4) 100%	(4/4) 100%
Specificity	(1/4) 25%	(2/4) 50%	(4/4) 100%	(4/4) 100%	(4/4) 100%
Accuracy	(4/28) 14%	(19/28) 67%	(27/28) 96%	(28/28) 100%	(28/28) 100%

El-Zarkouny et al. (2008) stated that it became feasible to detect pregnancy through TRU before day 20 PM, but with low Se (42%) and Sp (57%), and day 24 via TRU (45). Reza et al. (2019) also recorded high Se and Acc on day 30 of pregnancy (82%), higher than the present study (70%), the Se and Acc reach 100% on the 2nd and 3rd months of pregnancy.

In the present study, only one case was diagnosed incorrectly on the period (36-50) PM (Se 96% and Sp 100%). According to El-Zarkouny et al. (2008); the Se ranged from 86-90% and Sp 100% on day 45-55 PM. All detection parameters reached to 100% on day 75 PM. Several studies reported that Se and Acc in pregnancy diagnosis from gestational age 42nd and beyond was 100% using ULR (1, 30, 36), which is consistent with the present study.

A high false positive rate was identified in early pregnancy (17-20), which may be attributed to the presence of estrous fluids, CL and artifactual echoes like acoustic shadowing which led to a misdiagnosis. The false positive ratio decreases along with pregnancy progression, but several pathological changes may affect the Se. In period 23-29 PM, the false positive rate was 30%, it may attributed to some pathological changes such as endometritis and embryonic/fetal mortality. Karen et al. (2014) demonstrated that the accumulation of estrus fluid or endometritis in does may misdiagnosed as an allantoic fluid, and it could be a reasons for false positive diagnoses.

The efficiency of Echography to estimate embryo/fetal count

The earliest identification of multiple embryos was made on day 23 through the intrarectal scan. Out of the 17 does examined by TRU on period (23-29) PM, six singleton and seven twin pregnancies were detected with 77, 75 and 76% for Se, Sp and Acc, respectively. With the TAU technique, twin fetuses were first inspected at day 30 of pregnancy. The detection values were improved on the period (36-50) PM, 10 twin pregnancies out of 11 were detected correctly (Se 90%) and 12 singleton pregnancies out of 13 were diagnosed correctly (Sp 92%) and Acc was 91%. All pregnancy detection values were 100% on the period (60–70) PM, then the Se and Acc decreased to 72% and 87.5%, respectively on days 80-90 PM (Table 2).

According to Padilla-Rivas et al. (2005), the best period to diagnose the litter size in a goat is between the 28th and 40th day of pregnancy with a 7.5 MHz TRU. Additionally, Abdelghafar et al. (2009) demonstrated that the Acc to detect does bearing twins (Se) was 50% with 100% Sp in days (21-35 PM). The Se reached 100% during the pregnancy period (56-75 PM). While Dawson et al. (1994) reported 73% Acc for determining twins (Se) at 5 weeks of gestation and 89% at 7 weeks of gestation in Alpine does use TAU. Furthermore, El-Zarkouny et al. (2008) stated that Acc of using ultrasound scanning in twins identification (Se) was higher on days 55 and 75 compared to days 45 PM.

The pregnancy type identification by either TRU or TAU based on the appearance of the two gestational sacs within the uterus, was possible starting at day 23-29 of pregnancy with good diagnosis parameters (PV+, PV-, Sp, and acc) because both of embryonic vesicle appeared on the monitor during the scan. In other words, the detection in the earliest stage of pregnancy to differentiate between singles/twins was efficient using the TRU approach. Suguna et al. (2008) mentioned that it can differentiated between the doe-bearing singles and twins on days 35-42 by the TRU and TAU methods, respectively.

After that, the detection parameters improved in the period 36-50 PM because the ossification occurred after day 42 PM, and two groups of ribs and two vertebral columns showed during the scan for the twin, only one case was diagnosed as a false negative and one case diagnosed as a false positive at period 36-50 PM. These observation were agree with Younis and Hatif (2023), they mentioned that Se and Acc were very high in period 42-50 PM by using TAU in ewes. After day 50 (60-70 PM); the ribs and vertebral column become more prominent, also heartbeats can noticed readily. In the case of twins two beating hearts can detected in this period. All these indicators aid in distinguishing the does bearing twins from the singleton.

The increasing fetal size decreases the Se and Acc for detecting pregnancy type in gestational day 80 and beyond. According to Anilkumar et al. (2010), the Acc of identifying twin-bearing ewes was 92% by TAU in a confined period of 60–75 days PM in the Nilagiri ewes breed, the identification depended on the presence of two vertebral columns and two heart beats on a single screen, which was indicative that the ewe carries twin. Andrabi and Gulavane (2015) reported that in advanced pregnancy the Acc to detect twins was 80% because the bone shadow of the developing fetus makes it difficult to diagnose the fetus number, in addition, the foetuses sank deeply in the abdominal cavity.

Table 2: Efficiency of echography to estimate the pregnancy type in different pregnancy periods

Pregnancy status/Day	Pregnant/ 23–29 (n = 17)	Pregnant/ 36–50 (n = 24)	Pregnant/ 60–70 (n = 24)	Pregnant/ 80-90 (n = 24)
Predicted + value	(7/9) 77%	(10/11) 90%	(11/11) 100%	(11/11) 100%
Predicted - value	(6/8) 75%	(12/13) 92%	(13/13) 100%	(13/13) 100%
Sensitivity	(7/9) 77%	(10/11) 90%	(11/11) 100%	(8/11) 72%
Specificity	(6/8) 75%	(12/13) 92%	(13/13) 100%	(13/13) 100%
Accuracy	(13/17) 76%	(22/24) 91%	(24/24) 100%	(21/24) 87.5%

The efficiency of Echography to estimate embryo/fetal viability

First detection of embryo viability was at days 23-29 PM by noticing the sound of the embryo's heart pulses. By using the pulse wave Doppler approach, the sound was heard and the pulses diagram appeared on the monitor as a spike. The Se, Sp and Acc values as this time were 62, 50 and 60%, respectively with

high false positive and negative results. The viable fetus from days 36 to 90 was determined by pulse wave Doppler, and also by monitoring the fetal movement or heartbeat by B-mode. During Period 36-50 PM, the Se, Sp and Acc were 100, 75 and 96%. All detection values reached 100% after this period as the pulses (spikes) diagram became more prominent and the sound was more audiable, Additionally, the fetal movement and heart pulses could be readily monitored through detection by B-mode (Table 3).
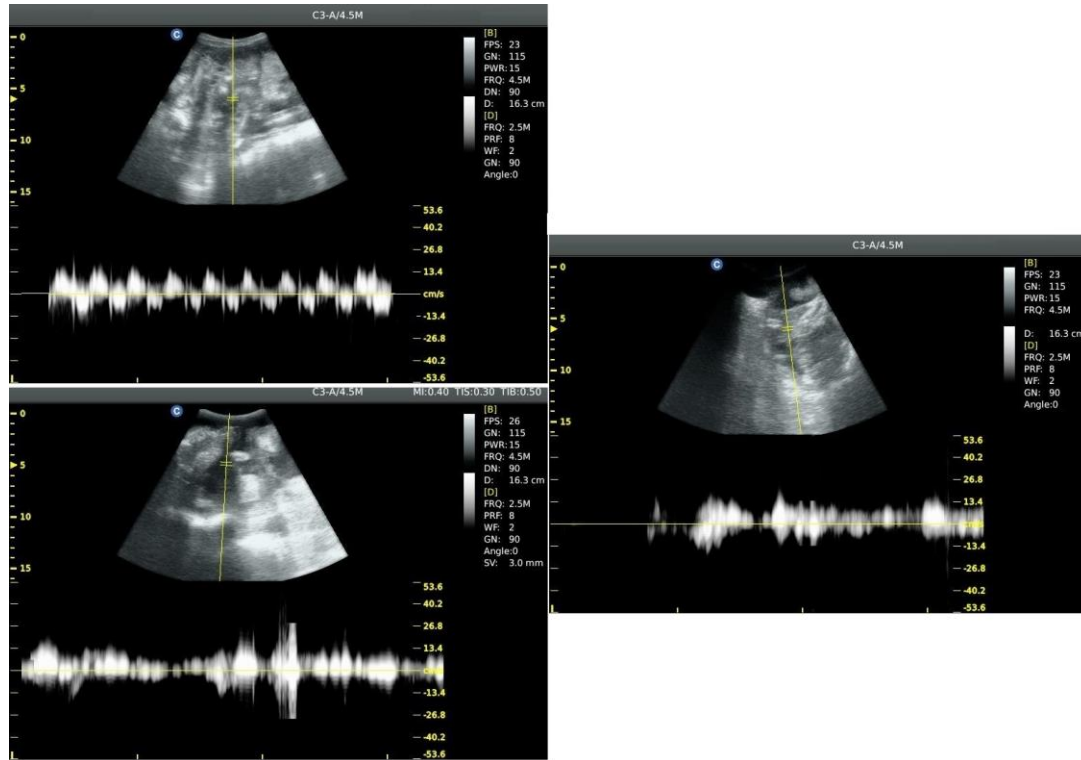

Figure 6: Transabdominal ultrasonography images showing the pulses of fetus suring different stages of pregnancy.

Table 3: Efficiency of ultrasonography to predict the embryo/fetal viability in different pregnancy periods

Pregnancy status/Day	Pregnant/ 23–29 (n = 28)	Pregnant/ 36–50 (n = 28)	Pregnant/ 60–70 (n = 28)	Pregnant/ 80-90 (n = 28)
Predicted + value	(15/17) 88%	(24/25) 96%	(24/24) 100%	(24/24) 100%
Predicted - value	(2/9) 22%	(3/4) 75%	(24/24) 100%	(24/24) 100%
Sensitivity	(15/24) 62%	(24/24) 100%	(4/4) 100%	(4/4) 100%
Specificity	(2/4) 50%	(3/4) 75%	(4/4) 100%	(4/4) 100%
Accuracy	(17/28) 60%	(27/28) 96%	(28/28) 100%	(28/28) 100%

The study of Verma et al. (2014) agreed with our findings, which observed that B-mode ULR is the best non-invasive technique to study the viability of developing conceptus depending on heartbeat, fetal movement and echoicity of fetal fluid. Additionally, Amer (2010) showed that viability was determined depending on pulse detection on day 21 PM with low Acc, but by day 28 PI, it was easily identifiable using TRU.

According to Cosentino et al. (2018), embryonic viability can be detected early (days 21-30) depending on Doppler ULR with approximately optimal Se and Sp 92%, therefore they considered that Doppler ULR was an efficient tool for early pregnancy diagnosis in does. Goel and Agrawal (1992) and Goel et al (1989) stated that Se ranged from 98% to 100% on 61 to 80 days of pregnancy. Devi et al. (2019) tested the fetal viability by using the pulse wave Doppler approach depending on the uterine arterial pulse in different pregnancy periods. The Se for uterine arterial pulse was very low from day 45 and 100% on day 70 onwards, while Umbilical arterial pulse, fetal pulses and movement could be detected with optimum Se from day 120 onward.

The efficiency of Echography to monitor the embryo/fetal development and landmarks

At early pregnancy evaluation, it was possible to visualize the gestational vesicle at day 23 PM by TRU and day 30 by TAU. Between days 23-29 PM, the gestational vesicle within the uterus appeared in front of the urinary bladder as an anechogenic structure enclosed by amniotic and allantoic membranes. During this period, the embryo also appeared as an echogenic patch inside the distal part of the embryonic vesicle. At this pregnancy stage, it was also possible to measure the embryo age by a specific scale (EVD). The same observations were recorded by Suguna et al. (2008), Padilla-Rivas et al. (2005) and Ali and Hayder (2007) studies, which describe the embryonic vesicle on days 21-25 by TRU and embryonic vesicle, allantoic fluid with embryo proper on day 28 by TAU.

The placentomes, it was first detected on day 25 PM, but they were monitored on day 30 and beyond, it had a distinct C or O format inside anechogenic fluid (allantoic fluid). At day 30 and beyond, the embryo/fetus became larger, the head and trunk appeared clearly and the heart was recognized as a small region inside the trunk that had rapid movement. At this gestational stage, it was also possible to determine the embryo/fetal age by measuring the crown-Rump Length. In the fifth week, the leg buds and ocular orbit were identified, but at the gestational age of 6-7 weeks; the ossification occurred and the vertebral column, ribs, thorax and head became prominent and could be recognized readily. At this point the EVD was no longer used because the embryonic vesicle was larger than the image, while CRL can used until 7th week. These findings agreed with Santos et al. (2018) and Abdullah and Mohd (2020).

Additionally, the femur, humerus, tibia, radius and metacarpus were visualized in the 6th week of gestation. The lung and heart (two anechogenic chambers) were easily identified between the vertebral

column and ribs (Fig. 5). From day 50 to 90 of gestation, it was possible to estimate the fetal age by measuring the Thoracic Diameter (TD) (Table 4) (Table 5).

Table 4: The first visualization of embryo/fetal and maternal structures by B-mode echography in Awassi ewes

Structure	Gestational age/ Day	Structure	Gestational age/ Day
Gestational sac	21	**Albow joint**	42
CL	17	**Tibia**	42
Proper embryo	24	**Humerus**	42
Placentomes	25	**Radius**	42
Heart	30	**Metacarpus**	42
Abdomen	30	**Thorax**	42
Umbilical cord	30	**Vertebral column**	50
leg buds	35	**Fetal movements**	50
Ocular orbit	35	**Femur**	42
Ribs	42	**Lung**	60-90
Rumen	60-90	**kidney**	60-90

The efficiency of Echography to determine embryo/fetal age by fetometry

Several scales were used for determining the embryo/fetal age. The ULR and EVD were first utilized from day 23 until day 42 PM when the embryonic vesicle became too large than the scan section with a very high correlation. The average value of EVD was 15 mm on day 23 days of gestation, the diameter increased progressively with the age of gestation to 29 mm on day 42 PM. Additionally, CRL was also utilized to estimate the age in a confined period (day 23- 60 PM) with values ranging from (18-63 mm). The statistical analysis recorded a very high correlation between the measurement of CRL and embryo/fetal age. Furthermore, the results showed that TD can be measured from 42-90 days of pregnancy, the TD increased along with pregnancy progression. It ranged from 11 mm on day 42 PM to 51 mm on day 90 PM (Table 5) (Fig.6).

The present study revealed a very high correlation between gestational age measurements and EVD, CRL and TD. The EVD showed a slightly higher correlation between EVD ($R^2 = 0.99$) and pregnancy age compared to the correlation of TD ($R^2 = 0.98$) and CRL ($R^2 = 0.97$).

Table 5: Comparison of EVD, CRL and TD in the estimation of fetal age until day 90 by echography

Actual Gestational age (Post mating)	EVD (mm)	CRL (mm)	TD
Day 23	15	18	Too small
Day 29	20.7	23	Too small
Day 36	25	32.3	Too small
Day 42	29	38	11
Day 50	Too large	56	17
Day 60	Too large	63	23
Day 70	Too large	Too large	31
Day 80	Too large	Too large	40
Day 90	Too large	Too large	51
Equation and Correlation	y = 0.7222x - 1.0463 $R^2 = 0.9903$	y = 1.2987x - 13.565 $R^2 = 0.9769$	y = 0.8161x - 24.487 $R^2 = 0.9896$
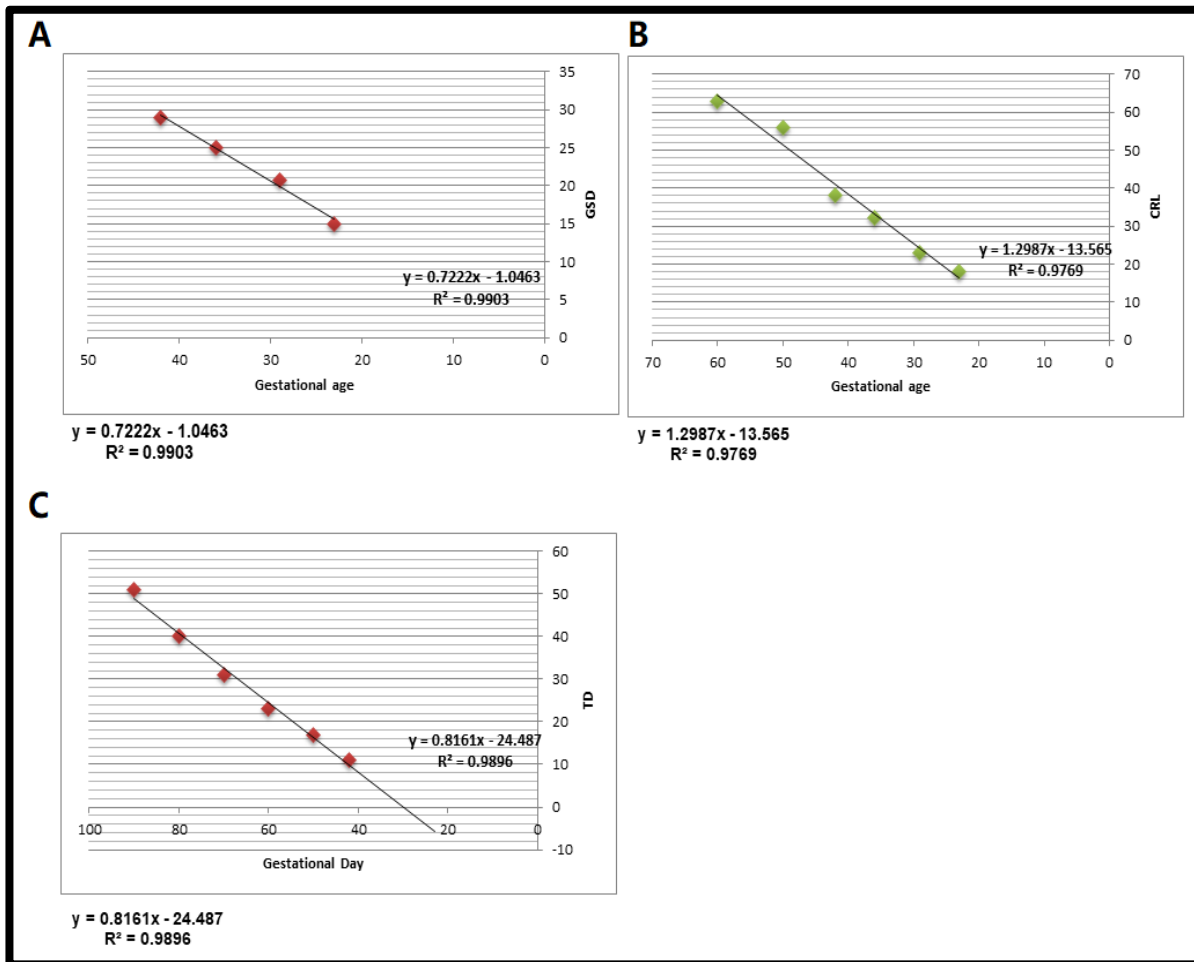
Figure 6: Measurements of the A. EVD, B.CRL and C. TD obtained by TRU and TAU through different gestational periods

By using the B mode of TRU with 5 MHz, Singh et al (2004) revealed a very high correlation coefficient between the fetal age and measurement of CRL and TD were R=0.92 and R=0.98, respectively (The correlation of TD was higher than CRL), that agreed with our findings. Ardakani et al. (2022) mentioned that GS showed a very low correlation factor ($R^2 = 0.318$) for age determining for ewes in days 17-21 PI, while a very high correlation coefficient $R^2 = 0.93$ on days 21-55. All these scales were efficient in the prediction of embryo/fetal age, but EVD used in the very early stage of pregnancy, Petrujkić et al (2016) agreed with our findings, they mentioned that EVD correlated with the gestational age when the TRU was utilized ($R^2 = 0.92$) in gestational days 23th-38th. Additionally, CRL was accurately used from day 30 onward, while TD was valuable throughout mid-gestation. Al-Rawi (2014) reported that CRL was used in ewes earlier than our findings (from day 18 until day 86 with the diameter with a very high correlation between this scale and embryo/fetal age ($R^2 = 0.97$), TD used between days 21-101 with very high correlation ($R^2 = 0.95$). According to Santos et al. (2018), the TD was 16 mm on gestational day

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57th (that agrees with present results) and CRL was 57 mm on day 58, which corresponds with our outcomes (56-63 mm on days 50-60).

In conclusion, Depending on the presence of positive signs of pregnancy, the pregnancy was first diagnosed by day 23 PM by TRU, the Acc increased and reached 100% by days 60 PM onward. The most suitable time to establish the fetus count and viability by ULR was in the period 60 – 70 days of pregnancy. The accuracy was 100% for single and twin pregnancies, and decreased significantly after that, while the viability was 100% in days 60 and beyond depending on pulse wave Doppler, heart beating and fetal movements. Several parameters (EVD, CRL and TD) were used to determine the embryo/fetal age. All of the scales represented significant correlation coefficients and illustrated linear regression in different pregnancy phases; EVD was used in period (23-42) PM, CRL in (23-60) PM and TD in (42-90) PM.

IV.   REFERENCES

1. Abdelghafar, R.M., Ibrahim, M.T., Abdelrahim, S.M. and Ahmed, B.H. (2010). Sensitivity and specificity of real-time ultrasonography for pregnancy diagnosis and litter size in Saanen goats (Capra hircus): proceeding of the 14th scientific conference. Assiut University, Egypt, 391-402.

2. Abdullah, R. and Mohd, F.M. (2020). The Foetal Ultrastructural Images Obtained from Pregnant and Non-Pregnant Does Using Transrectal and Transabdominal Probes of Ultrasound Scanner. Journal Of Agrobiotechnology, 11(1S).

3. Ali, A. and Hayder, M. (2007). Ultrasonographic assessment of embryonic, fetal and placental development in Ossimi sheep. Small Rum. Res., 73(1-3):277- 282.

4. Alkan, H., Kivrak, M.B., Satilmis, F., Tekindal, M.A. and Dinc, D.A. (2020). Detection of twin pregnancies in ewes by pregnancy-associated glycoprotein assay and transabdominal ultrasonography. Domestic Animal Endocrinology, 7: 106399.

5. Al-Rawi, H.M. (2014). Monitoring fetal development in sheep by using real-time ultrasonography. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 13(1):11-14.

6. Amer, H.A. (2010). Ultrasonographic assessment of early pregnancy diagnosis, fetometry and sex determination in goats. Anim. Reprod. Sci., 117 :(3-4):226-231.

7. Ammar R,M. and Baker T,J. (2021). Accuracy of Flock Pregnancy Diagnosis in Local Iraqi Ewes by A-mode Ultrasound Device.

8. Andrabi, S.E. and Gulavane, S.U. (2015). Real Time Ultrasonography for Determining Viable Foetal Numbers in Goats. Journal of Animal Research, 5(4):875-878.

9. Anilkumar, R., Iyue, M., Prema, N. and Sreekumar, C. (2010). Ultrasonographic diagnosis of pregnancy and estimation of fetal numbers in ewes. Indian Journal of Animal Sciences, 84(6): 640– 642.

10. Ardakani, M.S., Toosi, B.K., Azizzadeh, M. and Rajabioun, M. (2022). Estimation of gestational age using ultrasonography in Baluchi sheep. In Veterinary Research Forum, 13(2): 257-263.

11. Barbagianni, M.S., Ioannidi, K.I., Vasileiou, N.G., Mavrogianni, V.S., Orfanou, D.C., Fthenakis, G.C. and Valasi, I. (2017). Ultrasonographic examination of pregnant ewes: from early diagnosis of pregnancy to early prediction of dystocia. Small Ruminant Research, 152:41-55.

12. Bello, A.A., Voh Jr, A.A., Ogwu, D., Tekdek, L.B. and Ayo, J.O. (2023). Comparison of ultrasonography, progesterone assay, ballottement, and non-return-to-heat with digital rectal palpation technique for early pregnancy diagnosis in Red Sokoto goat. Reproduction in Domestic Animals, 58(2):263-271.

13. Brzozowska, A.,T. Stankiewicz, B. Błaszczyk, P. Chundekkad, J. Udała and N. Wojtasiak. (2022). Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep. BMC Vet. Res.18 (1):1-16.

14. Chama, T.H., Matthew, D.A., Chama, J.H. and Bala, D.B. (2019). Economic implications of small ruminant foetal wastage: A case study of Jalingo abattoir, Taraba State. Sokoto Journal of Veterinary Sciences, 17(2):73-76.

15. Cosentino, I.O., Balaro, M.F., Leal, F.S., Bade, A.L., Côrtes, L.R., Arashiro, E.K., da Fonseca, J.F. and Brandão, F.Z. (2018). Color Doppler ultrasonography for early pregnancy diagnosis in goats. Anim. Reprod, 15(1):1112.

16. Dawson, L.J., Sahlu, T., Hart, S.P., Detweiler, G., Gipson, T.A., Teh, T.H., Henry, G.A. and Bahr, R.J. (1994). Determination of fetal numbers in Alpine does by real-time ultrasonography. Small Ruminant Research, 14(3),:225-231.

17. Dana, O.I., Mukhtar, R.H., Mohammed M.O. and Dyary. H.O. (2021). Comparison of a rapid test with bPAG ELISA in pregnancy diagnosis in cows. Iraqi J. Agri. Sci.52 (6): 1475-1481. DOI: 10.36103/ijas.v52i6.1488.

18. Devi, N.B., Bhuyan, D. and Das, A. (2019). Application of ultrasonic doppler technique for pregnancy diagnosis in local Assam goat. J Pharm Innov, 8:1088-1091.

19. Elmetwally, M., Rohn, K. and Meinecke-Tillmann, S. (2016). Noninvasive color Doppler sonography of uterine blood flow throughout pregnancy in sheep and goats. Theriogenology, 85(6):1070-1079.

20. El-Zarkouny, S., Nour El-Din, A.N.M., Ghobashy, H. and AbdelGawad, E.I. (2008). Accuracy of early pregnancy diagnosis and detection of twins using real-time ultrasonography compared to progesterone concentration in Barki goats. Egyptian J. Anim. Prod., 45(2):131-138

21. Goel, A.k. (1990). Agrawal Kp. Pregnancy diagnosis in goats. Indian Veterinary Medical Journal, 14:77-78.

22. Goel, A.K. and Sinha, N.K. (1998). Agrawal KP. Pregnancy diagnosis in sheep. Indian Journal of Animal Science, 59:974-976.

23. Haibel, G.K. (1990). Use of ultrasonography in reproductive management of sheep and goat herds. The Veterinary clinics of North America. Food animal practice, 6(3):597-613.

24. Hussein, K.A., AL-Mutar, H.A. and Hassan, B.J. (2020). A clinical and ultrasonographical study to investigate the lhr receptor gene and embryonic death out season in sheep. Plant Arch., 20 (2):6329-6333.

25. Jones, A.K., Gately, R.E., McFadden, K.K., Zinn, S.A., Govoni, K.E. and Reed, S.A. (2016). Transabdominal ultrasound for detection of pregnancy, fetal and placental landmarks, and fetal age before day 45 of gestation in the sheep. Theriogenology, 85(5):939-945.

26. Karen, A., Samir, H., Ashmawy, T. and El-Sayed, M. (2014). Accuracy of B-mode ultrasonography for diagnosing pregnancy and determination of fetal numbers in different breeds of goats. Anim. Rep. Sci., 147(1-2):25-31.

27. Karen, A.M., Fattouh, E.S. and Abu-Zeid, S.S. (2009). Estimation of gestational age in Egyptian native goats by ultrasonographic fetometry. Animal Reproduction Science, 114(1-3):167-174.

28. Kumar, K., Chandolia, R.K., Kumar, S., Pal, M. and Sandeep, K. (2015). Two-dimensional and three-dimensional ultrasonography for pregnancy diagnosis and antenatal fetal development in Beetal goats. Veterinary World, 8(7):835.

29. Lianou, D.T., Vasileiou, N.G., Michael, C.K., Valasi, I., Mavrogianni, V.S., Caroprese, M. and Fthenakis, G.C. (2022). Patterns of Reproductive Management in Sheep and Goat Farms in Greece. Animal, 12(24):3455.

30. Mavrogenis, A.P., Antoniades, N.Y. Hooper, R.W. (2006). The Damascus (shami) goat of Cyprus. Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animales, 38:57-65.

31. Medan, M., Watanabe, G., Absy, G., Sasaki, K., Sharawy, S. and Taya, K. (2004). Early pregnancy diagnosis by means of ultrasonography as a method of improving reproductive efficiency in goats. Anim. Rep. Sci. Rep. Dev., 50(4):391-397

32. Muhammad, R.S. and Aziz, D.M. (2022a). Estimation of gestational age in Shami goats based on transabdominal ultrasonographic measurements of fetal parameters. Iraqi Journal of Veterinary Sciences, 36(4):839-846.

33. Muhammad, R.S. and Aziz, D.M. (2021b). Determination of gestational age in Shami goats based on transabdominal ultrasonographic measurements of placentomes and uterine diameter. Al-Anbar J Vet Sci, 14:30-41.

34. Okorie-Kanu, O.J., Ezenduka, E.V., Okorie-Kanu, C.O., Anyaoha, C.O., Attah, C. A., Ejiofor, T.E. and Onwumere-Idoloh, S.O. (2018). Slaughter of pregnant goats for meat at Nsukka slaughterhouse and its economic implications: A public health concern. Veterinary world, 11(8):1139.

35. Omontese, B.O., Rekwot, P.I., Ate, I.U., Rwuaan, J.S., Makun, H.J., Mustapha, R.A. and Lawal, M. (2012). Use of ultrasonography for pregnancy diagnosis in Red Sokoto goats. Scientific Journal of Biological Sciences, 1(5):101-106.

36. Osman, R.M. (2010). Ultrasonography as a diagnostic tool for fetal mortality in goats (Capra hircus) in the Sudan" two case reports". Assiut Veterinary Medical Journal, 56(127):316-322.

37. Omran, S.N. and K.A. Hussein (2007). Pregnancy diagnosis by ultrasonography in local awassi ewes treated with progesterone and eCG. Iraqi J. Agri. Sci.38 (4):111-117.

38. Padilla-Rivas, G.R., Sohnrey, B. and Holtz, W. (2005). Early pregnancy detection by real-time ultrasonography in Boer goats. Small Rumin. Res. 58:87–92.

39. Pagamici, C. and Stephan, R. (2022). Pregnancy in Slaughtered Lambs and Sheep—A Cross-Sectional Study in Three Abattoirs in Switzerland. Animals, 12(10):1328.

40. Petrujkić, B.T., Cojkić, A., Petrujkić, K., Jeremić, I., Mašulović, D., Dimitrijević, V., Savić, M., Pešić, M. and Beier, R.C. (2016). Transabdominal and transrectal ultrasonography of fetuses in Württemberg ewes: Correlation with gestational age. Animal Science Journal, 87(2):197-201.

41. Rasheed YM. (2017). Assessment of gestational age in goats by Real-Time Ultrasound measuring the fetal crown-rump length, and bi-parietal diameter. Iraqi J Vet Med., 41(2):106-112.

42. Reza, M.M., Parvez, M.A. and Faruk, M.O. (2019). Evaluation of pregnancy diagnosis using ultrasonography in goats. Bangladesh Veterinarian, 36(1-2):42-48.

43. Santos, V.J., Rodriguez, M.G., da Silva, P.D., Mariano, R.S., Taira, A.R., de Almeida, V.T., Uscategui, R.A., Nociti, R.P., Teixeira, P.P., Feliciano, M.A. and Vicente, W. R.R. (2018). B-mode ultrasonography and ecobiometric parameters for assessment of embryonic and fetal development in sheep. Animal reproduction science, 197:193-202.

44. Sayuti, A., Siregar, T. N., Melia, J., Rahmi, E., Abrar, M., Panjaitan, B. and Daud, R. (2019). Determination of Gestational Age and Observation of Kacang Goat Fetal Development during 60 Days of Pregnancy by Using Transcutaneous Ultrasonography. Jurnal Veteriner, 20(4): 534-540.

45. 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):760-768.

46. Suguna, K., Mehrotra, S., Agarwal, S.K., Hoque, M., Singh, S.K., Shanker, U. and Sarath, T. (2008). Early pregnancy diagnosis and embryonic and fetal development using real time B mode ultrasound in goats. Small Ruminant Research, 80(1-3):80-86.

47. Verma, S.K., Kailash, K. and Chandolia, R.K. (2014). Detection of fetal viability using real time B mode ultrasonography in Corriedale sheep. Haryana Veterinarian, 53(2):133-134.

48. Wani, N.A., G.M. Wani, A.M. Mufti and M.Z. Khan. (1998). Ultrasonic pregnancy diagnosis in gaddi goats. Small Rum. Res., 29 (2):239-240.

49. Yotov, S. (2005). Diagnostics of early pregnancy in Stara Zagora dairy sheep breed. Bulg. J. Vet. Med., 8(1):41-45.