The Efficiency of Real-time Ultrasonography to Assess Early Pregnancy and Fetal Number in First Trimester Period for Awassi Ewes

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Abstract

This article aims to predict the reliability of B-mode ultrasonography (ULR) for early pregnancy detection and determine gestational type in Awassi ewes. Two techniques were used through the first trimester, Transrectal (TRU) and Transabdominal (TAU) ultrasonography with frequencies of 6.5 MHz and 3.5-4.5 MHz, respectively. Thirty ewes were synchronized and exposed to five rams at estrus. The early gestation was detected by ULR scanning at different periods (15, 25, 35, and 50 days post mating). The findings indicated highly significant effect (P<0.01) of pregnancy stage on the Accuracy (Acc) of ULR for predicting pregnancy; the Sensitivity (Se), Specificity (Sp) and Acc was low (8%, 60% and 16.6%) at day 15, it began to rise at day 25 (68%, 80% and 70%) and at day 35 (92%, 80% and 90%) postmating, then it reached 100% at day 50. The correlation between fetal age and Gestational Sac Diameter (GSD) was higher (R² = 0.8809) between the period 21-35, and with Crown Ramp Length (CRL) (0.9852) during the period 30-50. The ultrasonography showed high efficiency to determine pregnancy type, it was significant (P<0.01) influenced by day of gestation; Se, Sp, PV+, PV- and Acc were high at day 30-35 and reached 100% at day 50 of gestation. Lastly, the pregnancy phase may impact significantly on ultrasound prediction of pregnancy, fetal age, and number. Since the prediction rate of UTR technique reach to 100% at day 50, it can be use as a gold standard for pregnancy and litter size detection on day 50.

Key words : Ewes, ultrasonography, Pregnancy, Fetal age, fetal number.

Determining pregnancy status early on is arguably the most important factor in determining reproductive efficiency; Karen et al (2001) reported that early pregnancy diagnosis has economic benefits to sheep producers. Early detection of pregnancy is an important tool in reproductive management; the earlier identification of non-pregnant ewes (a short period after mating) helps to decrease production loss and infertility through rapid intervention by appropriate treatment and then quickly rebreeding to barrens or culling (Jainudeen and Hafez, 2013), or to segregate the herd into non-pregnant and pregnant, that may minimize the losses (reproductive and production losses) such as decrease the abortions, stillbirths or weak lambs production (Wani et al. 1998).

There are various kinds of pregnancy identification in small ruminants; clinical (abdominal palpation and ultrasonography), Laboratory Methods; hormonal assays (such as Progesterone and Estrone Sulphate), detection of pregnancy-specific antigens (proteins), management methods (non-return to estrus) (Jainudeen and Hafez, 2013, Redden and Passavant, 2013; El Amiri et al., 2015).

The ultrasonography techniques allow the assessment of fetal count (type of pregnancy), estimation of foetal sex, age and viability of foetuses, and diagnosis of some foetal disorders (Dos Santos et al., 2010; Ochoa Cordero et al., 2019) and early embryonic death (Hussein et al., 2020). The objective of ultrasonography was to easily make use of accurate prediction of gesta-
The efficiency of pregnancy diagnosis by ultrasonography depends on several factors; operator experience, imaging technique, stage of pregnancy, body condition, age of gestation, and the number of foetuses (Jones et al. 2016; Mansoor et al., 2021).

In B-mode imaging, two-dimensional (2D) cross-sectional images are displayed in real-time on the screen (moving greyscale images of cross-section part), whereas ultrasound beams are emitted and received back in the same line (Mattoon and Nyland, 2020). Most ultrasound apparatuses use 256 grey shades, therefore, the imaging (grey-scale imaging) permits visual recognition of shades of grey, along with characterizing tissue details (Adams and Singh, 2011).

At an early stage of pregnancy (less than 20 days postmating), the detection is not consistently accurate until days 20 to 22 postmating (examined by transrectal real-time ultrasound, 7.5-MHz linear probe), while, reaching to the optimum between (days 25-40) (Schrick and Inskeep, 1993).

Pregnancy determination is essential at an early stage because a high percentage of early pregnant ewes were detected postmortem in an abattoir (One out of every 3 ewes or 4 slaughter was pregnant) (Muhammad et al., 2007). In Switzerland, 7.6% of the ewes that were being slaughtered were pregnant, and 74.5% of them were in the first trimester (Pagamici and Stephan, 2022), in addition, the early pregnancy determining is more important but difficult than advance pregnancy, therefore, this study aimed to determine the Acc from the earliest stage of gestation until the end of the 1st trimester (15-50 days) through TRU and TAU, and to examine the foetal number in the same period (15-50 days) post-mating in Awassi breed.

**Materials and Methods**

**Experimental Animals and experimental design**

A total of 30 multiparous local Awassi ewes (average age 3-4 years) without any clinical problems, were studied. All animals were housed in one flock with four breeding rams that were used for heat detection and mating. The experiment location was in Vet. college- Alfallujah university /Alfallujah, in latitude 33.37 and longitude 43.74). The experiment span extended from February to July 2022. The average age of ewes is between 3-4 years.

All ewes were examined by ultrasonography to ensure they were empty before synchronization (Pre insemination). The ewes were synchronized and inseminated by five breeding rams. The post-insemination ewes were examined by ultrasonographic in different gestation periods (days 15, 25, 35, and 50) after day 0.

**Estrus synchronization**

Thirty non-pregnant ewes were synchronized by MPA sponges 40 mg (Hipra Spain). According to Kuru et al. (2022), the sponges (one sponge/ ewe) were inserted through the intra-vaginal route by a special applicator, to persists for 14 days, then removed. At the time of sponge removal, half of flock was injected with PMSG (500 IU) and the rest with (750 IU). The heat was detected by observing the mating of the breeding ram (0 days).

**Ultrasonography**

All experimental ewes were examined by ultrasonography (Chison ECO2/ China) in pre-and post-insemination. The examination was performed with a real-time B-mode instrument equipped with a linear array 6.5 MHz transducer (linear probe) designed for applying transrectally, which was utilized to inspect the genital tract in the early pregnancy period (15, 25, and 35 days post insemination) including the uterus, gestational sac, embryo, extraembryonic membranes and placentome from the fifth gestation week. A convex transducer was used transabdominally (transcutaneously) via applying the probe (transducer) on the inguinal region or ventro-abdominal wall at frequencies 3.5 and 4.5 MHz to detect pregnancy on 35 and 50 days which included gestational sac, embryo, extraembryonic membranes, fetal head, vertebral column, heart, extremities, thoracic, trunk and placentome. Additionally, it was used to diagnose the fetal number.
A Ewe was designated pregnant by imaging the apparent conceptus (anechoic, elongated structure) within a uterine fluid. The gestational age was determined by comparing day 0 and by measuring the following structures (gestational vesicle diameter (GVD), Crown Rump Length (CRL), Biparietal diameter (BPD), and thoracic diameter (TD), according to statistical equations and equipment equations. The following diagnostic parameters were determined according to Yotov (2005):

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

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

Accuracy (Ac %) = Number of True diagnosis / Total Number of diagnoses (A+(C/D)) ×100.

Sensitivity (Se %) = Number of True Positive diagnoses (A)/Total Number of Positive diagnoses (A/ (A+D)) ×100.

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

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

Statistical analysis

All data were assessed via Statistical-Analysis System SAS (2018) program to determine the effect of day of pregnancy detection on study parameters. The Chi-square test also were used to make a significant comparison between the proportions in the study.

Coefficient of Determination (R²) calculation online by Coefficient of Determination Calculator according to the equation:

Correlation Coefficient (r) = \( N \times \sum XY - (\sum X)(\sum Y) / \sqrt{N \times (\sum X^2 - (\sum X)^2) \times N \times (\sum Y^2 - (\sum Y)^2)} \)

Coefficient of Determination (r²) = r x r.

Results and Discussion

The efficiency of ultrasonography to detect pregnant ewes

Results of Real-time ultrasonography technique showed different results according to the time of pregnancy observed by TRU and TAU. Among the 30 ewes, only two ewes were diagnosed as truly pregnant firstly on days 15-20 of pregnancy, it was imaged as the fluid that accumulated in a gravid uterus and CL using the transrectal technique, but it’s more readily imaged after day 21 (Fig.1). Additionally, 17 ewes out of 25 at days 21-25 (Figs 2 and 3), 23 ewes at days 35-30 (Figs 4, 5), and 25 at day 50 (Fig. 6). On the other hand, 23 ewes were determined incorrectly non-pregnant at an earlier stage of pregnancy (15-20 days), this false detection diminished along with pregnancy progression. Therefore, the pregnancy parameters improved.
with the progression of pregnancy, Se, Sp, and Acc were the lowest percents at 15-20 and 21-25 days, while they reached 90-100% at 30-35 and 42-50 days of pregnancy (Table I).

**Assessment of pregnancy characteristics and fetal age**

The gestational sac was first shown on day 21 of pregnancy. It appeared as an anechoic elongated structure within the uterus along with CL within uterine fluid (it was diagnosed in some cases). Before that (on day 15). Only 2/30 females were detected pregnant based on the presence of fluid and extra-embryonic membranes within the uterine horn and CL with central lacunae showed. All fetal landmarks before day 21 were lower than in another period. Along with pregnancy progress, the embryo and extra embryonic membranes showed up more prominently (at day 24). Placentome was easily noticed on day 35 as a O and C shape, it was visible in all cases (100%) in the confined period (45-50 days) of gestation. The leg buds were observed first at 35 days, but it was visualized 100% at day 50. Thorax, ribs, femur, humerus, tibia, radius, and metacarpus were imaged on day 42, and lastly, the vertebral column also was noticed primarily on day 50. The PV+, PV-, Se, Sp, and Acc increased steadily along with the advance of pregnancy, the best period for diagnosing pregnancy was 42-50 days (Table II).

Fetal age was determined by comparing the time of mating with fetal echobiometric variables including GSD and CRL. These parameters have been estimated as valuable pointers for estimating fetal age and lambing prediction in first trimester (Table II) (Fig. 7). It can be simply measured and it has shown the best association with intrauterine fetal age. A significantly higher correlation ($R^2 = 0.98$) was revealed in the study between the measurements of gestational age and the CRL, higher than the correlation between GSD and pregnancy age.
Identification of multiple pregnancies (Twins)

Sixteen singletons and 9 twin litters resulted from pregnant ewes, characterization of fetuses number at an early stage of gestation might be done successfully via TRU from 21th to the 25th day postconception. The Se, Sp, and Acc were recorded as 77.7%, 60%, and 70.5% respectively at these periods. Subsequently, the accuracy of detection parameters became higher than the earlier period (88.8%, 85.7% and 87% for Se, Sp, and Acc respectively). After that and until to 50th day, it can easily be carried out via the TAU technique (Se, Sp, and Acc reached 100% at the 50th day of pregnancy), as the fetuses are well separated from each other (Table III) (Fig 8 and 9).

Discussion

The efficiency of ultrasonography to detect pregnant ewes

The present study demonstrated that early pregnancy (15-35 days) identification in ewes
assisted by using TRU 6.5 MHZ and TAU 3.5 and 4.5 MHZ probes were used at 35-50 days when the uterus expanded from its intra-pelvic cavity towards the abdomen. The pregnancy identification based on the appearance of certain indicators of gestation was possible starting at day 15-20 of pregnancy with very low PV+, PV-, Sp, and acc because only a few examined ewes showed positive signs of pregnancy at this period. After that, all pregnancy detection parameters rose and reached maximum values at days 42-50 following mating. According to Romano and Christians (2008) study, there were no ewes detected pregnant at day 15 postmat- ing, while the percentages ranged from 26-100% between days 16 to 20.

The pregnancy detection at day 15 was dependent only on the presence of fluid and extra-embryonic membranes, it showed cranio or cranio-ventral to the bladder as hypo-echoic chambers and notable circular folds, but no embryonic vesicle was noted. Therefore, it should be confirmed by re-examination in the subsequent days of pregnancy.

Several studies reported that embryonic vesicles were considered the first definitive ultrasonographic finding for pregnancy determination; Ardakani et al. (2022), diagnosed the pregnancy when firstly detecting the embryonic vesicle between days 17 to 21. Additionally, the embryonic vesicle was observed on day 21(Santos et al., 2018).

The incorrectly non-pregnant detection (false negative) percent was high in ewes tested during less than 20 days post insemination. The false negative proportions progressively declined when the positive pregnancy signs became more

| Table III: Efficiency of ultrasonographic methods (Se, Sp, PV+, PV- and Acc) in the prediction of pregnancy type in different pregnancy periods. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Diagnosis status/Day            | Pregnant 21-25 (n = 17)         | Pregnant 30-35 (n = 23)         | Pregnant 42-50 (n = 25)         | Level of significance          |
| Diagnosis of correctly twin (A) | 7                               | 8                               | 9                               | -                              |
| Diagnosis of incorrectly twin (B)| 3                               | 2                               | 0                               | -                              |
| Diagnosis correctly singleton (C)| 5                               | 12                              | 16                              | -                              |
| Diagnosis incorrectly singleton (D)| 2                               | 1                               | 0                               | -                              |
| Predicted + value                 | 70%                             | 80%                             | 100%                            | 0.0078**                       |
| Predicted – value                | 71.4%                           | 92.3%                           | 100%                            | 0.0085**                       |
| Sensitivity                      | 77.7%                           | 88.8%                           | 100%                            | 0.0088**                       |
| Specificity                      | 60%                             | 85.7%                           | 100%                            | 0.0025**                       |
| Total Accuracy                   | 70.5%                           | 87%                             | 100%                            | 0.0057**                       |

** (P≤0.01).
evident as gestation advanced.

Ali and Hayder (2007) stated that three out of six does were detected pregnant depending on the appearance of the embryonic vesicle on 17-19 of gestation by TRU, but the embryonic vesicle was visible at day 26, therefore, all does was confirmed at day 26. The proper embryo in the doe was not revealed until day 29 and onwards. They also mentioned that Acc was minimum at periods (17 – 19 days), and it attained 100% on day 34. Additionally, the first embryonic vesicle detection was seen at 20-25 post-mating in doe (Hussein and Omran, 2007).

The significant increment in the Acc due to the rise in Se at period 30–50 postmating may be interpreted by the fast accumulation of fetal fluid in its membranes, also due to the increase in embryo size from day 30 onwards. The results agree with Karen et al (2004), who reported low Se percent in ewes for days 18–24 of TRU, and it recorded more than 90% for days 25-40, and higher than 96% for days 41–50 of pregnancy. Additionally, Ganaie et al. (2009) recorded that Se, Sp, and Acc ranged from 70-95%, 50-83%, and 68-94% in different periods from (15-30, 30-40, and 40-60 days) by using B-mode ultrasonography. Tasal et al. (2006) claimed that a slightly lesser range of accuracy (80% - 87.1%) between days 30- 60 of gestation than our findings came constant with previous studies; the detection values reach 100% at the 40-60 day of pregnancy (Romano and Christians, 2008; Ganaie et al., 2009)

The findings showed high correlation between CRL and gestational age, that give indication about accuracy of this fetometric measurement to precisely determine the fetal age. Several studies agreed with the present study, where they determine that the fetometric measurements correlated with pregnancy age. For instance, Ardakani et al (2022) stated that CRL, BPD, and other parameters were recommended for pregnancy estimation in sheep. Additionally, AL-Rawi (2014) recorded a very high correlation between GSD and CRL for determining fetal age in iraqi ewes. Furthermore, Rasheed (2017) declared that it is possible to predict pregnancy age by calculating CRL in goats. A recent study showed similar correlation (r=0.917) for CRL by TAU in shami goats (Muhammad and Aziz, 2022).

Identification of multiple pregnancies (Twins)

In the present study, the overall ability to determine correctly fetal numbers using TRU steadily improved from days 21-25 with good pregnancy parameter percentages, whereas very high Acc, Se, and Sp were consistently achieved after day 30 of gestation and became 100% at day 50 by using TAU. The B-mode was used successfully to measure the fetal number in different pregnancy periods [33]. Our outcomes came similar to that of Jones et al. (2016) research, which reported that Acc ranged from 68% at day 21 and rose regularly to reach 100% at day 39-42 of gestation.

It was observed that TRU and TAU were more precise in distinguishing twins from single pregnancies, the Acc increased along with pregnancy progress, Alkan et al. (2020) stated that higher Se, Sp, and Acc for TAU performed on days 40 to 60. According to the Yotov (2008) results, the overall Acc to detect twin pregnancy (PV+) was (64%), and singleton pregnancy (PV-) was 74%) at day 20-50 of TRU, lower than our findings in the same period. This is because the Yotov (2008) study depends only on a 5 MHz TRU (linear) probe until day 50, whereas the gravid uterus after day 30 of gestation fills with fluid and then displaced downward to the abdominal cavity which leads to a significant diagnostic errors (foetuses counting through TRU approach declined). This opinion is in agreement with that of Crilly et al. (2017), who mentioned that with pregnancy advancement, the uterus becomes intra-abdominal, therefore the Acc. was high for using TAU. Additionally, the results agreed with Tekin and Kose (2022), who believed that TAU was efficient for...
pregnancy diagnosis at day 35 and fetus number determining. Another method for determined the fetal number through ultrasonography depending on size and number of placentome, but it’s not reliable method (Hussein, 2017).

In the early pregnancy evaluation period (21-25 days), the Sp were reported low (60%) because false positives were more common than false negative predictions (three out of eight singletons). This result can be interpreted by the occurrence of early fetal loss. This observation was in agreement with Diskin and Morris (2008), who claimed that the survival rate of an ovine embryo declined along with an increase in ovulatory rate.

**Conclusion**

In conclusion, real-time (B-mode) ultrasonography is efficient for early pregnancy determining. Until day 35, TRU was used with high efficiency in the detection of pregnancy, fetal age, and litter size, but the optimum Acc of pregnancy judgment in Awassi ewes was achieved at day 42-50 by using TAU, while, before day 15 postmating, Acc in the ewes was not higher.

Until day 25, the pregnancy prediction was based majorly on monitoring the echotexture of the uterus and its place in the bladder, and CL instead of the conceptus monitoring, as the gestation advanced, the conceptus, fetal membranes, and fetal structures and placentome were demonstrated clearly.

Finally, the appropriate period to confirm the type of pregnancy (foetus no.) ethnographically is the period between the 42nd – 50th days of pregnancy.

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