



## THE IMPACT OF BIOTIN AND HCG ON THE LEVELS OF ESTRADIOL-17 $\beta$ , PROGESTERONE AND SHEEP PREGNANCY ASSOCIATED PLASMA PROTEIN B (PAPPB) IN LOCAL IRAQI EWES

A. M. F. Al-Mohammedy      Y. T. Abdul-Rahaman\*

College of Veterinary Medicine, University of Fallujah

\*Correspondence to: Y. T. Abdul-Rahaman, College of Veterinary Medicine, University of Fallujah, Iraq.

Email: [yassentaha@uofallujah.edu.iq](mailto:yassentaha@uofallujah.edu.iq)

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### Abstract

Twenty Iraqi ewes aged between 2-3 years were used in the current study. All animals were raised in an animal field in the Fallujah city/Saqlawiyah area during the period from August 2022 to February 2023. The animals were divided randomly into 4 equal groups. They were fed naturally on one diet. The control group (T1) received no additional treatment, the second group (T2) was given an hCG-COX 100 I.U./ewe injection (after sponge removal), the third group (T3) was given 10 mg of biotin per ewe daily (for 30 days), and the fourth group (T4) was given 10 mg of biotin per ewe daily (for 30 days) along with an hCG-COX 100 I.U./ewe injection (after sponge removal). Blood samples for estradiol-17 $\beta$  and progesterone measurement were collected on days 0, 18, 48, 79, and 110, and for PAPPB on days 48 and 79 after mating. The results showed a significant increase in the estradiol-17 $\beta$  hormone ( $P < 0.05$ ) on the 110th day of pregnancy in all the experimental treatments. The concentration of the progesterone hormone increased significantly ( $P < 0.05$ ) on the 79th day of pregnancy in all experimental treatments, with the T2 group outperforming the rest of the experimental treatments in progesterone concentration. No significant differences were observed between days 48 and 79 in the experimental ewes regarding the concentrations of

Sheep Pregnancy-Associated Plasma Protein B (PAPPB) (pg/ml). We conclude from the study that biotin has no effect on the level of sex hormones, while hCG (100 I.U.) alone affects the level of progesterone on day 79 of pregnancy.

**Keywords:** Biotin, Estradiol-17 $\beta$ , Progesterone, Sheep pregnancy- associated plasma protein B, Iraqi Sheep.

## تأثير البايوتين على الاستراديول، البروجستيرون وبيوتين البلازما المرتبط بالحمل في النعاج العراقية المحلية

احمد محمد فرحان المحمدي ياسين طه عبدالرحمن\* 

كلية الطب البيطري، جامعة الفلوجة

\*المراسلة الى: ياسين طه عبد الرحمن، كلية الطب البيطري، جامعة الفلوجة، العراق.

البريد الالكتروني: [yassentaha@uofallujah.edu.iq](mailto:yassentaha@uofallujah.edu.iq)

### الخلاصة

استخدمت 20 نعجة عراقية تراوحت اعمارها بين 2-3 سنوات. تم تربية جميع الحيوانات في حقل حيواني تابع لمدينة الفلوجة في منطقة الصقلاوية للمدة ما بين آب 2022 إلى شباط 2023. قسمت الحيوانات عشوائياً إلى 4 معاملات متساوية. وغذيت بشكل طبيعي وعلى نظام غذائي واحد وشملت مجموعة السيطرة (T1)، و(T2) hCG-COX 100 I.U. / حقنة نعجة (بعد إزالة الاسفنجية)، و(T3) أعطيت بيوتين 10 ملغم/ نعجة/ يوماً عن طريق الفم (30 يوماً) و(T3) بيوتين 10 ملغ/ نعجة/ يوماً عن طريق الفم (30 يوماً) و(T4) البيوتين 10 ملغ/ نعجة/ يوماً عن طريق الفم (30 يوماً) + hCG-COX 100 وحدة دولية/ حقنة نعجة (بعد إزالة الاسفنجية). تم جمع عينات الدم لقياس استراديول  $\beta$ 17 والبروجستيرون في الأيام 0 و18 و48 و79 و110 وبيوتين البلازما المرتبط بحمل الأغنام (PAPPB) بعد 48 و79 يوماً من التلقيح. أظهرت النتائج زيادة معنوية في هرمون الاستراديول 17 بيتا ( $P<0.05$ ) في اليوم الـ110 من الحمل في جميع المعاملات. ارتفع تركيز هرمون البروجستيرون معنوياً ( $P<0.05$ ) في اليوم 79 من الحمل في جميع معاملات التجربة. بينما تفوق الـ T2 على بقية المعاملات في مستوى تركيز هرمون البروجستيرون. لم تلاحظ فروق معنوية بين الفترتين 48 و79 يوماً في تركيز (PAPPB) (pg/ml). نستنتج من الدراسة أنه لا يوجد أي تأثير للبيوتين على مستوى الهرمونات الجنسية، في حين أن هرمون hCG (100 وحدة دولية) اثر على مستوى البروجستيرون في اليوم 79 من الحمل.

**كلمات مفتاحية:** البايوتين، الاستراديول، البروجستيرون، بروتين البلازما المرتبط بحمل الأغنام (PAPPB) B، النعاج العراقية.

## Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone originating from the trophoblast of the blastocyst (chorion) extracted from the urine of pregnant women, longer half-lives (hours to days). It has a similar effect to LH secreted by the anterior pituitary gland, used as superovulation hormone in female because their physiologic activity generally lasts a longer period (15). hCG used to induce oestrus, follicle growth, stimulate ovulation, reduce embryo mortality, improve pregnancy rates, treat follicular or luteal cysts, improvement of the weaning-to-oestrous interval (16). (3) indicated that the use of hCG (300 IU) at day 12 post-mating increased the plasma progesterone concentration, pregnancy rate, percentage of multiple ovulations, and fertility. Vitamins play an important role in blood formation, the function of lymphocytes, killer cells, neutrophils, and antibody production. It has an important role as antioxidant by reducing harmful reactive oxidative species (ROS). Dietary supplementation of antioxidants such as vitamins E, C, A, and B complex, may have beneficial effect to eliminate the damaged caused by ROS, embryonic development, cell and tissue differentiation, immune function and affecting ovarian steroids (1). According to (11) biotin have a saturable transport system dependent on Na<sup>+</sup> and actively collected within the placenta before releasing it more slowly into the fetal compartment. In comparison to placental buildup, the transmission of biotin through the placenta is sluggish. The availability of biotin influences the rates of cell division., progesterone secretion in chorionic carcinoma cells and biotinylation of carboxylases and histones (11). (6) found that giving 10 mg of biotin increased doe kidding twins, litter weight of kids, milk yield, dry matter intake, blood biochemical components, thyroid hormones, progesterone, and estradiol 17 $\beta$  and decreased significantly the time of return to estrous postpartum and cortisol levels. Therefore the present study was conducted to evaluate the effect of Biotin on Estradiol-17 $\beta$ , Progesterone, and Sheep Pregnancy Associated Plasma Protein B (PAPPB) levels in Local 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 and fed naturally 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. (T<sub>1</sub>) serve as a control group, (T<sub>2</sub>) was given hCG- COX 100 I.U./ ewe injection (after remove sponge), (T<sub>3</sub>) has been orally administrated with Biotin 10 mg/ ewe/ daily oral (30 days) and the (T<sub>4</sub>) was given Biotin 10 mg/ ewe/ daily oral (30 days) + hCG- COX 100 I.U./ ewe injection (after remove sponge). Estrous synchronized are unified by placing vaginal sponges for 14 days. Blood samples for estradiol-17 $\beta$  and progesterone measurement were collected on at days 0, 18, 48, 79 and, 110 and Sheep Pregnancy Associated Plasma Protein B (PAPPB) days 48 and 79 after mating. Estradiol-17 $\beta$  and Progesterone concentrations were measured by COBAS (E-411).

The kit provided by Roche, Inc., Switzerland, and (PAPPB) concentrations were measured by using ELISA technology. The kit is provided by Sunlong Biotech Co., Ltd, China. Statistical computations were performed using the General Linear Model (GLM) procedure in the SAS program (14). Differences among means were computed using the LSD.

### Results and Discussion

The results showed a significant increase in the concentration of estradiol-17 $\beta$  hormone ( $P < 0.05$ ) on the 110<sup>th</sup> day of pregnancy in all experimental groups, and the highest increase was in the control one  $6.0 \pm 0.8$  ng/ ml as compared with the other groups, While the lowest level of the estradiol-17 $\beta$  hormone was on the day 0 in the T<sub>3</sub>  $0.2 \pm 0.1$  ng/ ml (Table 1). No significant difference was observed between the experimental treatments in the level of oestradiol-17 $\beta$  hormone concentration. The concentration of the progesterone hormone increased significantly ( $P < 0.05$ ) at day 79<sup>th</sup> of pregnancy in all treatments of the experiment, and it was highest in the T<sub>4</sub>  $385.5 \pm 12.3$  ng/ ml as compared to the other treatments. While the lowest level of the progesterone hormone was at day 0 in the T<sub>4</sub>  $12.4 \pm 3.6$  ng/ ml (Table 2). While the T<sub>2</sub> outperformed the rest of the experimental treatments in the level of progesterone concentration. No significant differences were observed between the two periods (48 and 79) days in the experimental ewes of PAPPB concentrations (Fig 1 and Table 3).

**Table 1: Effects of the treatments on oestradiol-17 $\beta$  (ng/ ml) hormonal level in experimental ewes before and during treatment.**

Time (day)	0	18	48	79	110
<b>Treatments</b>					
T <sub>1</sub>	$1.7 \pm 0.6$ C	$3.3 \pm 0.8$ BC	$3.8 \pm 0.4$ B	$3.7 \pm 0.5$ B	$6.0 \pm 0.8$ A
T <sub>2</sub>	$1.2 \pm 0.6$ C	$2.7 \pm 0.9$ BC	$3.6 \pm 0.7$ AB	$3.7 \pm 0.4$ AB	$5.2 \pm 0.6$ A
T <sub>3</sub>	$0.2 \pm 0.1$ C	$3.3 \pm 0.1$ AB	$2.9 \pm 0.7$ B	$4.0 \pm 0.2$ AB	$5.8 \pm 0.9$ A
T <sub>4</sub>	$0.6 \pm 0.4$ C	$3.8 \pm 1.3$ AB	$2.2 \pm 1.2$ BC	$3.3 \pm 0.3$ B	$5.7 \pm 0.9$ A

LSD=2.04

The different capital letters refer to significant differences between time at ( $P \leq 0.05$ ).

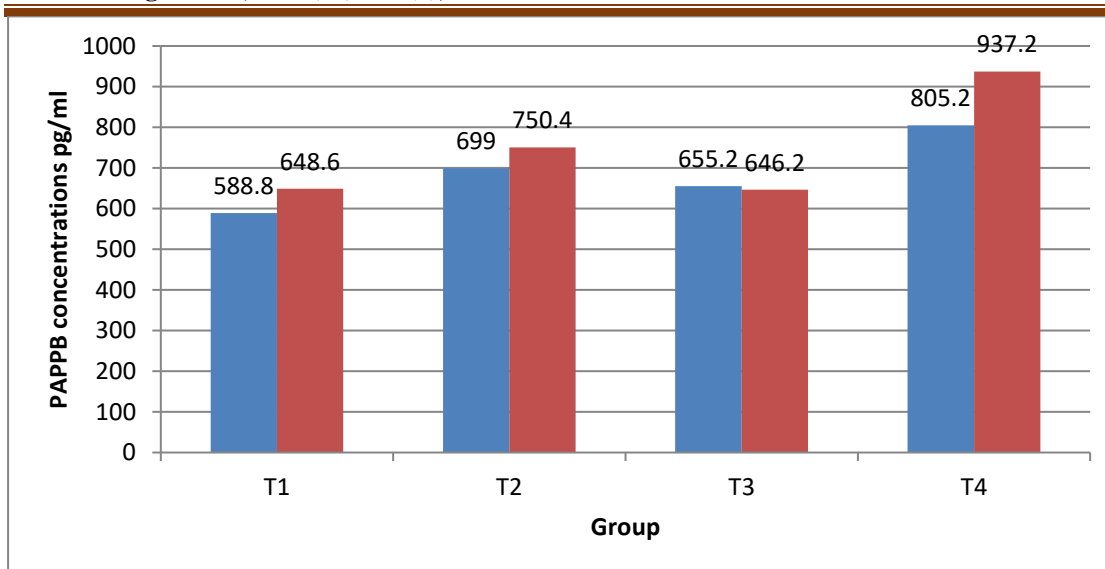
**Table 2: Effects of the treatments on Progesterone (ng/ ml) hormonal level in experimental ewes before and during treatment.**

Time (day)	0	18	48	79	110
<b>Treatments</b>					
T <sub>1</sub>	$12.9 \pm 3.6$ D	$182 \pm 10.3$ B	$84.9 \pm 4.7$ C	$413.5 \pm 9.9$ A a	$27.7 \pm 2.8$ D
T <sub>2</sub>	$31.7 \pm 16.5$ D	$180.3 \pm 8.4$ B	$84.1 \pm 6$ C	$415.6 \pm 40.6$ A a	$20.1 \pm 4$ D
T <sub>3</sub>	$17.8 \pm 6.1$ D	$185.6 \pm 22.5$ B	$78.2 \pm 3.2$ C	$375.6 \pm 21.7$ A b	$20.8 \pm 4.7$ D
T <sub>4</sub>	$12.4 \pm 3.6$ D	$171.6 \pm 6.2$ B	$81.8 \pm 5.5$ C	$385.5 \pm 12.3$ A ab	$17.9 \pm 2.7$ D

LSD= 37.4.

The different capital letters refer to significant differences between time at ( $P \leq 0.05$ ).

The different small letters refer to significant differences between group at ( $P \leq 0.05$ ).



**Fig. 1: PAPPB concentrations (pg/ml) of ewes at days 48 and 79 PM.**

**Table 3: PAPPB concentrations of ewes during pregnant.**

Treatments	Time (day)	
	48	79
T <sub>1</sub>	588.8±150.5	648.6±161.4
T <sub>2</sub>	699.0±175.1	750.4±201.3
T <sub>3</sub>	655.2±160.9	646.2±158.8
T <sub>4</sub>	805.2±39.4	937.2±121.9

No. significant.

The result of the study agreed with (19), as he noticed a decrease in the concentration of the progesterone hormone during mating, and it increased during pregnancy due to the activity of the corpus luteum, and decreased at the end of pregnancy. The result also agreed with (7), as he noticed that the estrogen level decreased in the second month, reached its lowest level in the fourth month, and increased in the fifth month of pregnancy. While the progesterone level increased in the second and third months and decreased in the fifth month of pregnancy.

Estrogen and progesterone are a steroids hormones (biosynthesized from cholesterol). Estrogen is produced in the granulosa and theca cells lining the follicle and surrounding the oocyte, and progesterone produced by the corpus luteum and placenta. Estrogen controls the development of genital tissues and the estrus cycle. Additionally, it has a role in the fecundation process, including ovulation, oocyte implantation, pregnancy, and parturition. Progesterone has a variety of functions during the estrus cycle, embryogenesis, and particularly pregnancy. It intervenes also in the metabolism of corticosteroids. It also has an important link with estrogen. Progesterone prepares the uterus to facilitate the implantation and decreases maternal immune response to allow acceptance of the embryo (5).

The rise in progesterone is also responsible for the so-called (progesterone block), which prevents uterine contractions. In sheep, the corpus luteum is important in the early days of progesterone production, but after 50 days of pregnancy, the placenta secretes the bulk of the hormone. Estrogen is essential during the 30 days of pregnancy. It modifies the gene expression of the uterus, and the placenta is an

important source of estrogen production, especially in the last stages of pregnancy (15).

The increased steroidogenic activity of the fetal adrenal results in increased dehydroepiandrosterone sulfate (DHEAS) synthesis which, is converted by the placenta into estrogen. Estrogen stimulates uterine contractility directly and through increased prostaglandin production in the fetal membranes. The estrogen hormone also plays an important role in the process of exchanging nutrients and waste between the mother and the fetus through the capillaries of the placenta in the second half of pregnancy (12).

(6) recorded elevated levels of estrogen and progesterone on the 10 days before parturition and the lowest values on day 2 and in the first month post-partum, and increased at 60 and 90 days after parturition. Also, giving 10 mg of biotin increased the level of progesterone and estrogen.

Nutrition affects the level of hormones, as (10) noticed that giving corn oil in the diet increases the levels of progesterone and estradiol in ewes. Also, giving 10 mg of biotin increased the level of progesterone and estrogen (6).

High environmental temperature changes follicular and oocyte development by altering progesterone, LH and FSH hormones' secretion and dynamic during the estrous cycles (8). Cycle progesterone during the luteal phase is increased in high temperatures, suggesting decreased progesterone production and/or increased clearance, which affect the timing and development of preovulatory follicle growth (18).

The age of the ewe also affects the level of the progesterone hormone, as (2) noticed an increase in the level of the progesterone hormone with age and considered it a discovery evidence of the age of puberty in the ewes.

The results of the current study did not agree with previous studies on the PAPPB protein level

(13) indicated that it is possible to diagnose pregnancy by Pregnancy-Associated Glycoprotein (PAGs) on the 21- day PM in Corriedale ewes and Ile de France x Texel crossbred ewes.

(4) showed that it is possible to distinguish pregnant and non-pregnant in Sarda and Lacaune ewes by the Pregnancy-Associated Glycoprotein (PAG) method from day 18 of gestation. While some researchers noted that pregnancy could be detected by PAGs in urine or serum on day 16 of gestation in Osseimi ewes (9).

Pregnancy Associated Plasma Protein B is produced from binucleate cells of the trophoctoderm of the conceptus. Diagnoses of these proteins in blood are direct evidence of a recent viable pregnancy. They can be found in the mother's blood from week three of pregnancy until two to three weeks following lambing (17).

It is clear from previous studies that it is possible to diagnose pregnancy in sheep using the PAPPB method at an early age between 16-21 days of gestation

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## Conclusions

We conclude from the study that there is no effect of biotin on the level of sex hormones, while hCG (100 I.U) alone has an effects the level of progesterone on day 79 of pregnancy.

### Supplementary Materials:

No Supplementary Materials.

### Author Contributions:

Author A. M. F. Al-Mohammedy; methodology, writing—original draft preparation, Author Y. T. Abdul-Rahaman writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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