



## Diagnosis of Pregnancy in Iraqi Awassi Ewes Through Progesterone Hormone Measurement and Ultrasonography Following Induction of Fertile Estrus with Sulpiride



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**T**HE aim of this study was to diagnose pregnancy in Iraqi Awassi ewes by assessing progesterone (P4) levels and using ultrasonography after inducing fertile estrus during the anestrus season with Sulpiride, a dopamine antagonist for improvement of reproductive efficiency. This study was conducted from December 2022 to June 2023, with ewes randomly divided into two groups, each consisting of 6 ewes. The treatment group received a dose of 0.6mg/kg of Sulpiride, while the control group received normal saline. The results showed a significant ( $P < 0.05$ ) estrus response in the treated group. P4 concentrations were significantly higher ( $P < 0.05$ ) in the treatment group on days 14, 21, and 28. However, the results of ultrasonography for pregnancy detection were non-significant in both the treatment and control groups at 30%, 60%, and 75% on days 30, 60, and 75, respectively, due to a high rate of early embryonic mortality. In conclusion, the use of Sulpiride proved to be a successful method for inducing fertile estrus in ewes, serving as a non-hormonal intervention during the seasonally anestrus period. The Seasonal anestrus is considered one of the main causes important problems in sheep, which causes Poor reproductive efficiency. P4 hormone measurement and ultrasonography were found to be rapid, safe, and reliable methods for diagnosing early pregnancy for improvement of reproductive efficiency in Iraqi Awassi ewes.

**Keywords:** Induction, Fertile estrus, Progesterone, Sulpiride, Ultrasonography.

### Introduction

Awassi is dominant breed in Iraq 60% of ewes, it is at most raised for the consumption from human as milk, meat and wool [1]. Awassi breed, having good reproductive performance, conception rate and litter size [2, 3]. Iraqi Awassi ewes considered as seasonal ewes, Iraqi researchers approved 40% seasonality of Iraqi Awassi ewes [4, 5]. The seasonal anestrus is considered one of the main causes important problems in sheep, which causes poor reproductive

efficiency [6]. The management methods in ruminants as estrous synchronization is successfully to enhance reproductive efficiency [7]. The sulpiride (dopamine antagonists) used as pharmacological treatments in veterinary medicine for the estrus synchronization in animals such as melatonin, prostaglandins and P4. The sulpiride is may be substitute correct alternative to the current medication used for the synchronization [8, 9]. Sulpirid, has been successfully used for induction of oestrus in sheep [10, 11]. The studies explained, administration of

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dopamine in axons synapsing on GnRH terminals in the median eminence act inhibits GnRH release [12]. The P4 assay is a very valuable method for pregnancy determining in early, mid and late gestational stages [13, 14]. The ultrasonography is method rapid, safe and non-invasive used for pregnancy diagnosis in sheep [15, 16]. The obstetric ultrasound has been widely used in small ruminants, administered transrectally or transabdominal for diagnosing pregnancy, fetal viability, sexing, and quantifying fetus numbers [15, 17, 18].

The aim of this study, pregnancy diagnosis in Iraqi Awassi ewes by measurement of P4 hormone and ultrasonography after induction of fertile estrus during anestrus season by sulpiride (dopamine antagonist).

### **Material and Methods**

#### *Experimental animals:*

This study involved 12 Iraqi Awassi ewes within the anestrus season, spanning from December 2022 to June 2023, conducted at the Animal Field of the College of Veterinary Medicine, University of Baghdad. The ewes had an average age ranging from 2.5 to 4 years and weighed between 25 and 33 kg.

Heat detection was two time a day by two fertile male on the ewes and measurement of P4 concentration to assure estrus. The P4 concentration in ewes of less than (1ng/mL) were anestrus ewes, also used transabdominal and transrectal ultrasonography (Chison ECO2/ China) for pregnancy diagnosis in Awassi ewes.

#### *Experimental procedure:*

Seasonally anestrus, 12 Iraqi Awassi ewes were divided to two groups (6 ewes for each group): the first group, take the sulpiride (Solarbio Life Science, China) at the dose of (0.6 mg/kg) subcutaneously in back side of the neck, two time a day (7 AM and 5 PM) till the occur of estrus, whereas the second group, take of normal saline. Sulpiride was diluted in 1ml (DMSO) and continue to 25 ml by normal saline daily. Blood samples were collected before treatment and weekly for month on Days 7,14, 21, and 28 after estrus. Heat detection ewes was done two time a day (3 hours morning and 3 hours evening) using two male. the estrus signs in ewes was such as ram-seeking behavior, urination and stands to be mated.

#### *Assessment of progesterone assay:*

The blood was collected via vena puncture and evacuated into Gel tubes on days 0 before sulpiride

treatment and weekly for month after treatment to determine P4 concentration. Serum was collected after centrifugation with 3000 rpm for 10 minutes and stored by Eppendorf tube at -20°C until assays of hormonal concentrations. P4 kit (RIA P4, direct)®. Immunotech, A Beckman Coulter Company (Czech Republic).

#### *Ultrasonography*

##### *B-mode Ultrasonography*

All experimental ewes were examined by Ultrasonography in pre Insemination and Post Insemination. The examination Procedure was by used ultrasonography B-mode diagnostic approach. The examination technique chosen depends on the pregnancy stage. Ultrasonography tool equipped with a linear probe 6.5MHz designed for applying transrectally in 30 days Post Insemination and (convex probe) 4.5 MHz designed for applying transabdominal in 60 and 75 days. Ultrasonography examination was including gestational sac, embryo, fetal head, vertebral column, extremities, thoracic, trunk and placentome. The transabdominal examination, animals are inspected while standing, starting at the fleece less inguinal area caudo-ventral to the udder, and moving the transabdominal probe towards as gestation progresses. The gel was placed on the tip of the transducer and the examinations were performed on longitudinal and transverse sections to identify the pregnancy details. The ewe was particular pregnant by imaging apparent conceptus (elongated structure, anechoic) within a uterine fluid.

#### *Statistical analysis*

The analysis of data was by using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were complete to assess significant differences among means.  $P < 0.05$  is considered statistically significant [19].

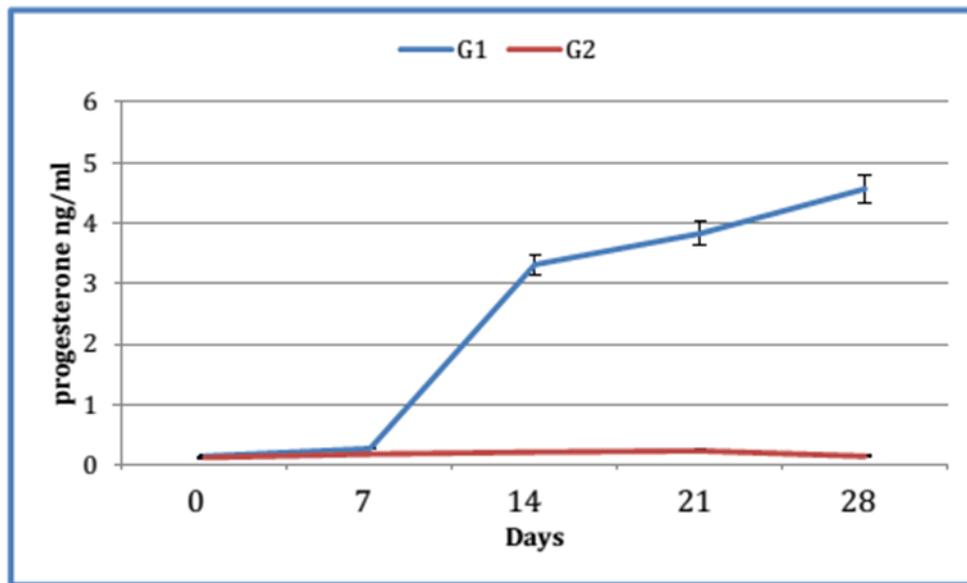
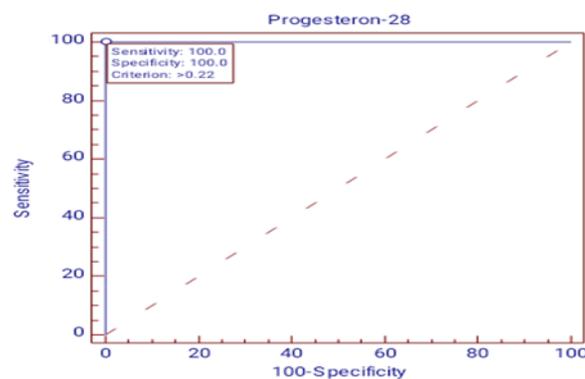
### **Results and Discussion**

Progesterone was measurement in the first groups table (1) and figure (1 and 2), the P4 level was a significant higher ( $P < 0.05$ ) on Day 14, 21, 28 compared with Day 0, 7 and also with second group.

The determination of fetal at an early stage of gestation was effectively accomplished using ultrasonography. The detection was made at 30, 60 and 75 days post-conception, during which five, two and one respectively out of six pregnant ewes were correctly diagnosed, table (2) and figure (3, 4 and 5).

**TABLE 1.** The mean levels ( $\pm$ SE) of P4 concentrations (ng/ml) in pregnant/non pregnant ewes at different gestational periods.

Days	0	7	14	21	28
<b>Groups</b>					
G1	0.15 $\pm$ 0.05 <sup>Ca</sup>	0.28 $\pm$ 0.05 <sup>Ca</sup>	3.32 $\pm$ 0.37 <sup>Ba</sup>	3.83 $\pm$ 0.33 <sup>Ba</sup>	4.57 $\pm$ 0.38 <sup>Aa</sup>
G2	0.13 $\pm$ 0.03 <sup>Aa</sup>	0.19 $\pm$ 0.08 <sup>Aa</sup>	0.22 $\pm$ 0.08 <sup>Ab</sup>	0.24 $\pm$ 0.08 <sup>Ab</sup>	0.15 $\pm$ 0.02 <sup>Ab</sup>
LSD	0.59				

**Fig. 1.** Show a significant higher in the (P4) level ( $P < 0.05$ ) on Day 14, 21, 28 compared with Day 0, 7 in the first group and also with second group.**Fig. 2.** Receiver operating characteristic (ROC) curves for Progesterone between first group and second group on days 0, 7, 14, 21, 28. The typical test has a ROC curve that is fully deflected to the left, and achieved 100% for both Sensitivity and Specificity.

**TABLE 2.** The diagnosis pregnancy by ultrasonography in Iraqi Awassi ewes, during different time after insemination.

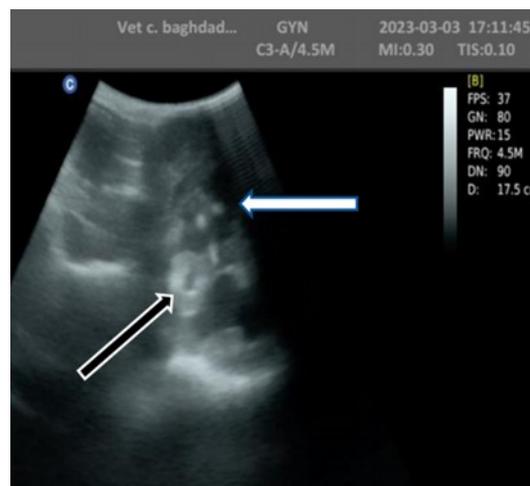
Days	No	0	30	60	75
<b>Groups</b>					
G1	6	0	5(83.3%)	2(33.3%)	1(16.7%)
G2	6	0	0(0.0%)	0(0.0%)	0(0.0%)
P-value		0.00	<0.01	0.12 NS	0.29 NS

**TABLE 3.** Incidence rate of early embryonic mortality using ultrasonography during different period in ewes after estrus induction by sulpiride 0.6 mg/kg.

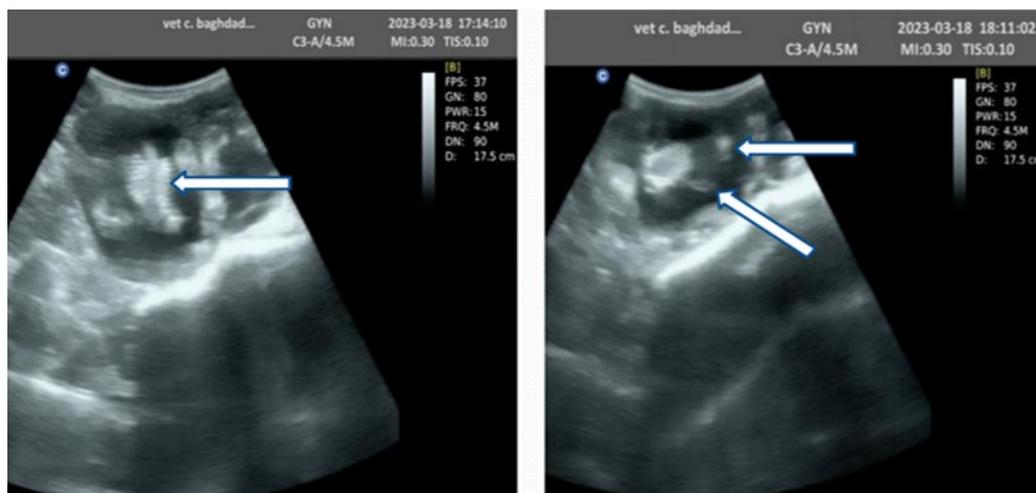
Days	No	0	30	60	75
<b>Groups</b>					
G1	6	0	1(16.7%)	2(33.3%)	5(83.3%)
G2	6	0	0(0.0%)	0(0.0%)	0(0.0%)
P-value		0.00	<0.01	0.12 NS	0.29 NS



**Fig.3.** The Ultrasonography image of day 30 after mated by using Transrectal probe (6.5MHz). The white arrow indicate to embryonic mass in pregnant uterus of ewe.



**Fig.4.** The Ultrasonography image of day 60 after mated by using Transabdominal probe (4.5MHz). The white arrow indicate to fetal fluid and black to placentome in pregnant ewe.



**Fig.5. Transabdominal (4.5MHz) Ultrasonography image of ewe pregnant uterus in day 75 after mated, white arrows indicate to fetal head, legs and rib.**

The concentration of P4 was significant differences ( $P < 0.05$ ) between treatment and control group on days 14, 21 and 28 respectively. This study agreement with Rahman *et al.* [20], who tested the accuracy of pregnancy detection in different pregnancy intervals depended on P4 level, their finding demonstrated that accuracy were ranged from 80% to 85% in conjunction with pregnancy progression. This study agreement with results of plasma P4 concentration can be used to detect ewes pregnancy on 18 days [21, 22]. There is satisfactory agreement between the present study and Younis and Hatif (2023) study [23], they observed that the threshold value of  $>4.9$  ng/mL for progesterone assay can be used to differentiate between pregnant and empty ewes from day 25 onwards [23]. There is satisfactory agreement between the present study and sensitivity, accuracy of pregnancy diagnosis by P4 assay on day 17 post breeding were higher with moderate specificity in threshold 4ng/ml [24]. The finding of Mohammed *et al.* [25] illustrated that accuracy for P4 assay method was 88.5% in Iraqi local ewes at threshold 4.5 ng/mL at day 21 for pregnant ewes and lower than 1.9 ng/mL for non-pregnant ewes. Alexander *et al.* [26] has already noted that mean P4 levels increased from less than 0.3 ng/mL around ovulation to more than 3 ng/mL on day 7 in both bred and non-bred ewes. Also, the hormone level deviations might also exist because of differences in the ewes age, sampling and environmental factors (like climate or season). The P4 level reach greater than 3ng/mL in pregnant ewes and less than 0.4 ng/mL in all non-pregnant ewes at day 19. Then, the level increased gradually to 7 ng/mL on day 50 in pregnant ewes. The present study agreed with DeNicolo *et al.* [27], who observed that mean P4 concentration reach to 5ng/mL in pregnant ewes at day 14. These deviations between

P4 concentrations of pregnant ewes and between the proposed discriminatory levels as compared with other authors' results may be attributed to small differences of the reference range existing between RIA and EIA [28], although both methods have been proved accurate for P4 determination.

High incidence of early embryonic death recorded in ewes, ranging from 5-22% in FGA- intravaginal sponges treatments [29] and about 20% from total pathological lesions of the ewes experienced embryonic and fetal loss [30].

In small ruminants, ultrasonography is a safe, fast accurate, cost effective, and practical method that can be used to detect pregnancies at early stages [31]. This study agreement with result used the transabdominal ultrasonography is 100% of accuracy for pregnancy diagnosis in Awassi ewes, after 40 days of mated [17, 18]. Possible, early pregnancy diagnosis at 22 or 24 days of gestation by using transabdominal probe [32, 33]. This study agreement with Goel and Agrawal [34] reported that accuracy (90%) in days 40-45 of gestation was slightly lower than our results in days 30-40 of gestation. While, Yotov. [35] demonstrated that accuracy of pregnancy detection was high (87%) at week 4 post-breeding, and it increased to 98% at day 35 post-mating using a 5 MHz transabdominal probe in Satara Zagora sheep breed. The ultrasonography examination method on day 50 can be considered a gold standard for pregnancy diagnosis, given the high diagnostic criteria and reliability recorded (100%) [36]. This finding is consistent with previous studies, where detection values reached 100% at the 40-60 days of pregnancy Romano and Christians. [37] and 39-42 [38]. Furthermore, B-mode ultrasonography conducted by Ganaie *et al.* [39] showed accuracy ranging from 70-95%, 50-83%, and 68-94% in

different gestational periods 15-30, 30-40, and 40-60 days, respectively.

The loss of ewe lambs occurs in the 1st month of pregnancy. In healthy ewes, the loss of early embryo is often high (30% to 60%) [40, 41]. The reasons of embryonic fetal loss was greater after 30 days of pregnancy [42, 43]. When not connected between the embryo and uterine epithelium, and the Losses before day 18th of gestation occur is called early embryonic death [44, 45, 46]. The early embryonic loss is (from fertilization to day 27) and late (from day 28 to 42) [47, 48, 49]. Also other researchers define late embryonic or fetal death is used when the loss occurs after 18th, 30th or 40th days of gestation, respectively In the period between the 22 and 31 days, the embryos were categorized as developing or dead according to crown-rump length and presence of cardiac activity [30,50,51,52]. This study agreement with result of observed early embryonic loss rate was 10% between 30-40 days [53]. The ewes embryonic losses occur in the 1st month of pregnancy, the percentage of losses at 30 days is (40%-60%) [54]. This study agreement with result, the percentage of 42% of embryonic losses occur on 20 days after insemination in Merino ewes, with embryonic losses of 52.6% was for Katahdin ewes [42, 55]. The adult and lamb ewes, the significant embryonic losses was (15–25%) after 30 days of pregnancy [40,54, 56].

### **Conclusion**

We can conclude that using sulpiride is successful treatment in induction of fertile estrus ewes as non-hormonal intervention in seasonally anestrus. The P4 hormone and ultrasonography is rapid, safe and dependable for the diagnosis of early pregnancy, and Assessing Progesterone Levels in Awassi Ewes: A comparison between pregnant and non-pregnant, bearing twins, and singletons during the first trimester in Iraqi Awassi ewes.

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### **Conflict of interest**

There are no conflicts of interest to be declared.

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### **Author contributions**

Conceptualization, study design, sample

collection, and Ultrasonography: Ali Aziz.

Data analyses, Manuscript drafting, and

Manuscript finalization: Najlaa S. Ibrahim.

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## تشخيص الحمل بواسطة قياس هرمون البروجسترون والفحص بالموجات فوق الصوتية في النعاج العواسي العراقية بعد استحداث الشبق الخصب خارج موسم التناسل بواسطة السليرايد علي عبد<sup>1</sup> \* ونجلاء ابراهيم<sup>2</sup>

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تهدف هذه الدراسة إلى تشخيص الحمل في النعاج العواسي العراقية عن طريق قياس هرمون البروجسترون الفحص بالموجات فوق الصوتية بعد تحفيز الشبق الخصب خارج موسم التناسل بواسطة مادة السليرايد (مضاد الدوبامين). أجريت الدراسة خلال الفترة من ديسمبر 2022 حتى يونيو 2023. تم تقسيم النعاج بشكل عشوائي على مجموعتين بواقع 6 نعاج لكل مجموعة. تلقت مجموعة المعاملة دواء السليرايد بجرعة (0.6 ملغم/كغم)، في حين تلقت مجموعة السيطرة محلول ملحي عادي. أظهرت نتائج استجابة الشبق معنوية ( $P < 0.05$ ) في المجموعة المعالجة. كان تركيز البروجسترون أعلى معنويًا ( $P < 0.05$ ) في مجموعة العلاج في الأيام 14 و 21 و 28. وكانت نتيجة التصوير بالموجات فوق الصوتية المستخدمة للكشف عن الحمل غير معنوية في مجموعة العلاج والسيطرة 83.3، 33،3 و 16،6٪ خلال 30 و 60 و 75 يوما بسبب ارتفاع معدل الوفيات الجنينية المبكرة. يمكننا أن نستنتج أن استخدام السليرايد هو علاج ناجح في استحداث الشبق الخصب للنعاج كندخل غير هرموني خارج الموسم التناسلي. إن هرمون البروجسترون والتصوير بالموجات فوق الصوتية سريع وآمن ويمكن الاعتماد عليه لتشخيص الحمل المبكر في النعاج العواسية العراقية.

**الكلمات الدالة:** التحريض، الشبق الخصب، البروجسترون، السليرايد، الفحص بالموجات فوق الصوتية.