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*Article*

# Comparison of the three methods for pregnancy detection in Iraqi Sheep

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**ABSTRACT**

Twenty Iraqi ewes aged between 2-3 years were used in the current study. All animals were raised in animal field belong to the Fallujah in the Saqlawiyah area during the period between August 2022 to February 2023. It was fed naturally and on one diet and T1 Control, T2 was given hCG- COX 100 I.U./ ewe injection, T3 was given Biotin 10 mg/ ewe/ daily oral and T4 was given Biotin 10 mg/ ewe/ daily oral + hCG- COX 100 I.U./ ewe injection. Blood samples for progesterone and Sheep Pregnancy Associated Plasma Protein B (PAPPB) measurement were collected on Days 48 and 79 after mating. The results of the study showed the accuracy when diagnosing pregnancy by the PAPPB method. Where it was 100% during the 48th and 79th day of pregnancy. Followed by the method of diagnosis by ultrasonography. Hence the progesterone assay method. While specificity was superior in the PAPPB method. The accuracy of pregnancy diagnosis between PAPPB and ultrasonography on day 48 of pregnancy was equal. While there were no results during the diagnosis by means of progesterone assay. In conclusion, the pregnancy diagnosis by PAPPB is considered one of the most accurate and modern methods.

**Keywords:** Pregnancy detection; progesterone; ultrasonography; PAPPB; Iraqi Sheep.

## INTRODUCTION

Livestock contributes a large percentage to the agricultural sector, around 50% most of the world's countries and this wealth is important in the national income of many countries of the world, and livestock is meant to be raised cattle, sheep, goats, buffaloes, camels, horses, poultry and fish. With regard to sheep, they have an important role in the economies of many countries of the world, past and present, and are mainly raised for the production of meat, wool, milk, and leather[3]. Early diagnosis of pregnancy in sheep has an important role in the economy of countries to reduce increased expenses on the herd and exclude sick animals [4]. The method of diagnosing progesterone is one of the primary methods for diagnosing pregnancy in animals, including sheep, as the high concentration of the hormone in the blood or milk is considered an indication of the presence of pregnancy due to the presence of the corpus luteum. But sometimes the diagnosis cannot be considered correct in this way due to early embryonic death and the survival of the corpus luteum in the secretion of the progesterone. Therefore, the diagnosis of pregnancy by ultrasonography method was very important in detecting pregnant females on the 21$^{st}$ day of pregnancy, but this method requires an experienced person and a high cost. The PAPPB method is a modern and accurate method for detecting pregnancy in sheep at an early stage of pregnancy through placenta formation. Early detection of pregnancy works to reduce the expenses, costs and effort of the farmer, while excluding sick animals, and this increases the productivity and fertility of the herd[1,2,11,15,16,17,20]. The aim of this field study was to compare accuracy of progesterone, ultrasonography and PAPPB test for early pregnancy detection in Iraqi Sheep.

## MATERIALS AND METHODS

Twenty Iraqi ewes aged between 2-3 years with body weight between 34-48 kg were used in the current study. All animals were raised in animal field belong to the Fallujah in the Saqlawiyah area during the period between August 2022 to February 2023. The animals were isolated for 30 days before the study began and were examined by the ultrasonography apparatus to ensure that it was not pregnant. The animals were divided randomly into 4 equal groups. It was fed naturally and on one diet and the first group ($T_1$) Control, the second group ($T_2$) was given hCG- COX 100 I.U./ ewe injection, the third group ($T_3$) was given Biotin 10 mg/ ewe/ daily oral (30 days) and the fourth group ($T_4$) was given Biotin 10 mg/ ewe/ daily oral (30 days) + hCG- COX 100 I.U./ ewe injection. Estrous synchronization are unified by placing vaginal sponges for a period of 13 days.
Blood samples for progesterone and Sheep Pregnancy Associated Plasma Protein B (PAPPB) measurement were collected on Days 48 and 79 after mating. The blood was centrifuged at 4000 rpm for 15 minutes. The plasma was collected in sterilized plastic tubes and kept at -20°C. Progesterone concentrations were measured by COBAS (E-411). The kit provided by Roche, Inc., Switzerland, and Sheep Pregnancy Associated Plasma Protein B (PAPPB) concentrations were measured by using ELISA technology. The kit provided by Sunlong Biotech Co., Ltd, China. The indices were determined as: 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.

## RESULTS

Between days 48 PM (16 ewes) and 79 PM (20 ewes), were declared pregnant using the progesterone assay. The sensitivity of progesterone assay for detecting pregnant ewes increased from 76.47 to 100% during the experimental period. The sensitivity of progesterone assay for detecting pregnant ewes was decreased accurate (65%) at day 48 PM and increased accurate (85%) during the 79 PM. The specificity of the progesterone test in detecting non-pregnant ewes on days 48 and 79 was equal (0%). Similar trend was observed for positive predictive values which were decreased progressively from 81.25% at day 48 PM, at a time of increased again at day 79 PM to 85%. In the contrary, negative predictive value of this test for detection non-pregnant females was equal at day 48 PM and day 79 PM. Better results were obtained for detecting pregnant and non-pregnant ewes using ultrasonography technique Fig (1). The sensitivity for detecting pregnant ewe was highly (94.12%) at day 79 PM and moderately accurate (76.47%) at day 48 PM Fig (2). The specificity (%) for detecting non- pregnant ewe obviously from 66.67% at day 79 PM Fig (3), and 100% during day 48 PM. The positive predictive value (%) for detecting the pregnant ewe was increased (100%) at day 48 PM and lower at day 79 PM (94.12%). In contrast, negative predicting values were lower (42.86%) at day 48 PM and higher (66.12%) at day 79 PM. Accuracy were decreased (80%) at day 48 PM and increased (90%) at day 79 PM. The results of the current study showed the compatibility between accuracy, specificity, sensitivity, predictive value of positive and negative test during the test periods days 48 and 79 using the PAPPB method (100%) (Table 1).

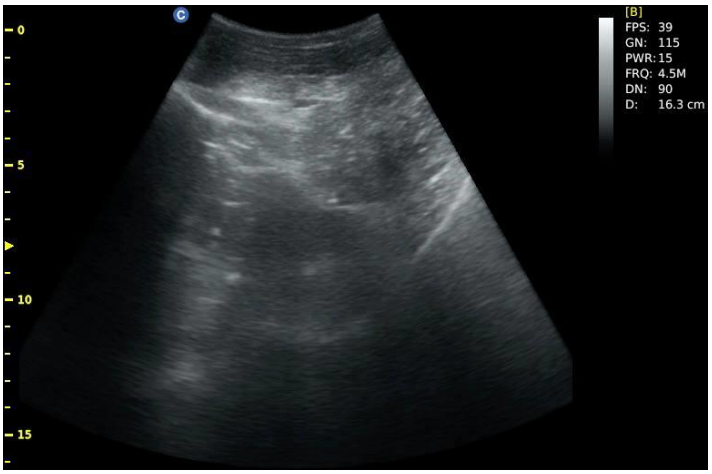


**Figure 1**. Ultrasonogram of the ewe at before treatment

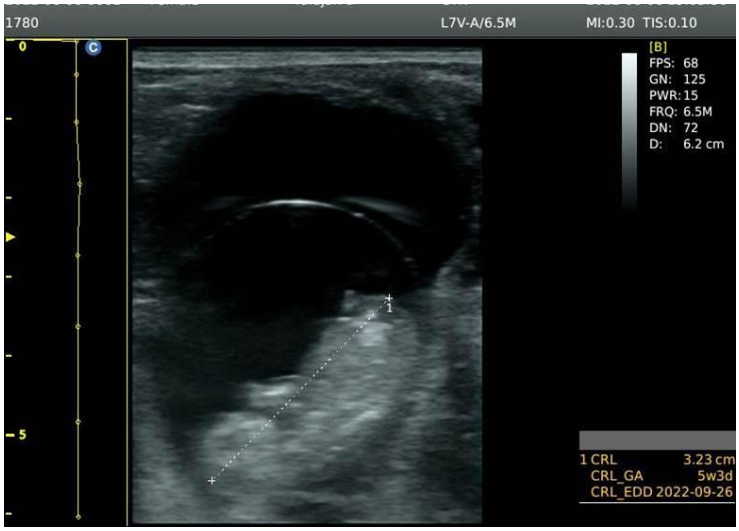


**Figure 2.** Ultrasonogram of the ewes at day 48 PM


**Figure 3.** Ultrasonogram of the ewes at day 79 PM

| Evaluation / Days post-mating | Progesterone assay | | Ultrasonography | | PAPPB | |
|---|---|---|---|---|---|---|
| | **48** | **79** | **48** | **79** | **48** | **79** |
| **Predictive Value of Positive Test** | 81.25% | 85% | 100% | 94.12% | 100% | 100% |
| **Predictive Value of Negative Test** | 0 | 0 | 42.86% | 66.67% | 100% | 100% |
| **Sensitivity (%)** | 76.47% | 100% | 76.47% | 94.12%% | 100% | 100% |
| **Specificity (%)** | 0 | 0 | 100% | 66.67% | 100% | 100% |
| **Accuracy** | 65% | 85% | 80% | 90% | 100% | 100% |

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) x100

**Table 1.** Early pregnancy detection of ewes using three different methods

## DISCUSSION

The plasma progesterone assay is often the method of choice for early detection of pregnancy. This method is accurate and can be done for the second week of pregnancy[15].
Karen et al.[11] found that sensitivity (100%), specificity (95.4%), positive (81.6) and negative (100%) predictive values for progesterone at day 18 after AI.
Ganaie et al.[7] noted that accuracy of detecting pregnancy 100 % and non-pregnancy 83.3% was same for days 15-30 and 31-45 of pregnancy, And the accuracy of 98%, prediction values of 97.7 and 100% for positive and negative detection, at days 18.23±0. 78. The researcher also showed that the determination of progesterone hormone by ELISA on the 18th day after fertilization is considered one of the reliable methods for diagnosing pregnancy in Corriedale ewes.
The difference in results may be due to the difference in the sensitivity of the progesterone test. Affects: irregularity of the estrous cycle, early embryonic deaths, ovarian and ovarian pathological conditions like hydrometra, pyometra and luteal cysts on the result of hormone concentration[8].
The results of the current study did not agree with[1] as it was found that sensitivity (95.2%), specificity (100%), positive predictive value (100%), negative predictive value (80%) and accuracy (96%) and [19], noted that sensitivity 91.30%, specificity100%, positive predictive value 100%, negative predictive value ration 83.3% for pregnancy examination findings by transabdominal ultrasonography on days 34- 35.
Ultrasonography is used to detection pregnancy in a variety of domestic species, such as ewes. The use of ultrasonography has proven effective for the examination of ovarian follicles, corpora lutea and pregnancy. Genetic factors and nutrition affect the process of diagnosing pregnancy in ewes by ultrasound, and the gestational age is determined by the placenta and its development[6].
Studies have shown that optimum time for transabdominal or transrectal ultrasonography in ewes ranges from 12- 23 days PM may correctly predict approximately 85% of the pregnant sheep, and by 40 days using external trans-abdominal. The sex is determined between 60-69 days, as the accuracy was (100%) for male but only (76%) for females fetuses. The number of foetuses can be counted accurately from about 45- 100 days of gestation. So detection is normally undertaken between the 12- 13 weeks after insemination[5, 14]. The diagnosis was made on the day 16 the accuracy was 50%, whereas at day 23 (72.73%) and at day 30 (90.91%)[12].
Age affects the sensitivity of the test by ultrasonography, as it increased in young ewes and decreased in older ewes on the 40th day of pregnancy, In addition, the examination method, whether external or internal, affects the accuracy of the ultrasonography result[17,18].
Embryo development can be divided into three periods: First period initial attachment of the blastocyst, second period, The embryonic period extends from day 12 to day 34 in the ewes. In this period, growth and differentiation occur, during which the major tissues, systems and organs are established and the major features of external body form. Third period, fetal period extends from about day 34 of gestation in ewes, until birth. Growth and changes in the form of the fetus characterize this period. Species, breed, and strain differences in fetal size are due to differences in the rate of cell division, which is determined genetically. Thus, there is close integration between the feed supply to the fetus (environmental factors), the rate of cell division (genetic factors), and hence, the rate of growth[9].
The current study agreed with the study of [13] where it was shown that pregnancy can be diagnosed by PAPPB method on the 45th day of pregnancy in ewes.
PAPPB are significantly higher in sheep carrying twin than in those carrying singles, because the PAPPB are secreted by binucleate cells which represent 15–20% of fetal placental cells, and the total placental masses was higher areas of contact between the fetal placenta and maternal caruncles than those in sheep carrying singles[10].

## CONCLUSIONS

In conclusion, the pregnancy diagnosis by PAPPB is considered one of the most accurate and modern methods, followed by ultrasonography and progesterone assay. Pregnancy can be clearly and accurately diagnosed by ultrasound on the 48$^{th}$ day of pregnancy in sheep.

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