

Comparison of the Effectiveness of Two Different Pregnancy Detection Methods in Awassi Ewes

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Abstract

Aim of Study: This study aimed to compare several methods of pregnancy diagnosis in local Iraqi ewes including progesterone (P4) assay and the Ultrasonography (ULR) to identify the Sensitivity(Se), Specificity(Sp) and Accuracy(Acc) of the methods of diagnosis. **Materials and Methods:** This study was carried Sixty of local Iraqi ewes with 3-5 years old. The experimental animals were subjected to an estrus synchronization. Blood samples were collected at 25 days of insemination, in order to measurement the level of P4 the first method. Pregnancy was diagnosed with Transrectal ULR (TRU) (second method) via TRU and transabdominal (TAU) –ULR, respectively. **Results:** The result showed that the Se, Sp, Acc of the P4 measurement at 25 days post-examination using the COBAS test were 95.1%, 42.1%, and 78.3% with ($P < 0.05$), respectively. The Se, Sp, and Acc at 30 days of examination by ULR were 85.4%, 78.9%, and 83.3% respectively, with ($P < 0.05$). The Se, Sp, and Acc at 60 days of examination by ultrasonography were 100%, 100%, and 100% respectively, with ($P < 0.05$). The findings demonstrated that Se and Acc for P4 assay in day 25 were higher than ULR at day 30, but the Sp was lower than ULR method. **Conclusion:** It can be concluded that diagnosing pregnancy Depending on P4 assay at day 25 in sheep by measuring P4 concentration on day 25 after insemination and performing TR_ULR on day 30 and TA-ULR on day 60, are very effective methods for identifying pregnant sheep. Furthermore, the parameters for evaluating these methods (Se, Sp, Acc) improved as the pregnancy progressed, reaching their maximum on day 60 after insemination.

Keywords: Hormones, pregnancy diagnosis, progesterone, ultrasonography

INTRODUCTION

Pregnancy detection in ewes is important for reproductive planning and results in minimised economic costs of eliminating or rebreeding non-pregnant ewes. It can therefore cause significant financial losses in milk and lamb production because of long intervals between lambing. Therefore, there is need to identify early pregnancy in sheep(1). However, in the larger animals, rectal palpation of the genital tract is possible but cannot be employed for the small stock.

There are various techniques used for pregnancy diagnosis in sheep, the most important of which include A-scan ultrasonography(2), Doppler ultrasound(3), real-time B-mode ultrasound(4,5), oestrone sulphate(6), and progesterone(7). Ultrasonography for diagnosing pregnancy in small ruminants offers multiple benefits compared to traditional techniques such as rectal palpation and serum hormone testing. Standard methods tend to be invasive, take more time and generally less accurate than ultrasound(8). Ultrasonography is safe,

non-invasive and practical diagnostic tool in the early first-trimester pregnancy assessment, embryonic/foetal number, age, sex, viability and several pregnancy-related disorders(9-11). It can also predict the timing of parturition and decrease foetal losses from dystocia(12), as well as early foetal death(13,14). Due to sensitivity and specificity of over 90% and 95% respectively, ultrasonographic evaluation of early pregnancy can be performed as early appears 24 days post-breeding(15). Measuring progesterone levels in blood plasma is one of the diagnostic methods used shortly after insemination(16), with levels around 4–5 ng/mL on day 21 indicating pregnant ewes. AL-Mansoury(17) find that

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measuring progesterone levels 21 days after breeding can accurately detect pregnancy in ewes with 90% accuracy. In addition, ultrasonography has emerged as a new, safe method for detecting pregnancy in domestic animals without causing harm to body tissues. Based on previous studies, this study hypothesised a comparison of the efficiency of three pregnancy detection methods: measuring progesterone concentration on day 25, ultrasound on day 30 and ultrasound on day 60 after mating.

MATERIALS AND METHODS

Experimental animals

The study included 60 ewes reared locally in Iraq that were aged between 3 and 5 years and whose weight ranged between 50 and 70 kg. Furthermore, 12 healthy breeding rams aged between 3 and 4 years and mean body weight of 60–90 Kg were included in the study. All ewes were from the lambing and weaning of their last-born lamb classes before January 2020. This study was carried out in Abu Ghraib area, west of Baghdad, during breeding season, from 7 October 2023 to 24 January 2024. The ewes were separated from the rams for 30 days before the start of the experiment and examined with an ultrasound device to ensure that they are not pregnant. All animals were housed in one flock, in a private field. The ewes were housed in medium management conditions. All the ewes were received vaccines and treated with anthelmintic, and during the study period, concentrate grains, alfalfa and hay straw were given.

Oestrus synchronisation protocol

The ewes were synchronised by intravaginal polyurethane sponges device contain 60 mg MAP (MSD, Holland). The sponges (one sponge/ewe) were inserted through the intra-vaginal by a special applicator. The vaginal sponges remained in the ewes for 12 days then received intramuscularly 330 IU of Pregnant Mare Serum Gonadotropin after withdrawal of sponges.

Blood collection

Blood samples were collected at 25 days after insemination(6). The samples were then transferred to a laboratory and separated by centrifugation of the samples (3000 RPM for 15 min) and stocked under -20°C until assay.

Pregnancy diagnosis

Hormonal measurement (plasma progesterone assay)

The *P4* kit (*P4* reagent) was used to determine the *P4* level by using full automated COBAS E 411 analyser, The samples were examined by Enzyme-Linked Immunosorbent assay (ELISA) using ELIAS kits (Human Kit, Germany). Progesterone measurements were recorded in ng/mL(18).

B-mode ultrasound examinations

The ULR examination was performed with a B-mode. The examination technique chosen depends on the pregnancy stage. Ultrasonography instrument equipped with a linear-array 6.5 MHz transducer (linear probe) designed for applying transrectally; it was utilised to inspect the genital tract in

the early pregnancy period (30 days) including the uterus, gestational sac, embryo, extra-embryonic membranes and placenta. The uterine horns that are not gravid or in early pregnancy are frequently detected around the bladder, maybe in the cranial and sometimes in ventral positions or even laterally to the bladder. Furthermore, a convex transducer was used transabdominal by applying a probe on the inguinal region and/or ventral abdomen at frequencies 3.5–4.5 MHz to detect the pregnancy including foetal head, vertebral column, heart, extremities, thoracic, trunk and placentoma.

Statistical analysis

The Statistical Analysis System(19) program was used to detect the effect of different factors in study parameters. Chi-square test was employed to compare percentages significantly, with probability levels of 0.05 and 0.01. In addition, the correlation coefficient was estimated between the variables.

Ethical approval

The Ethical Committee of Fallujah University (No. 196 in 11/12/2022) provided ethical approval for this study on 17 September 2024.

RESULTS AND DISCUSSION

Comparison of sensitivity, specificity and accuracy among all methods on pregnancy diagnosis

The finding of the study demonstrates that the effectiveness of pregnancy diagnosis varies depending on the method utilised and the stage of pregnancy.

Table 1 showed that Se, Sp and Acc of *P4* measurement at 25 days post-examination using the ELISA test were 95.1%, 42.1% and 78.3%, respectively. The Se, Sp and Acc at 30 days of examination by ULR are 85.4%, 78.9% and 83.3%, respectively.

The Se, Sp and Acc at 60 days of examination by ultrasound were optimum (100%).

The efficiency of each pregnancy diagnosis method relies on its individual mechanism, resulting in significant variations between them. These methods also differ in Acc depending on the stage of pregnancy. Certain methods become more precise as pregnancy progresses, while others only exhibit high Acc during early pregnancy(20).

We observe from the results that the specificity of this test for diagnosing non-pregnancy (negative pregnancy) was low, at 42.1%. This may be attributed to a technical error (as a diagnostic kit specific to humans was used), early embryonic mortality, ovarian luteal cysts or cases of endometritis/pyometra, which can lead to the persistence of the corpus luteum, resulting in elevated progesterone levels above 4 ng/mL.

False-negative and false-positive results are common during this period of pregnancy. Accuracy at these early stages is relatively low for two main reasons. First, false-negative

diagnoses occur when the trophoblastic vesicle or embryo is not detected. Second, false-positive diagnoses can arise due to the accumulation of intrauterine fluids from non-pregnancy-related causes(21) or the misidentification of embryonic vesicles with intestinal loops, blood vessels or pathological conditions(12). In such cases, a positive diagnosis may be erroneous if the procedure is performed before 30th day of gestation and subsequent embryonic or foetal death or abortion. The effect of inadequate visualisation of ewe's reproductive tract can also lead to false negative result; this May be mainly because of imaging constraints during early stages of gestation Caused by upper abdominal fullness from fluids and/or gas in the bowel. This is particularly relevant in multiparous ewes, especially when administrating using the transrectal method. As a precaution against obtaining a negative result from a pregnancy ultrasound, it is recommended to use both the transrectal and transcutaneous approaches if the gravid uterus is situated over the brim of the pelvis(22). Ultrasonography is effective for diagnosing pregnancy in ewes [Figure 1], but false positives and negatives were observed at 30 days, reducing diagnostic accuracy during this period(23). However, as pregnancy progressed, the rates of false negatives and positives gradually decreased, with clearer signs of pregnancy becoming more evident by day 60 of ultrasound examination.

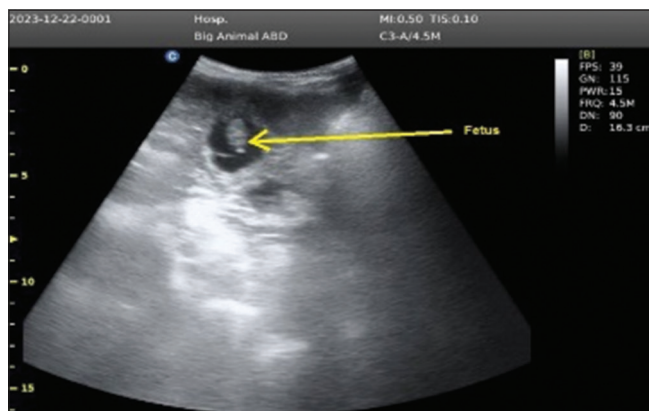


Figure 1: TAU image on day 30 of pregnancy ewe showing the fetus

The Acc of both techniques progressively increased, reaching 100% [Figure 2] from days 60 to 90 of intrauterine age(24). These findings align with earlier research by(25, 26,27) which also reported higher sensitivity, specificity and accuracy for transabdominal ultrasound performed between days 40 and 60. The sensitivity and accuracy showed a significant increase during the 60 days post mating, making this period more effective than earlier stages. These results are consistent with findings from(28), who reported low sensitivity percentages for days 24–30 of transrectal ultrasound, which then increased to over 90% by day 50 and surpassed 96% by day 60 of pregnancy.

In this study, the sensitivity rates for pregnancy diagnosis using progesterone concentration and ultrasound at days 30 and 60 post-mating were 95.1%, 85.4% and 100%, respectively. Specificity was 42.1%, 78.9% and 100%, while accuracy was 78.3%, 83.3% and 100%, respectively. These results align with(29), who reported that ultrasonography was significantly more accurate compared to the progesterone level method.

Nevertheless, all parameters improved as the pregnancy progressed and reached their maximum on the sixtieth day of pregnancy [Table 1]. Ultrasound sensitivity improves as

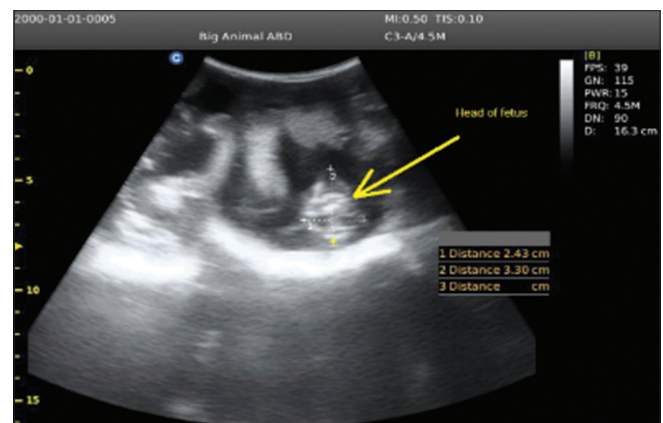


Figure 2: TAU image on day 60 of pregnancy ewe showing the head of fetus

Table 1: Table showing the Sensitivity, specificity and accuracy according to results

Parameters	Range of days			P
	P4-25 days	Ultrasound - 30 days	Ultrasound - 60days	
a: Correct positive	39	35	41	-
b: Incorrect positive	11	4	0	-
c: Correct negative	8	15	19	-
d: Incorrect negative	2	6	0	-
n: Total number of animals	60	60	60	-
Positive predictive value (%)	78	89.7	100	0.0285*
Negative predictive value (%)	0	71.4	100	0.0001**
Sensitivity (%)	95.1	85.4	100	0.679 (NS)
Specificity (%)	42.1	78.9	100	0.0016**
Accuracy (%)	78.3	83.3	100	0.0272*

* $P \leq 0.05$, ** $P \leq 0.01$. NS: Not significant

pregnancy advances(24). Reported that the transabdominal method exhibited a sensitivity of 40% on day 21 of pregnancy, reaching 100% by day 39. Typically, sensitivity begins to rise after day 40, when the uterus shifts into the abdominal cavity. Therefore, the best period for diagnosing pregnancy using the transabdominal method is between days 40 and 75(1,30).

CONCLUSION

It can be concluded that the P4 assay at day 25 is better than TR-ULR in day 30 in pregnancy diagnosis, but lower efficiency for the detection of non-pregnant ewes; in addition, the TAU at day 60 is a perfect method for pregnancy detection, because all the pregnancy diagnosis parameters reach to 100%.

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Conflicts of interest

There are no conflicts of interest.

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