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Comparative study between progesterone, ultrasonagraphy, and Pregnancy Associated Glygoprotein for pregnancy diagnosis in local Iraqi ewes

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ABSTRACT

This study aimed to detect pregnancy by ultrasonography, progesterone and *PAG* in local Iraqi ewes. This experiment was conducted in the breeding season and region at Saqlawiya/ Al-Anbar province, period from 1/9/2023 until 1/3/2024. Thirty-seven polyparous nonpregnant ewes aged 2-4 years and twelve matur rams were with used, their weight ranged between 45.3±3 kg. The animals were examined with Trans-Rectal Ultrasonography (*TRU*) and trans-abdominal (*TAU*) to ensure that thay were non pregnant. Estrus synchronization was achieved by applied intra vaginal sponges for 14 days. Twelve breeding rams used to estrus detection and breeding. Ewes underwent ultrasonic examination at day 34 post-mating (*PM)* by TAU. Hormonal assay were conducted at day (34) *PM*, Ovine Pregnancy Associated Glycoprotein (*PAG*) from day 34 PM estemal. All ewes showed Estrus. Moreover, the diagnosis of pregnancy by ultrasound examination was better at days 34 of pregnancy followed by the *PAG* and then the progesterone hormone. Additionlly, ultrasonography method recorded higher accuracy for pregnancy detection in days 34 PM comparison *PAG* assay and progesterone assay. In conclusion the ultrasonography is regarded as one of the most precise and up-to-date techniques for diagnosing pregnancy followed by progesterone assay and PAG and pregnancy could be accurately detected by ultrasound on the 34th day of pregnancy in ewes.

دراسة مقارنة بين استخدام البروجسترون، الموجات فوت الصوتية والجليكوبروتين المرتبط بالحمل في النعاج العراقية المحلية

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الخلاصة

هدفت هذه الدراسة إلى تشخيص الحمل بواسطة الموجات فوق الصوتية والبروجيستيرون و PAG، أجريت هذه التجربة في محافظة الانبار/ مدينة الصقلاوية للمدة من 2023/9/1 إلى 2024/3/1. تم استخدام 37 نعجة متعددة الولادة غير حامل بعمر 2-4 سنوات وكباش ناضجة عدد 12، بمعدل وزن (45.3 ± 3) كجم. تم فحص الحيوانات بالموجات فوت الصوتية عبر المستقيم (TRU) وعبر البطن (TAU) للتأكد من عدم وجود الحمل. تم اجراء تزامن الشبق عن طريق وضع اسفنجات مهبلية لمدة 14 يومًا، كل الحيوانات كانت في مجموعة واحدة. تم إدخال الكباش ال 12 للكشف عن الشبق والاخصاب. خضعت النعاج للفحص بالموجات فوت الصوتية في اليوم 34 بعد التزاوج. تم إجراء التحاليل الهرمونية والتي شملت قياس PAG والبروجستيرون في اليوم (34) بعد الاخصاب. وقد أظهرت النتائج المتحصل عليها ما يلي: كان معدل الشبق متساوياً حيث أظهرت جميع النعاج شبقاً متساويا. لوحظ بان تشخيص الحمل بواسطة الموجات فوق الصوتية كان افضل في اليوم 34 من الحمل مقارنة PAG والبروجستيرون. بالإضافة إلى ذلك، سجلت طريقة الموجات فوق الصوتية دقة أعلى في الكشف عن الحمل في اليوم 34، مقارنةً بتحليل PAG وتحليل البروجسترون. في الختام، يُعدّ التصوير بالموجات فوق الصوتية من أكثر التقنيات دقةً وحداثةً لتشخيص الحمل، يليه تحليل البروجسترون وPAG. ويمكن الكشف عن الحمل بدقة باستخدام الموجات فوق الصوتية في اليوم 34 من الحمل. نستنتج من الدراسة ان الموجات فوت الصوتية تعتبر من أكثر التقنيات دقة وحداثة لتشخيص الحمل تليها تحليل البروجسترون واختبار PAG ويمكن الكشف عن الحمل بدقة عن طريق الموجات فوت الصوتية في اليوم 34 من الحمل في النعاج.

الكلمات المفتاحية: البروجستيرون، الموجات فوق الصوتية، الجليكوبروتين المرتبط بالحمل، تشخيص الحمل، النعاج العراقية.

INTRODUCTION

Sheep estrus cycle induction is done to control ewes reproduction and lambing during or out of season. This induction management is on several methods: ram effect, photoperiod, exogenous hormones (prostaglandin F2α, melatonin, progesterone, prostaglandins, equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) (Gonzalez-Bulnes *et al.*, 2020; Hameed *et al.*, 2023; Alhimaidi *et al.*, 2023).

In order to facilitate single and timed insemination without the need for behavioral estrous detection, The main goal of controlling corpus luteum function and follicular growth is to create a software that will more precisely synchronize estrous and control the timing of ovulation. Estrous synchronization boosts an animal's productivity for greater financial gain. Synchronization may shorten the females' 21-day breeding time to a fewer than five days, depending on the treatment strategy. The production of homogeneous calf crops is another important advantage of this technique (Aray *et al.*, 2023). Early pregnancy diagnosis is critical for maximizing herd productivity in the cattle industry, current methods for pregnancy diagnosis include: (ultrasonographic; vaginal biopsy, vaginal smears and Laboratory methods (progesterone, pregnancy specific proteins, oestrone sulphate assay) (Akköse, 2020).

The progesterone (P4) is an important for hormone pregnancy diagnosis (Çebi & Akköse, 2024). In sheep, P4 is detectable in the blood as of the 18 day of pregnancy and increase to a level of 2-3 ng/ml at day 50 of pregnancy, displaying a rapid increase after the first 3 months of gestation to reach a peak level of 12-20 ng/ml (Kaplan Bilmez, 2018). While a increase level of success is achieved in the diagnosis of pregnant ewes with progesterone measurement, cases of early embryonic death, CL persistence, the prolongation or shortening of the oestrus cycle, faulty insemination, and pathologies of uterus and ovaries may cause misdiagnosis (Karen *et al.*, 2001). Furthermore high P4 levels observed during certain phases of the oestrus cycle may lower the success of diagnosing non-pregnant ewes (Karen *et al.*, 2004).

Transabdominal ultrasonography could used with a sensitivity of 100% as from the 39 day, and accuracy of 100% as from the 40 day of pregnancy (Aziz & Lazim, 2012; Jones *et al.,* 2016). Despite its increase efficiency in the detection of pregnancy in ewes, regarding the facilities needed for examination, ultrasonography also has certain practical restrictions, availability, portability of equipment, positioning of ewes for examination, cost of equipment, type of transducer and frequency of transducer (Roberts *et al.,* 2019). During pregnancy, the ruminant placenta's fetomaternal interface produces pregnancy-associated glycoproteins (PAGs), which are then discharged into the intercotyledonary region (Sousa *et al.,* 2006). Sheep can be diagnosed with early pregnancy when PAGs are present in the mother's bloodstream (Uçar *et al.,* 2018; Alkan *et al.,* 2020).

The hCG alone and with P4 could improve the reproductive performance in ewes (Rostami *et al.,* 2017). Improving reproductive performance represented b uniformity of estrus rate, conception rates, fertility rates, pregnancy rate, lambing rates, litter size, etc., considered as one of the special advantages in increasing number of animals and improving the offsprings (Đuričić *et al.,* 2019). Because of low fertility rate, pregnancy rate in local Iraqi ewes many protocols used to rise the reproductive performance. The data regarding this aspect (Estrus synchronization with *P4+hCG*) is scanty. The aims of study to detect the pregnancy by used *PAG*, Progesterone assay and ultrasography method and compare between the relability of different methods in first pregnancy detection.

MATERIALS AND METHODS

37 multiparous ewes, along with 12 breeding rams aged between 2- 4 years on average, were observed during the study. The animals had an average weight of 45.3 ± 3 kg. The experiment was conducted at Saqlawiya/ Al-Anbar Governorate during the period 1/9/2023 until 1/3/2024. Animals were isolated for 30 days before the start of the experiment and examined with an ultrasound device to ensure that there was no pregnancy. All necessary supplies were available in the field, including sheds and tools to conduct the experiment. The animals housed in medium management condition and barley was given. A therapeutic and preventive program and a vaccination program were installed. The animals were examined. They were treated with anthelmintics and injected with ivermectin to prevent internal and external parasites as well as a vaccine was injected to prevent enterotoxemia caused by bacterial infection. The period between vaccination and sponge placement was 30 days.

The ewes were synchronized by intravaginal polyurethane sponges device contain 60 mg MAP. Additionally, eCG (vial contain a dispersible tablet) with concentation 5000 IU accompined with 50 mL solvent. The eCG was prepared through solved the dispersible tablet by the solvent. The small quantity of the solvent was aspired by syringe and evacuated in the eCG vial and shaked well to completly disloved. Then, the mixture was aspired and added to the whole solvent to prepared the the concentration 5000 IU in ~ 50 mL. After that, the hormone been ready for injection. Thirty nonpregnant ewes were synchronized according to Kuru *et al*. (2022). The sponges (one sponge/ ewe) were inserted through the intra-vaginal root by a special applicator. It persists for 14 days, then withdrawal. The heat was detected by observing the mating of the breeding ram. Each ewe was considered in estrus when observed to accept a service from a ram. The day of breeding was considered day 0.

Blood samples (10 ml) were collected from the jugular vein into anticoagulant tubes on day 0, before the insertion of sponges, and again on day 34 after sponge removal. The samples were transported to a laboratory where they were separated using centrifugation at 4000 RPM for 15 minutes and stored at -20°C until further analysis. Progesterone concentrations were determined using the COBAS (E-411) method, with the kit supplied by Roche, Inc., Switzerland. Additionally, Sheep Pregnancy Associated Glycoprotein (PAG) concentrations were measured using ELISA technology with kits provided by Sunlong

Biotech Co., Ltd, China. Ultrasound examination was conducted using a B-mode diagnostic approach, tailored to the stage of pregnancy. On day 0, the genital tract was assessed to confirm that the ewes were not pregnant. The uterine horns, typically located cranially and occasionally found ventrally or laterally to the bladder, were inspected. Beginning at the fifth week of gestation, a convex transducer was employed transabdominally by placing the probe on the inguinal region and/or ventral abdomen at frequencies of 3.5-4.5 MHz to detect pregnancy by day 34, identifying key structures such as the gestational sac and embryo. During this procedure, the ewe was lightly restrained in a standing position. For a more detailed examination, the linear probe lubricated with specialized ultrasound gel was gently inserted along the rectal floor after cleaning out fecal residues. Advancing the probe approximately 15 cm cranially allowed visualization of the bladder, identified as a fluid-filled (non-echogenic) structure with distinct walls. A diagnosis of pregnancy was confirmed upon imaging an evident gestational sac and conceptus—an elongated, anechoic structure within the uterine fluid.

Statistical analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference groups in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Sensitivity and Specificity of variables in this study. The analysis of variance statistical model was: $Yij = \mu + Pi + eij$

Where:

Yij = dependent variable (P4, Ultrasonography and PAG).

μ = overall mean.

Pi= effects of the pregnancy detection (P= Day 34 PM for P4, Ultrasonography and PAG).

eij = error term

The following detection traits were determined according to Yotov (2005):

- Predicted positive Value (PV+) = Detection true positive (A)/ Detection pregnant (True and false positive) (A+B) × 100.
- Predicted Negative Value (PV-) = Detection true Negative (C)/ Detection non- pregnant (true and false negative) (C+D) × 100.
- Sensitivity (Se %) = Number of True Positive diagnoses (A)/ Total Number of Positive detection (A/ A+D) ×100
- Specificity (Sp %) = Number of True Negative detection/ Total number of negative detection (B/B+C) ×100.
- Accuracy (Acc %)= Number of True detection/ Total Number of detection (A+C/N) ×100

A. True positive (pregnant)

B. False positive (non-pregnant)

C. True Negative (non-pregnant)

D. False negative (pregnant)

N. Total number of animals (A+B+C+D)

The results of pregnancy detection depend on the lambing results, it considered a golden standard to compared with the pregnancy detection methods.

RESULTS AND DISCUSSION

Estrous synchronization is an important reproductive technique to improve the utilization of ewes by shortening the lambing interval. The majority of ewes (100%) treated with 250 IU of *hCG* exhibited estrus within 72 hrs of I.M. injection. Depending on observed estrus and on (TAU and TRU) examinations at day 34, the conception or pregnancy rates were 78.3%. The hCG treatment resulted in follicular growth patterns, even though there was a greater synchrony in the time of ovulation with use of the hCG treatment, there were greater

serum P4 concentrations in ewes after the removal of the sponges from the vagina and administration of the hCG treatment. The result of current study demonstrated that application of vaginal sponge with *hCG* administration resulting in an advancement of the onset of estrus and time of ovulation, since serum P4 levels greater than 1 ng /ml are enough to control LH pulsatility and ovulation in ewes, thus concentration of P4 in sponge does not or slightly effect to reproductive performance, this results supported by Vilarino *et al.,* (2010), agreed with my current study. The duration of response (from progesterone removal until beginning of estrous symptoms) in current study varies from 39 to 72 hrs this results close to some authors (Gungor *et al.,* 2009). While my results disagree with (Kaya *et al.,* 2013) that found the onset of estrus symptom was from 50.3 hrs to 59 hrs this variation depending on many factors, one of them breed of ewes also show close to our results in percentage of estrus response in treated ewes.

Using ultrasonography enables to distinguish between pregnant and non-pregnant ewes with better *ACC*. The *SE* for diagnose pregnant ewe was increase (93.1%) at day 34 PM. Additionally, the results proved depending on result of parturition, predictive value of positive test, predictive value of negative test, sensitivity, specificity and accuracy is (100%, 80%, 93.1%, 100% and 94.59% respectively) at day 34 of pregnancy as presented in (Table1), Fig (1, 2, 3).

Table 1. Pregnancy detection of ewes using ULR method at day 34 P.M.

Pregnancy status	Day 34
Diagnosis correctly pregnant (A)	27
Diagnosis incorrectly pregnant (B)	0
Diagnosis correctly non-pregnant (C)	8
Diagnosis incorrectly non-pregnant (D)	2
Total number	37
Predicted + value	100%
Predicted – value	80%
Sensitivity	93.1%
Specificity	100%
Accuracy	94.59%

($P \leq 0.05$), ($P \leq 0.01$), NS: Non-Significant Positive predictive value: 100 a/ a+ b; Negative predictive value: 100 c/ c + d; Sensitivity: 100 a/ a + d; Specificity: 100 c/ c + b; Accuracy (%) = (a+c/a+b+c+d) ×100

This finding aligned with earlier research (Tekin & Köse, 2022; Hameed *et al.,* 2024) observied that transabdominal ultrasonographic examination on 35 day for early pregnancy detection in ewes is increased effective in determination of pregnant ewes. And these results are agreed with those of Younis & Hatif (2023) that showed the Se at day 30 of pregnancy was 92% and Karen et al. (2004), who found that the Se percentage was increased to over 90% for days 25-40 and above 96% for days 41-50 of pregnancy. While these results were disagreed with Ganaie et al. (2009a) that showed Se and Sp ranging from 50-83% in the same gestational period 30-40 days.

It has been shown that when transabdominal ultrasonography is used in a sitting position as opposed to a standing position. providing a better results for early pregnancy detection (Gürler & Kaymaz, 2011).

Transrectal ultrasonography can detect anechoic fluid in the uterine lumen in sheep as early as 17–19 days of gestation, or transabdominally between 25–28 days. Pregnancy confirmation should not be based only on fluid in the uterus lumen. A fetal heartbeat and the identification of the amniotic vesicle are necessary for a conclusive diagnosis. By 27–30 days of gestation a fetal heartbeat may be consistently found in ewes using a transabdominal scanning technique (Jones & Reed, 2017; Roberts, 2022). After 30 days of mated the

transabdominal ultrasonography is 90% of accuracy for pregnancy diagnosis in Awassi ewes (Younis & Hatif, 2023) this research agreed with the results, and the accuracy (90%) in days 40-45 of gestation was slightly lower (Tasal *et al.*, 2006; Abd & Ibrahim, 2024). While, that accuracy of pregnancy detection was increased to 98% at day 35 post-mating using a 5 MHz transabdominal probe in Satara Zagora ewes breed (Yotov, 2005). At day 34 after mating, Akköse (2020) found that the transabdominal ultrasonography's sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were, respectively, 95.2%, 100%, 100%, 80%, and 96% in Awassi ewes, my results were similar to this research.

According to Tekin & Köse, (2022) ultrasonography results sensitivity, specificity, positive predictive value, negative predictive value ratios for pregnancy examination findings data by transabdominal ultrasonography on day 35 were determined as 91.30%, 100%, 100%, 83.3%, respectively, this research agreed with current results.

Fig. 1. TRU images at day 0 showed the Empty ewe at before treatment.

Fig. 2. TRU images at day 34 of pregnancy ewe showed the fetus.

Fig. 3. TAU images at day 34 of pregnancy ewe showed the fetus.

In days 34 PM were declared pregnant using the P4 assay. According to the cutoff point <3 (according to youdin index), the Se of P4 assay for pregnant detecting in ewes 84.3% during the experimental time at day 34 PM. The Sp of the progesterone test in detecting non-pregnant ewes at days 34 was equal (40%). Similar trend was observed for Predicted Positive Values (PV+) which were 93.1% at day 34 PM. In the contrary negative predictive value of this test for diagnosis non-pregnant ewes was equal (37.5%) at day 34 PM (Table 2).Blood P4 levels are a good way to tell if the placent or CL is functioning, this approach can be used for the full 18 days of pregnancy and is accurate false positive cases could be caused by early embryonic demise (Karen *et al.*, 2001; Purohit, 2010).Ganaie et al. (2009 b) observed that for days 15–30 and 31–45 of pregnancy.The accuracy of 98%, prediction values of 97.7 and 100% for positive and negative detection at days 18.23±0.78, and the accuracy of 83.3% for non-pregnancy detection remained the same. The study also demonstrated that one of the most accurate ways to diagnose pregnancy in Corriedale ewes is to measure the progesterone hormone by ELISA on the 35 day following fertilization.Rahman et al. (2020) found that the accuracy of progesterone-based early pregnancy diagnostic was 80 - 85% was higher than Barium chloride solution gave 67 - 70.5% for days (15- 75). Abd & Ibrahim (2024) noted that the progesterone level was a higher at days 14, 21, 28 compared with days 0, 7. The progesterone test's varying sensitivity could be the cause of the discrepancy in the results. Impacts include luteal cysts, hydrometra, pyometra, early embryonic mortality, and irregularities in the estrous cycle on the outcome of hormone concentration (Ishwar, 1995).

Table 2. Pregnancy detection of ewes using plasma progesterone assay

Pregnancy status	Day 34
Diagnosis correctly pregnant (A)	27
Diagnosis incorrectly pregnant (B)	2
Diagnosis correctly non-pregnant (C)	3
Diagnosis incorrectly non-pregnant (D)	5
Total number	37
Predicted + value	93.1%
Predicted – value	37.5%
Sensitivity	84.3%
Specificity	40%
Accuracy	81%

($P \leq 0.01$), NS: Non-Significant. Positive predictive value: 100 a/ a+ b; Negative predictive value: 100 c/ c + d; Sensitivity: 100 a/ a + d; Specificity: 100 c/ c + b; Accuracy(%)= (a+c/a+b+c+d) ×100

- **Sheep Pregnancy Associated Glycoprotein (PAG).**

The current study's results demonstrated the predictive value of both positive (87.1%) and negative tests (33.3%) during test period day 34, as well as accuracy (78.4%), specificity (66.6%), and sensitivity (87.1%) (Table 3). During pregnancy, the ruminant placenta's fetomaternal interface produces Sheep Pregnancy Associated Glycoprotein (PAG), which is then discharged into the intercotyledonary gap (Sousa *et al.,* 2006). A recent, viable pregnancy is directly demonstrated by the presence of these proteins in the blood. From week three of pregnancy until two to three weeks after lambing, they are present in the mother's blood (Uçar *et al.,* 2018; Taverne & Noakes., 2019; Alkan *et al.,* 2020).

Numerous factors, such as breed, fetal sex, fetal number, birth weight, environmental conditions, maternal nutritional status, and the type of test employed for detection, might influence maternal concentrations of PAG throughout gestation. PAG tests have limitations as compared to ultrasound. However they can be useful when ultrasound is not available for pregnancy diagnosis, these drawbacks include the inability to detect uterine pathology or assess fetal viability, as well as the fact that PAG remains in maternal serum for variable periods of time following fetal demise, potentially leading to a false-positive diagnosis (Roberts *et al.,* 2017; Roberts, 2022).

Sheep Pregnancy Associated Glycoprotein (PAG) assay accurately 100% diagnosis pregnancy from day 26 after mating (Karen *et al.,* 2001). Akköse (2020) observed that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of transabdominal ultrasonography was 97.6%, 62.5%, 93.2%, 83.3% and 92% respectively during day 34 post-mating in Awassi ewes.

Meshref *et al.,* (2022) observed that the PAG is more accurate for early pregnancy diagnosis in Osseimi ewes' blood at 16 days of gestation. Additionally, the amount of feti is indicated by the PAG concentration. The PAG values in East Friesian ewes showed increase between days 25- 35 after AI with higher mean values in pregnant than non-pregnant groups at day 35 (Yotov & Sinapov, 2023).

Table 3. Pregnancy detection of ewes using PAG method

Pregnancy status	Day 34
Diagnosis correctly pregnant (A)	27
Diagnosis incorrectly pregnant (B)	4
Diagnosis correctly non-pregnant (C)	2
Diagnosis incorrectly non-pregnant (D)	4
Total number	37
Predicted + value	87.1%
Predicted – value	33.3%
Sensitivity	87.1%
Specificity	66.6%
Accuracy	78.4%

($P \leq 0.01$), NS: Non-Significant. Sensitivity: 100 × a/ a + d; Specificity: 100 × c/ c + b; Positive predictive value:100 × a/ a+ b; Negative predictive value: 100 × c / c + d; Accuracy (%) = (a+c/a+b+c+d) ×100

According to the study's findings, the ACC for using the ULR method to diagnose pregnancy was 94.59%. It happened on the 34th. The progesterone assay method was developed in response to the PAG diagnostic approach. However, the ULR method's specificity (100%) was better. As for the *PV*- test it constituted the highest rate of diagnosing pregnancy using the ULR method (Dempsey, 2021). It was followed by the PAG method and percentage was shown using the progesterone assay method. The highest predictive value of positive test was diagnosed by ULR method. This was followed by progesterone assay and PAG (Table 4).

Ewe growers profit economically from early pregnancy diagnosis, fetal number assessment, and pregnancy detection, pregnancy detection techniques should be precise, easy, quick, useful, affordable, and safe for both humans and animals. P4 and PAG are capable of accurately detecting pregnancy, but they are costly and their ability to distinguish between single and twins is not thought to be that enough to be useful. Although ULR is a rapid, easy, and inexpensive approach it has a poor accuracy rate in identifying multiple pregnancies and may result in abortion (Karen *et al.*, 2001).

Table 4. Pregnancy detection of ewes using three different methods

Pregnancy status/Day	P4 day 34	ULR day 34	PAG day 34	P-value
Predicted +ve	93.1%	100%	87.1%	0.407 NS
Predicted -ve	37.5%	80%	33.3%	0.0001 **
P-value	0.0001 **	0.0395 *	0.0001 **	---
Sensitivity	84.3%	93.1%	87.1%	0.0001 **
Specificity	40%	100%	66.6%	0.0002 **
Accuracy	81%	94.59%	78.4%	0.0001 **

* ($P \leq 0.05$), ** ($P \leq 0.01$), NS: Non-Significant. Positive predictive value: 100 a/ a + b; Negative predictive value: 100 c/ c + d; Sensitivity: 100 a/ a + d; Specificity: 100 c/ c + b; Accuracy (%) = (a+c/a+b+c+d) ×100.

Akköse (2020) noted that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of rapid visual PAG were 97.6%, 62.5%, 93.2%, 83.3%, 92% and transabdominal ultrasonography were 95.2%, 100%, 100%, 80%, 96%.

Al-Rawi & Hussain (2024) showed that the predictive value of positive and negative test, accuracy, sensitivity and specificity of the PAG and P4 were 100%, 100%, 100%, 100% and 100% on both 30 and 60 of pregnancy, compared to ultrasonography which were 100%, 62.5%, 83.3%, 76.9% and 100% at day 30 and 100%, 100%, 100%, 100%and 100% at day 60 of pregnancy.

CONCLUSION

The Ultrasonography is regarded as one of the most precise and up-to-date techniques for diagnosing pregnancy followed by progesterone assay and PAG. Pregnancy could be accurately detected by ultrasound on the 34th day of pregnancy in ewes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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