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Accuracy of INF-tau assay for early pregnancy diagnosis in Awassi ewes compared to progesterone assay and transrectal ultrasonography

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Abstract

The present study evaluated the accuracy of interferon-tau (INF-tau) assay for early pregnancy diagnosis in Awassi ewes compared to progestogen assay and transrectal ultrasonography. Twenty-four non-pregnant Awassi ewes were used in this study. The early pregnancy diagnosis in 22 ewes was evaluated using INF-tau assay, 18 ewes were evaluated using progesterone assay, and 24 ewes were evaluated using transrectal ultrasonography. The accuracy, sensitivity, specificity, and predicted positive and negative values for each method were calculated according to the results of confirmation transabdominal ultrasonography on day 40 of pregnancy. At 15 and 25 days of gestation, the accuracy, sensitivity, specificity, and positive and negative predictive values of INF-tau assay were 77.27, 68.75, 100, 100 and 54.55%, respectively. A significant correlation was observed between the values of INF-tau assay that were recorded at 15 and 25 days of pregnancy. There was a significant correlation between the values of INF-tau and progesterone assay, which were obtained at 25 days of pregnancy. The study results conclude that the INF-tau assay has a good accuracy that can be relied upon as an early pregnancy marker (less than 20 days of pregnancy). However, suppose the duration of pregnancy increases to 25 days or more. In that case, it is preferable to use and depend on transrectal ultrasonography because the INF-tau assay may lead to the culling or slaughter of several pregnant ewes due to incorrect non-pregnant diagnosis.

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Introduction

Early pregnancy diagnosis is one of the basics to the success of ewe's reproductive management because it reduces the economic losses caused by culling or breeding non-pregnant ewes. Therefore, early identification of non-pregnant ewes gave a chance to rebred the ewes and prevented the extension of lambing intervals (1). Different methods were used to identify pregnancy in small ruminants, including clinical (abdominal palpation and ultrasonography), laboratory methods, hormonal assays (such as progesterone and estradiol sulphate), detection of pregnancy-specific antigens (proteins), and management techniques (non-return to estrus) (2-6). Ultrasonography techniques are considered the best method for pregnancy diagnosis in small ruminants (4,7,8), as this method enables the identification of early embryonic death (9,10) and some fetal disorders (11), as well as the assessment of fetal count, determination of fetal sex, age, and viability of fetuses (12). Utilizing an accurate gestational prediction as early as feasible was the goal of ultrasonography (13). The effectiveness of ultrasonography in diagnosing pregnancy depends on various criteria, including the number of fetuses, gestation age, body condition, operator expertise, and imaging method (14). The ultrasonography for early pregnancy diagnosis is ineffective until 20 to 22 days of pregnancy (when using transrectal real-time ultrasonography with a 7.5-MHz linear probe), while the best results can be

obtained between 25 and 40 days of pregnancy (15). Many studies depended on estimating progesterone concentration in serum as a standard and most commonly performed method for early pregnancy diagnosis in ewes (5,6,16-19). One of the main factors that affected the accuracy of the progesterone assay is the close concentration of progesterone during the dioestrus period before corpus luteum luteolysis in mated non-pregnant ewes compared with pregnant ewes (16). Therefore, assaying progesterone levels on the 20th day of pregnancy was recommended for successfully diagnosing pregnant and non-pregnant ewes (5). Maternal recognition of pregnancy in ruminants, including sheep, occurs through the effect of interferon tau secreted by the ovine embryo's elongating. Interferon tau has an inhibitory effect on the endometrial luteolytic mechanism (20); it is produced by the embryo during the period 10 to 21 days of pregnancy and peaks secretion during the 14 and 16 days of pregnancy (21,22). Therefore, INF tau was used as a marker for early pregnancy diagnosis. Very limited studies estimated the concentration of INF tau as an early pregnancy indicator in cows (23,24) and buffalo (25). However, there was no information about estimating INF-tau level in peripheral blood as a marker for early pregnancy diagnosis in sheep. In this context, we found some studies that used the INF-tau as a marker for early pregnancy in ewes by assessing interferon-stimulated gene expression (26-28).

The present study was designed to assess the INF-tau level in peripheral blood and evaluate its accuracy for early pregnancy diagnosis in Awassi ewes compared to progestogen assay and transrectal ultrasonography.

Materials and methods

Ethical approval

The current study was approved by the Committee of Ethics in the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq (No. P.G./1662 on 11/9/2024).

Animals

The present study was conducted between June and December 2023 in the sheep experimental farm of the Office of Agricultural Researches, Department of Agricultural Researches, Nineveh. Twenty-four mature, healthy, multiparous, dry, and non-pregnant Awassi ewes (aged 2-4 years and having a body weight of 45-55 kg) and six Awassi rams for heat detection and breeding (that were separated from the ewes for six weeks before treatments), were used in this study. The animals were placed in semi-opened shade shelters and provided with a concentrate feed mixture and roughages, and freshwater was available ad libitum.

This study was part of the PhD research project, where the estrus cycle of ewes was synchronized by intravaginal sponges containing 60 mg of medroxyprogesterone acetate/Sponge (ESPONJAVET, Laboratories Hipra, Girona, Spain) for 12 days. Ewes were injected with 500 IU of equine Chorionic Gonadotropin (OVISER, Laboratories Hipra, Girona, Spain) at the time of the sponge removal. At the end of estrus synchronization protocols, the ewes were mated naturally by rams, and the date of mating was considered the zero-day of pregnancy.

Blood collection

After fixation of the zero-day pregnancy of ewes, 5 ml of blood samples were collected from the jugular vein at 15 and 25 days of pregnancy. At room temperature, the collected blood samples were allowed to clot. The serum was separated by centrifugation of blood samples at 3000 rpm for 10 min. Serums were harvested into Eppendorf tubes and stored at -20 °C until laboratory analysis.

Hormonal assay

The serum samples collected at 15 and 25 days of pregnancy were evaluated for INF-tau and progesterone levels at the ELISA laboratory of the College of Veterinary Medicine, University of Mosul. The level of INF-tau was assayed by enzyme immunoassay using a sheep INF-tau ELISA kit (Shanghai Ideal Medical Technology, China) with an analytical assay range of 30 - 1200 ng/L. The progesterone level was assayed by enzyme immunoassay using a sheep progesterone ELISA kit (Shanghai Ideal Medical Technology, China) with an analytical assay range of 1.5 - 48 ng/ml. The data of INF-tau and progesterone that were measured at 15 and 25 days of pregnancy were included in this study (out of 24 ewes, the early pregnancy in 22 ewes was evaluated using INF-tau assay and 18 ewes was evaluated using progesterone assay).

Receiver operating characteristic (ROC) curves were used to estimate the optimal cut-off value for INF-tau and progesterone to assess whether these two hormonal assays could adequately discriminate between pregnant and non-pregnant ewes. The point at which the product of sensitivity and specificity reaches the maximum was regarded as the optimal cut-off value (29).

Ultrasonography

A real-time B-mode portable Ultrasonographic machine (KAIXIN, KX5100, China) was used for early pregnancy diagnosis in ewes. The machine was equipped with a linear array transrectal multi-frequency transducer 5.5 to 7.5 MHz. The ewes were scanned transrectally according to the technique of Hariom (30) for early pregnancy at two periods: 15-20 and 25-30 days of gestation. Observation of an echogenic area of the fetal fluid dilled the uterus was depended upon for pregnancy diagnosis (31). To confirm the pregnancy, ewes were subsequently scanned on day 40 by transabdominal ultrasonography using a 3.5 MHz convex array transducer according to the approach used for sheep and goats (32,33).

Analysis of data

Based on the transabdominal ultrasonography of pregnancy diagnosis on day 40 of pregnancy, the result of pregnancy diagnosis in ewes, which was achieved by transrectal ultrasonography, INF-tau and progesterone assay, were arranged as follows: correct positive diagnosis, incorrect positive diagnosis, correct negative diagnosis, and incorrect negative diagnosis. From these values, the accuracy, sensitivity, specificity, predicted positive value and predicted negative values were calculated (34,35).

Statistical analysis

The Chi-square test was used to compare the results of accuracy, sensitivity, and specificity, as well as predicted positive and negative values. All statistical analysis, including calculating cut-off point values of INF-tau and progesterone, was performed by SPSS software (IBM SPSS Statistics, Version 29.0.2.0). P values equal to or less than 0.05 were considered significant.

Results

The concentration of INF-tau that recorded in this study at 15 and 25 days of pregnancy ranged between 0.698 - 2.301 and 0.940 - 3.078 ng/ml, respectively. While the progesterone concentration ranged between 7.553 - 10.420 and 7.332 - 11.579 ng/ml, respectively.

Figure 1 presents the ROC curves for INF-tau values at 15 and 25 days of gestation. At 15 day of gestation, the area under the ROC curves was 0.802±0.094 (with a 95% Confidence Interval for the area being between 0.618 and 0.986). The cut-off value of 0.948 ng/ml was discriminated between pregnant and non-pregnant ewes. The area under the ROC curves for INF-tau values at 25 day of gestation was 0.766±0.101 (with a 95% Confidence Interval for the area being between 0.567 and 0.964). The cut-off value of INF-tau between pregnant and non-pregnant ewes was 1.199 ng/ml. According to these two cut-off values, 11 ewes were diagnosed as pregnant, and 11 ewes were diagnosed as non-pregnant at 15 and 25 days of gestation (Table 1).

Figure 2 presented the ROC curves for progesterone values at 15 and 25 days of gestation. At 15 day of gestation, the area under the ROC curves was 0.875±0.083 (with a 95% Confidence Interval for the area being between 0.712 and 1.038). The cut-off value of 9.508 ng/ml was discriminated between pregnant and non-pregnant ewes. The area under the ROC curves for progesterone values at 25 day of gestation was 0.889±0.078 (with a 95% Confidence Interval for the area being between 0.736 and 1.042). The cut-off value of progesterone between pregnant and non-pregnant ewes was 9.344 ng/ml. According to these two cut-off values, 15 ewes were diagnosed pregnant, 3 ewes were diagnosed non-pregnant at 15 day of gestation, 10 ewes were diagnosed pregnant, and 8 ewes were diagnosed non-pregnant at 25 day of gestation (Table 1).

The transrectal ultrasonographic evaluation of pregnancy at the period of 15-20 day of gestation revealed that 17 ewes were diagnosed pregnant and 7 ewes were diagnosed non-pregnant, while 16 ewes were diagnosed pregnant and 8 ewes were diagnosed non-pregnant at the period of 25-30 day of gestation (Table 1). The results of pregnancy diagnosis confirmation which was obtained by transabdominal ultrasonography at 40 day of gestation showed that 16 ewes were pregnant and 8 ewes were non-pregnant (Table 1).

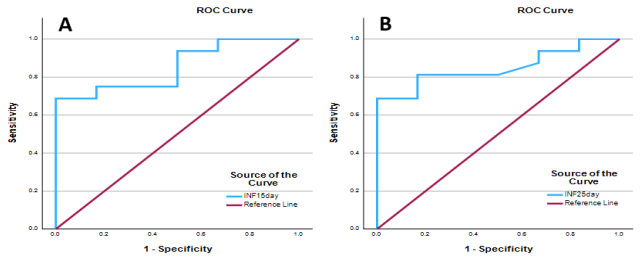

Figure 1: the ROC curves for INF-tau values at 15 (A) and 25 (B) days of gestation in ewes.

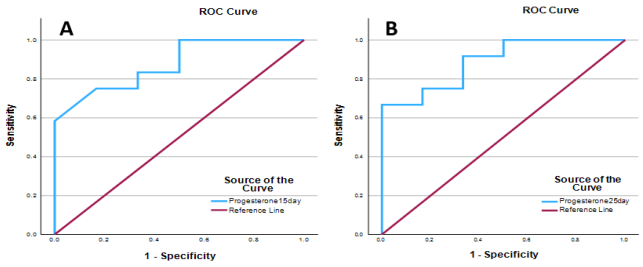

Figure 2: the ROC curves for progesterone values at 15 (A) and 25 (B) days of gestation in ewes.

Table 1: Results of early pregnancy diagnosis in ewes by transrectal ultrasonography, INF-tau and progesterone assay at 15 and 25 days of gestation

	Methods of early pregnancy diagnosis						Confirmation transabdominal ultrasonography
	IFN-tau		Progesterone		Ultrasonography		
Day of gestation	15	25	15	25	15	25	40
Pregnant	11	11	15	8	17	16	16
Non-pregnant	11	11	3	10	7	8	8
Total	22	22	18	18	24	24	24

Table 2 summarizes the accuracy, sensitivity, specificity, predicted positive value, and predicted negative value of transrectal ultrasonography, INF-tau, and progesterone assay, which were calculated according to the pregnancy diagnosis confirmation which was obtained by transabdominal ultrasonography at 40 day of gestation.

At 15 and 25 days of gestation, the same results were obtained by INF-tau assay for early pregnancy diagnosis, in which 11 ewes were correctly pregnant diagnosed, and no ewes were incorrectly pregnant diagnosed. Six ewes were correctly diagnosed as non-pregnant, and only five ewes were incorrectly non-pregnant diagnosed. The accuracy, sensitivity, specificity, positive and negative predictive values of the INF-tau assay were 77.27, 68.75, 100, 100 and 54.55%, respectively (Table 2).

The results of the progesterone assay for early pregnancy diagnosis at 15 day of gestation showed that 12 ewes were correctly pregnant diagnosed, and only three ewes were incorrectly pregnant diagnosed. Three ewes were correctly diagnosed as non-pregnant, and none were incorrectly diagnosed as non-pregnant. Different results were obtained at 25 day of gestation; eight ewes were correctly pregnant diagnosed, no ewes were incorrectly pregnant diagnosed, six ewes were correctly non-pregnant diagnosed, and four ewes were incorrectly diagnosed as non-pregnant. The accuracy, sensitivity, specificity, and positive and negative predictive values of progesterone assay at 15 and 25 days of gestation were 83.33, 100, 50, 80, 100%, and 77.78, 66.67, 100, 100, 60%, respectively (Table 2).

Different results were obtained at 15 and 25 days of gestation by transrectal ultrasonography; at 15 day of gestation, twelve ewes were correctly pregnant diagnosed, five ewes were incorrectly pregnant diagnosed, three ewes were correctly non-pregnant diagnosed, and four ewes were incorrectly non-pregnant diagnosed. At 25 day of gestation, the results showed that fourteen ewes were correctly pregnant diagnosed, only two ewes were incorrectly pregnant diagnosed, six ewes were correctly non-pregnant diagnosed, and two ewes were incorrectly diagnosed as non-pregnant. The accuracy, sensitivity, specificity, positive and negative predictive values of transrectal ultrasonography at 15 and 25 days of gestation were 62.5, 75, 37.5, 70.6, 42.9%, and 83.3, 87.5, 75, 87.5, 75%, respectively (Table 2).

Table 2: Results of early pregnancy diagnosis in ewes by transrectal ultrasonography, INF-tau and progesterone assay at 15 and 25 days of gestation

Values	Methods of early pregnancy diagnosis					
	IFN-tau assay		Progesterone assay		Transrectal ultrasonography	
	15 day	25 day	15 day	25 day	15 day	25 day
Total number of ewes	22	22	18	18	24	24
Ewes correct pregnant diagnosed (A)	11	11	12	8	12	14
Ewes incorrect pregnant diagnosed (B)	0	0	3	0	5	2
Ewes correct non-pregnant diagnosed (C)	6	6	3	6	3	6
Ewes incorrect non-pregnant diagnosed (D)	5	5	0	4	4	2
Accuracy (A+C)/(A+B+C+D)*100 (%)	77.3 a	77.3 a	83.3 a	77.8 a	62.5 b*	83.3 a
Sensitivity A/(A+D)*100 (%)	68.8 a	68.8 a	100 b*	66.7 a	75 a*	87.5 b
Specificity C/(B+C)*100 (%)	100 a	100 a	50 b*	100 a	37.5 b*	75 b
Positive predictive value A/(A+B)*100 (%)	100 a	100 a	80 b*	100 a	70.6 b*	87.5 b
Negative predictive value C/(C+D)*100 (%)	54.6 a	54.6 a	100 b*	60 a	42.9 a*	75 b

a,b different red small letters refer to a significant variation between the values that obtained at 15 day of pregnancy. a,b different blue small letters refer to a significant variation between the values that obtained at 25 day of pregnancy. * Refers to a significant variation between the values of each method that obtained at 15 and 25 day of pregnancy.

When comparing the results of the interferon tau assay at 15 day of pregnancy, the interferon assay had a very high positive predictive value compared to the progestogen assay and transrectal ultrasonography (100 vs. 80 and 70.6%, respectively), as there were no ewes incorrect pregnant diagnosed using the INF-tau assay. However, the result was inversed in the negative predictive value of the INF-tau assay as it was very little compared to its value in the progestogen assay (54.6 vs. 100%), and this result was obtained because five ewes were incorrectly non-pregnant diagnosed. While there was no significant difference in the negative predictive values between INF-tau assay and transrectal ultrasonography (42.9 vs. 100%).

At 25 day of pregnancy, both positive and negative predictive values were identical to their values at 15 day of pregnancy. However, the positive and negative predictive values were varied in progestogen assay and transrectal ultrasonography. When comparing the results, we found that the positive predictive value was identical in the INF-tau assay with the progestogen assay; both were 100%, while the positive predictive value of the transrectal ultrasonography was lower (87.5%). There was also no significant difference

in the negative predictive value between the INF-tau and progestogen assays (54.6 vs. 60%). In comparison, the negative predictive value of the transrectal ultrasonography was significantly higher (75%) than its value in INF-tau and progestogen assay.

At 15 days of pregnancy, the study's results showed that the accuracy of the INF-tau assay was significantly higher than that of transrectal ultrasonography (77.3 vs. 62.5%). However, there was no significant difference in the accuracy value between INF-tau and progestogen assay (77.3 vs. 83.3%). At 25 day of pregnancy, there was no significant difference between the accuracy of the INF-tau assay and the accuracy of the progestogen assay and transrectal ultrasonography (77.3 vs. 77.8 and 83.3%).

A significant ($P<0.001$) correlation ($r = 1.00$) was observed between the accuracy, sensitivity, specificity, positive and negative predictive values of the INF-tau assay that were recorded at 15 day of pregnancy and the same values which were recorded at 25 day of pregnancy. Also, a significant ($P<0.001$) correlation ($r = 0.98$) was recorded between the values of the transrectal ultrasonography that were calculated at 15 and 25 days of pregnancy. However, there was no significant correlation between the values of progesterone assay, which were obtained at 15 and 25 days of pregnancy. Comparing the results of accuracy, sensitivity, specificity, positive and negative predictive values of the three methods of early pregnancy diagnosis used in this study showed only one significant ($P<0.001$) correlation ($r = 0.99$), it was between the values of INF-tau and progesterone assay which were obtained at 25 days of pregnancy.

Discussion

In this study, progestogen assay and transrectal ultrasonography were used as two standard methods for early pregnancy diagnosis in sheep, which have been relied on in many published studies. We can compare and evaluate the results of the interferon-tau assay for early pregnancy diagnosis in ewes.

Evaluation of interferon-tau concentration in peripheral blood was used for early pregnancy diagnosis in cows (23,24) and buffalo (25). According to our knowledge and internet search, there is no publication assessing the level of INF-tau in peripheral blood for pregnancy diagnosis in sheep. However, many studies evaluated the INF-tau gene expression as evidence of early pregnancy diagnosis in sheep (26-28) and cows (36).

This study calculated the cut-off value of INF-tau between pregnant and non-pregnant ewes using the ROC curve. According to the results of transabdominal confirmation ultrasonography and the cut-off value of INF-tau, the accuracy, sensitivity, and specificity of the INF-tau assay for early pregnancy diagnosis in ewes were achieved.

A good accuracy value of the INF-tau assay was recorded in this study (77.3%). However, it is somewhat lower than the accuracy value of the INF-tau assay (87.5%) recorded by Amri et al. in cows. At the same time, the accuracy of the INF-tau assay in our study was considered higher than the accuracy (66.7%) recorded by Panjaita et al. in cows. Results of our study indicated that INF-tau assay is a more efficient method for diagnosing non-pregnant ewes than pregnant ewes. The specificity of the INF-tau assay was 100% (this value reflects the correctness of the non-pregnant ewe diagnosis). This result was obtained because the non-pregnant ewes have no embryo to produce the INF-tau, which can be detected in the peripheral blood (37); therefore, no ewes were incorrectly diagnosed, so we got a specificity value of 100%. In cows, a similar specificity value (100%) of INF-tau was recorded at 15 and 16 day of pregnancy (23). Contrary to the results of the specificity of the INF-tau assay, we recorded incorrect non-pregnant diagnoses of five out of 16 pregnant ewes; therefore the sensitivity of the INF-tau assay was 68.8% (this value reflects the correctness of the pregnant ewe diagnosis), the reason for obtaining this result may be due to the high cut-off value of INF-tau that adopted in this study, which led to diagnosis of some pregnant ewes as non-pregnant ewes. A similar value but slightly higher (80%) for the sensitivity of INF-tau assay was observed in cows at 15 and 16 days of pregnancy (23).

The study's results also showed that the parameters of INF-tau assay at 15 and 25 days of gestation were identical. This is due to the same reason mentioned above since the non-pregnant ewes have no embryo to produce the INF-tau, which can be detected in the peripheral blood (37). In addition, the concentration of INF-tau in the pregnant ewes is at a high level between 15 and 25 days of gestation and then decreases to a low level (22).

As expected and recorded in previous studies (4,5,18,38), our study found at the age of 15 ay of gestation an accuracy of 83% for the progestogen assay with high sensitivity (100%) since all pregnant ewes were correctly pregnant diagnosed and at the same time there were no pregnant ewes diagnosed as non-pregnant (incorrect non-pregnant). However, the specificity of this assay was weak (50%) due to the incorrect diagnosis of three out of six ewes as pregnant while they were non-pregnant. These results were obtained because the corpus luteum remains active at this period in pregnant and non-pregnant ewes. Therefore, the level of progestogen in non-pregnant ewes appears equivalent to its level in pregnant ewes (39). The values of the parameters were changed at the age of 25 days of pregnancy, the accuracy was decreased to 77%, and the sensitivity was decreased to 66%, while the specificity was increased to 100% since all non-pregnant ewes were diagnosed as non-pregnant and there were no non-pregnant ewes were diagnosed as pregnant. This result was observed because the level of progestogen was decreased and returned to its low level in non-pregnant ewes due to corpus luteum regression (16,39).

Transrectal and transabdominal ultrasonography have a high accuracy for pregnancy diagnosis in sheep; the ultrasonographic diagnosis depends on detecting and observing an embryonic vesicle that appears as an echogenic area inside the uterine horns. In our transrectal ultrasonography results, we found that the sensitivity values at 15 day of gestation were lower significantly than those recorded at 25 day of gestation since many ewes were true pregnant diagnosed, while few ewes (4 ewes at the age of 15 days and 2 ewes at the age of 25 days) were false non-pregnant diagnosed. On the contrary, there was a highly significant difference between the values of transrectal ultrasonography specificity recorded at 15 and 25 days of gestation; the value of specificity at 15 days of pregnancy was low, 37.5% (five out of eight ewes were false pregnant diagnosed), but this result was reflected at the age of 25 days of pregnancy, the value of specificity was increased to 75% (only two out of eight ewes were false pregnant diagnosed). As a result of the high sensitivity and specificity of transrectal ultrasonography at 25 day of gestation, the accuracy also increased significantly from 62.5% at 15 day of pregnancy to 83.3% at 25 day of pregnancy. These results confirmed the results of previous studies, which found that the accuracy of ultrasonographic pregnancy diagnosis increases with the advance of pregnancy because the embryonic vesicle becomes easier to identify by progress of the pregnancy (15,31,40-41).

Through the correlation values between the parameters (accuracy, sensitivity, specificity, positive and negative predictive values) of the early pregnancy diagnosis methods obtained in this study at 15 and 25 days of pregnancy, the INF-tau assay parameters were 100% identical, transrectal ultrasonography parameters were 98% identical, while no conformity was recorded between the parameters of progestogen assay. This may be because the INF-tau assay and transrectal ultrasonography are dependent on the presence of the fetus to give a positive result. In contrast, the progestogen assay depended on the presence of the corpus luteum, which led to obtaining a false positive result at 15 days of pregnancy due to the delayed regression of the corpus luteum (39). Also, through the correlation values, we found only a 99% conformity between the INF-tau parameters and progestogen assay achieved at 25 day of pregnancy. These results can be explained by the high level of INF-tau and progestogen in the peripheral blood, which cannot occur after 25 days of mating without pregnancy (16,22). A similar close association was recorded between IFN-tau and progesterone assay in buffaloes at 28 day of pregnancy (25).

Conclusion

The study's results conclude that the INF-tau assay is accurate and can be relied upon as an early pregnancy marker (less than 20 day of pregnancy). However, suppose the duration of pregnancy increases to 25 day or more. In that case, it is preferable to use and depend on transrectal ultrasonography because the INF-tau assay may lead to culling or slaughtering several pregnant ewes due to incorrect non-pregnant diagnosis.

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Conflict of interest

The manuscript has no conflict of interest.

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دقة قياس الإنترفيرون تاو للتشخيص المبكر للحمل في النعاج العواسي مقارنة بقياس البروجستيرون والتصوير بالأمواج فوق الصوتية عبر المستقيم

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الخلاصة

صممت هذه الدراسة لتقييم دقة قياس إنترفيرون تاو للتشخيص المبكر للحمل في النعاج العواسي مقارنة بقياس البروجستيرون والتصوير بالأمواج فوق الصوتية عبر المستقيم. استخدمت ٢٤ نعجة عواسية غير حامل في هذه الدراسة. تم تقييم التشخيص المبكر للحمل في ٢٢ نعجة باستخدام قياس إنترفيرون تاو، وتم تقييم ١٨ نعجة باستخدام فحص البروجسترون، وتم تقييم ٢٤ نعجة باستخدام التصوير بالأمواج فوق الصوتية عبر المستقيم. تم حساب الدقة والحساسية والخصوصية والقيم الإيجابية والسلبية المتوقعة لكل طريقة وفقا لنتائج التصوير بالموجات فوق الصوتية عبر جدار البطن في اليوم ٤٠ من الحمل. بعمر ١٥ و ٢٥ يوما من الحمل، كانت قيم الدقة والحساسية والخصوصية والتنبؤ الإيجابي والسلبي لقياس إنترفيرون تاو ٧٧,٢٧ و ٦٨,٧٥ و ١٠٠ و ١٠٠ و ٥٤,٥٥٪ على التوالي. لوحظ وجود علاقة كبيرة بين قيم قياس إنترفيرون تاو التي سجلت في ١٥ و ٢٥ يوما من الحمل. كان هناك ارتباط كبير بين قيم قياس إنترفيرون تاو وقيم قياس البروجسترون التي تم الحصول عليها في عمر ٢٥ يوما من الحمل. استنتج من نتائج الدراسة أن قياس إنترفيرون تاو له دقة جيدة يمكن الاعتماد عليها في التشخيص المبكر للحمل في النعاج (أقل من ٢٠ يوما من الحمل). أما إذا زادت مدة الحمل إلى ٢٥ يوما أو أكثر، فمن الأفضل الاعتماد على التصوير بالأمواج فوق الصوتية عبر المستقيم، لأن قياس إنترفيرون تاو قد يؤدي إلى استبعاد أو ذبح عدد من النعاج الحوامل بسبب التشخيص الخاطئ لها على أنها غير حوامل.