

Detection of changes in some hematological, biochemical, and hormonal parameters during different stages of pregnancy in Ewes

Dnia Hazem , Jawad Kadhim Taher 

Physiological department, veterinary medicine college, AL-shastra university

E-mail: Dounai.h.ali@vet.edu.iq

E-mail: jawad.kadhim@shu.edu.iq

Abstract

Aim: the study was designed to investigate the effects of different stages of pregnancy on selected hematological, biochemical, and hormonal parameters in Awassi ewes.

Materials and methods: The present study was conducted in a sheep field located in Suq Al-Shuyukh district, south of Dhi-Qar province, southern Iraq. This study was carried out during the period from 1 September 2025 to February 2026. A total of (100) clinically healthy pregnant ewes were included in this study. The animals ranged in age from 2 to 5 years and had body weights between 40-50 kg. The ewes were categorized into three groups according to the stage of pregnancy and four groups control non – pregnant ewes. Pregnancy stage were determined using transabdominal ultrasound XF50 with a 3.5 MHZ convex probe. Ultrasonographic examination was performed according to standard reproductive diagnostic procedures in small ruminants.

Results: The statical analysis was appeared white blood cells significant differences ($P \leq 0.05$) in control groups (non – pregnant) compare with second trimester and while no significant differences between first and late trimester pregnancy also lymphocyte was showed higher significant differences between late stage of pregnancy and other stages of pregnancy and non-pregnant ewes. The current study was showed high significant differences between late trimester of pregnancy and other stages in pregnant and non – pregnant ewes in neutrophil levels.

The results of current study were appeared high significant differences in Red Blood Cell between second trimester of pregnancy and other groups non- pregnant, first and late trimester of pregnancy also, the current study was appeared no significant differences in MCH between control groups non pregnant ewes and first trimester of pregnancy while recoded decrease significant differences ($P \leq 0.05$) in second trimester compare with non- pregnant and first trimester pregnancy. A mean corpuscular Hemoglobin concentration blood test (MCHC) was appeared high significant differences between control and other stages of pregnancy while no significant differences between first and second trimester of pregnancy.

The results of the current study were recorded significant differences in cholesterol levels between control groups non – pregnant compare with different stages of pregnancy while no significant differences between first and late pregnancy. The level of glucose was appeared high significant differences between non – pregnant (control) and different stages of pregnancy no significant differences between first and second trimester of pregnancy. Aspartate aminotransferase high significant differences in second and late stages compare with non-pregnant and first trimester of pregnancy. The statical analysis of Alanine aminotransferase levels appeared higher significant differences between first trimester and different stages of pregnancy and control while no significant differences between control groups and second trimester of pregnancy. The current study was appeared high significant differences in total protein between second trimester compared with Control groups (non-pregnant) and different stages of pregnancy while no significant differences between control and first stages of pregnancy. The level of globulin was appeared

high significant differences between control groups (non- pregnant ewes) and different stages of pregnancy, while no significant differences between first and second trimester of pregnancy.

The stational analysis was appeared the level of placental lactogen hormones increase significant differences between different stages of pregnancy compare with control (non – pregnant) while no significant differences between first, second and late trimester of pregnancy. The level of cortisol was appeared high significant in late stage compare with non-pregnant, first and second trimester of pregnancy while no significant differences in non – pregnant, first and second trimester. The results of current study were showed high significant in first and second trimester compare with non-pregnant and late pregnancy, progesterone levels in the current study was recorded significant differences in non – pregnant, first and second trimester compare with late trimester of pregnancy.

Conclusion: The present study confirmed that pregnancy stages significantly influence on some hematological, biochemical, and hormonal parameters in pregnant ewes. The present study also documented an important finding indicating that placental lactogen in non- pregnant ewes exhibits functional cross-reactivity with prolactin and growth hormone, likely due to structural and genetic similarities among these hormones.

I. Introduction

Sheep production plays an important role in the agriculture industry, because it is considered a source of meat, wool, and other by-products. Sheep in Iraq are seasonally poly estrus and thus can reproduce the year round (Alwan,1999; Abd-Alrahman,1998). Pregnancy is one of the physiological conditions leads to remarkable and dramatic changes in hormonal, hematological, and biochemical variable in all animals' species. (Khaton and Wani, 2011; Khazaal et al., 2023; Medan et al., 2015).

Hematological and biochemical parameters in sheep and goats exhibit significant variability influenced by litter size, age, sex, physiological state, fetal growth malnutrition and genotype of the animals (Seiax, et al., 2021; Yokus et al., 2006). The identification of changes in biochemical parameters in sheep metabolic through the evaluation of the blood metabolic profile during gestation is a useful tool for the management and nutritional decision making.

Glucose is the primary fuel used by sheep fetus, the increased fetal demand in pregnant ewes is not accompanied by increased endogenous glucose production, resulting in lower glucose levels during pregnancy. Therefore, low glucose levels are considered predictive factors for diseases such as pregnancy toxemia (Haffaf and Benallou 2016; Raoofi et al., 2013; Yokus et al.,2006 ; Swetha et al.,2018)

Placental lactogen hormone is usually secreted in both fetal and maternal compartments (Taylor et al.,1980). Previous studies have shown an increase in placental lactogen concentration as pregnancy progresses (Bosc et al.,1982)

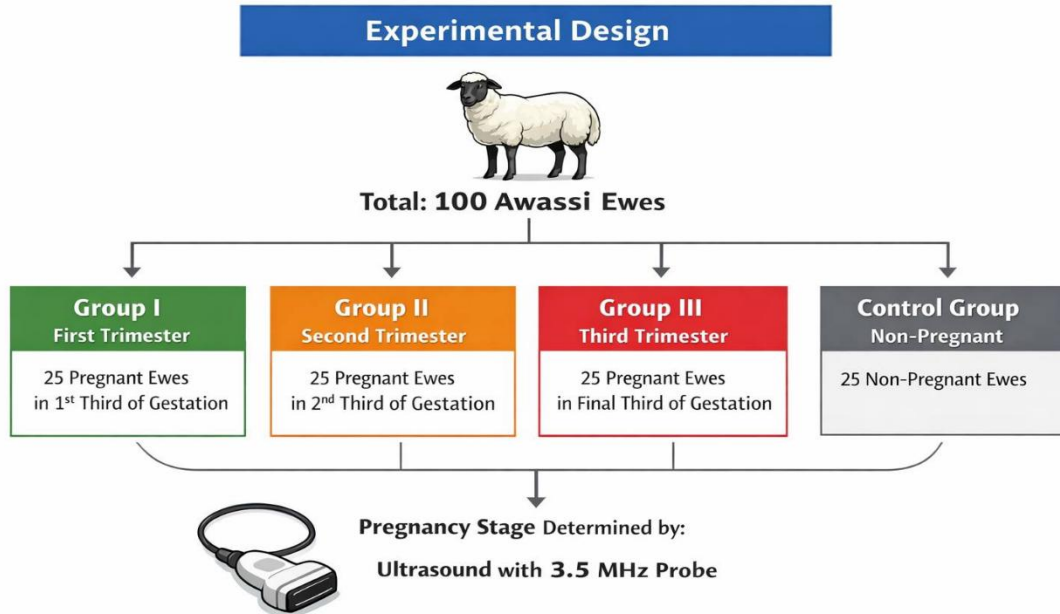
The structure of the endometrium and uterine immune system are significantly affected by endocrine alteration, including variations in estrogen and progesterone profiles (Abu Nasar, 2006). White blood cells, lymphocyte, monocytes, basophils, eosinophils, and neutrophils are responsible for fighting infections in the animal's body (Etim.2015). Hematological profile guide farmers and sheep breeders in taking proper measures to improve the health and productivity of their animals.

Therefore, this study was designed to investigate the effects of different stages of pregnancy on selected hematological, biochemical, and hormonal parameters in Awassi ewes.

II. Material and methods

Study area:

The present study was conducted in a sheep field located in Suq Al-Shuyukh district, south of Dhi-Qar province, southern Iraq. The study area is situated at approximately city 130.89 N latitude and 46.46 E longitude, with an average elevation of about 5 meters above sea level.



This study was carried out during the period from 1 September 2025 to February 2026.

Animal and management

A total of (100) clinically healthy pregnant ewes were included in this study. The animals ranged in age from 2 to 5 years and had body weights between 40-50 kg. All ewes put at same environmental and managements conditions throughout the experimental time to decrease external influences on the studies parameters. The ewes were allowed to graze freely on natural pasture, and each animal received 1 kg of concentrated feed per day as a dietary supplement.

Pregnancy diagnosis

Pregnancy stage were determined using transabdominal ultrasound XF50 with a 3.5 MHZ convex probe. Ultrasonographic examination was performed according to standard reproductive diagnostic procedures in small ruminants. The ewes were categorized into three groups according to the stage of pregnancy and four groups control non – pregnant ewes

Experimental Design

Figure 1: Experimental design illustrating the distribution of ewes according to non- pregnant and pregnancy stages.

Blood samples and Laboratory Analysis

A total (7ml) of blood was collected from jugular vein of each ewe using a 10 ml syringe. Samples were divided as follows: 2ml into an EDTA- containing tube for complete blood count (CBC) analysis. 5ml into a plain tube without anticoagulants, serum for biochemical and hormonal analysis. The blood samples for biochemical and hormonal assay were centrifuged at 3000rpm for 10 minutes to separate the serum. The obtained serum was then collected by Eppendorf tube and stored at -20 C until analysis to preserve sample integrity

Ethical approval

The study was conducted following approved guidelines of the institutional animals' ethics committee, veterinary medicine of Al- Shatra university, Iraq. All efforts ewe made to ensure animal welfare, minimize stress, and avoid unnecessary discomfort during ultrasonographic examination and blood samples collection.

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups/ factors in study parameters. LSD-Least significant difference used to significant compare between means in this study.

Table 1: The values of white blood cells types parameter of non-pregnant and pregnant in different stages in ewe

Parameters	Non- pregnant (control) No=25	First trimester From 1 to 2 days No=25	Second trimester From 3 to 4 months No=25	Late pregnancy 4.5 to 5 months No=25	L.S.D.
WBC (cell $\times 10^3$)	10.20 \pm 0.56 a	9.73 \pm 0.41 ab	9.24 \pm 0.47 b	10.76 \pm 0.65 ab	1.307 *
Lym.	5.11 \pm 0.32 b	5.68 \pm 0.39 ab	5.20 \pm 0.25 b	6.81 \pm 0.43 a	1.519 *
Mon.	0.17 \pm 0.02	0.20 \pm 0.04	0.20 \pm 0.04	0.26 \pm 0.05	0.126 NS
Neu.	5.73 \pm 0.24 ab	5.68 \pm 0.34 ab	5.10 \pm 0.27 b	6.81 \pm 0.42 a	1.366 *
Eso.	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.013 NS
Baso.	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.013 NS

Means having with the different letters in same row differed significantly.

* ($P \leq 0.05$), NS: Non-Significant.

In table 1 the statical analysis was appeared white blood cells significant differences ($P \leq 0.05$) in control groups (non – pregnant) compare with second trimester and while no significant differences between first and late trimester pregnancy also lymphocyte was showed higher significant differences between late stage of pregnancy and other stages of pregnancy and non-pregnant ewes. The current study was showed high significant differences between late trimester of pregnancy and other stages in pregnant and non – pregnant ewes in neutrophil levels. Also, the results were showed no significant differences between monocyte, eosinophil, and basophil levels between control groups non – pregnant and different stages of pregnancy.

Table2: The effect of different gestation stages of ewe on erythrocyte constituents

Parameters	Non – pregnant (control) No=25	First trimester No=25	Second trimester No=25	Late pregnancy No=25	L.S.D.
RBC (cell x10 ⁶)	2.80 ±0.08 ab	2.53 ±0.06 b	3.00 ±0.08 a	2.82 ±0.03 ab	0.428 *
HEM (g/dL)	8.23 ±0.57	8.02 ±0.48	8.38 ±0.51	7.85 ±0.29	0.791 NS
MCH (pg)	31.63 ±1.04 a	31.72 ±1.56 a	27.71 ±1.05 b	28.27 ±1.13 ab	3.775 *
MCHC (g/dL)	77.35 ±3.58 a	69.82 ±2.97 b	66.74 ±2.89 b	73.26 ±3.75 ab	8.021 *
RDW (%)	6.04 ±0.34	5.43 ±0.28	5.64 ±0.31	5.54 ±0.27	0.893 NS

Means having with the different letters in same row differed significantly.
* (P≤0.05), NS: Non-Significant.

In table 2 the results of current study were appeared high significant differences in Red Blood Cell between second trimester of pregnancy and other groups non- pregnant, first and late trimester of pregnancy also, the current study was appeared no significant differences in MCH between control groups non pregnant ewes and first trimester of pregnancy while recoded decrease significant differences (P≤0.05) in second trimester compare with non- pregnant and first trimester pregnancy .Hemoglobin and Red Cell Distribution levels were appeared no significant differences between different stages of pregnancy and control groups (non – pregnant). A mean corpuscular Hemoglobin concentration blood test (MCHC) was appeared high significant differences between control and other stages of pregnancy while no significant differences between first and second trimester of pregnancy

Table 3: The effect of different gestation periods on some biochemical parameters.

Parameters	Non – pregnant N0 = 25	First trimester N0 = 25	Second trimester No = 25	Late pregnancy No = 25	L.S.D.
Cho2 (mg/dL)	60.72 ±2.47 a	48.40 ±2.39 b	52.80 ±2.86 ab	49.52 ±2.41 b	10.705 *
Glu. (mg/dL)	32.11 ±1.55 a	30.96 ±1.26 ab	30.92 ±1.35 ab	25.78 ±1.08 b	6.019 *
AST (U/L)	64.35 ±3.08 ab	67.13 ±2.78 ab	61.45 ±2.55 b	73.15 ±3.27 a	7.467 *
ALT (U/L)	13.52 ±0.67 ab	15.06 ±0.72 a	13.9 3 ±0.67 ab	11.87 ±0.54 b	3.267 *
albumin (U/L)	31.60 ±1.05	31.63 ±0.97	33.17 ±1.29	30.59 ±1.09	4.552 NS
Total protein (g/L)	66.96 ±2.84 ab	68.05 ±3.34 ab	70.08 ±2.66 a	61.64 ±2.45 b	7.431 *
Globulin (g/L)	38.05 ±2.14 a	36.11 ±1.61 ab	36.41 ±1.94 ab	33.39 ±1.03 b	4.270 *

Means having with the different letters in same row differed significantly.
* (P≤0.05), NS: Non-Significant.

The results of the current study in table 3 were recorded significant differences in cholesterol levels between control groups non – pregnant compare with different stages of pregnancy while no significant differences between first and late pregnancy. The level of glucose was appeared high significant differences between non – pregnant (control) and different stages of pregnancy no significant differences between first and second trimester of pregnancy. The biochemical parameters of liver enzyme in this study were appeared Aspartate aminotransferase high significant differences in second and late stages compare with non-pregnant and first trimester of pregnancy. The stational analysis of Alanine aminotransferase levels appeared higher significant differences between first trimester and different stages of pregnancy and control while no significant differences between control groups and second trimester of pregnancy. The results of Albumin levels were appeared no significant differences between control and different stages of pregnancy in ewes. The current study was appeared high significant differences in total protein between second trimester compared with Control groups (non-pregnant) and different stages of pregnancy while no significant



differences between control and first stages of pregnancy. The level of globulin was appeared high significant differences between control groups (non- pregnant ewes) and different stages of pregnancy, while no significant differences between first and second trimester of pregnancy.

Table 4: The effect of different gestation periods on some Hormonal parameters.

Parameters	Non – pregnant No = 25	First trimester No = 25	Second trimester No = 25	Late pregnancy No = 25	L.S.D.
Placental lactogen (mg/L)	30.05 ±1.05 b	45.48 ±2.54 a	46.45 ±1.98 a	51.65 ±2.56 a	7.410 *
Cortisol (nmol/L)	222.90 ±13.72 b	199.87 ±10.94 b	218.70 ±12.06 b	345.72 ±18.73 a	42.773 *
E2 (pg/ml)	163.39 ±8.66 b	199.39 ±10.02 a	200.47 ±12.43 a	188.70 ±10.35 ab	29.640 *
P4 (ng/ml)	32.10 ±1.08 a	30.96 ±0.95 a	30.92±0.85 a	25.78 ±1.04 b	4.502 *
Means having with the different letters in same row differed significantly. * (P≤0.05).					

The present study focused on some important hormonal parameters in different stages of pregnancy and control groups (non – pregnant ewes), the statical analysis was appeared the level of placental lactogen hormones increase significant differences between different stages of pregnancy compare with control (non – pregnant) while no significant differences between first, second and late trimester of pregnancy. The level of cortisol was appeared high significant in late stage compare with non-pregnant, first and second trimester of pregnancy while no significant differences in non – pregnant, first and second trimester. The results of current study were showed high significant in first and second trimester compare with non-pregnant and late pregnancy, progesterone levels in the current study was recorded significant differences in non – pregnant, first and second trimester compare with late trimester of pregnancy.

III. Discussions

Hematological profile in different stages of pregnant ewes

The current study appeared that gestation in sheep induces significant differences (P≤0.05), stage -dependent hematological changes, influence both leukocyte and erythrocyte parameters. These variations reflect adaptation of the physiology to pregnancy, including immunomodulation and changes of oxygen demand

Leukocyte parameters profile (immune response)

White blood cells values appeared significant differences in non – pregnant ewes while showed a slight decrease during second trimester of pregnancy followed by an increase in late trimester gestation, previous studies confirm fluctuate leukocyte level counts during gestation due to hormonal changes and stress – related responses, particularly near lambing (Turini et al., 2024).

Neutrophils higher significant differences in late trimester pregnancy compare with non- pregnant and other stages of trimester pregnancy. this increase in late stage may attributed to elevated cortisol levels or preparation for labor – associated inflammatory processes. (Olatunji et al., 2023) who showed that increase neutrophil in late pregnancy is a common physiological response in ruminants.

Lymphocytes higher significant differences in late trimester pregnancy compare with non- pregnant and other stages of trimester pregnancy, this reflects immune tolerance during pregnancy or activation of immune system before labor, previous studies showed mechanism to protect the fetus while maintaining maternal defense (Deniz et al., 2024). The present study demonstrates no significant differences between non pregnant



and different stages of pregnancy in other leukocyte cells such as Monocyte, Eosinophils, Basophils. These results indicate that these cells are less sensitive to physiological changes during gestation, which is agreement with recent hematological studies in small ruminants (Olatunji et al., 2023)

Erythrocyte parameters profile (oxygen transport system)

Red blood cells high significantly during second trimester compare with non- pregnant and other stages of pregnancy, this increase attributed to increase erythropoiesis during second trimester. previous studies confirm that red blood cells variations are linked to embryo growth demands and circulatory adaptation (Turini et al.,2024). **The statical analysis of present study was appeared no significant differences between non – pregnant and different stages of pregnancy in hemoglobin and RDW. This stability may be attributed to maintenance of oxygen carrying capacity and stable erythrocyte size distribution, similar results were reported in previous studies, indicating Hb and RDW stability under normal condition of pregnancy (Deniz et al., 2024). Both MCH and MCHC showed significant decrease during different stages of pregnancy compare with non – pregnant sheep, previous studies confirm that decrease in MCH and MCHC are typical adaptive responses during pregnancy (Olatunji et al., 2023)**

Biochemical profile in different stages of pregnant ewes

The present results appeared that different pregnancy stages significantly influence several biochemical parameters in ewes, reflecting physiological and metabolic adaptation during geatation. Cholesterol levels were signifaantly higher in non – pregnant ewes compared to gestation groups, especially late trimester of pregnancy, cholesterol lower during gestation is mainly because of increased utilization of cholesterol for steroid hormone synthesis. (Nazifi et al., (2002) demonstrated that cholesterol levels decrease during gestation due to enhanced steroidogenesis, while Piccione et al., (2009) reported increase fatty metabolic activity in gestation ewes.

Glucose levels appeared a significant decrease in late trimester of pregnancy compared to non- gestation ewes, the decline of glucose is attributed to increased fetal demand for glucose, maternal glucose is sources of fetus energy. Bell, (1995) showed increase fetal glucose utilization in late trimester of pregnancy also Rook (2000) explained glucose consumptions toward fetal growth.

Aspartate Aminitransferase levels increased significantly in late trimester pregnancy, this elevation due to increased hepatic metabolic activity during late pregnancy, previous studies such as Antunovic et al., (2011) and Kaneko et al., (2008) reported increased liver activity during late trimester pregnancy in ewes. Alanine Aminotransferase was significantly higher in the first trimester and decreased in late trimester of gestation, the variation of ALT levels may relate with hormonal changes during gestation, these results agreement with Mbassa and Poulsen (1991) which observed variation in hepatic enzymes during physiological states.

The current study was appeared no significant differences in albumin levels between pregnant and non -pregnant ewes at different stages of pregnancy, Kaneko et al., (2008) showed albumin usually stable unless severe metabolic disturbance occurs. Total protein increased significantly in the second trimester pregnancy and decrease in late gestation, the increase may due to enhanced peotein synthesis while the decrease in late pregnancy due to transfer of amino acids to the fetus. El-Sjherif and Assad (2001) reported protein fluctuation during pregnancy. Globulin levels high significant in non – pregnant ewes compare with different stages of pregnancy while decrease significantly in late pregnancy compare with first and second trimester pregnancy, this reduction may be to immunoglobulin transfer toward colostrum formation and increased fetal demand, Jain, (1993) showed redistribution of immunoglobulin in late stage of gestation.

Hormonal profile in different stages of pregnant ewes

The current study was focused on investigating placental lactogen levels during different stages of gestation and comparing them with non-gestation sheep, which were considered as a control group. However, the results of the study revealed the presence of measurable levels of this hormone in non-gestation animals.



this finding can be attributed to cross – reactivity between placental lactogen, growth hormone, and prolactin. The underlying causes of this cross- reactivity is the structural and genetic similarity among these hormones, as they belong to the same family and share homologous sequences. Lesniak et al., demonstrated that ovine prolactin and growth hormone can interact with receptors associated with placental lactogen due to structural similarities among these hormones. The result of present study was appeared no significant differences between different stages of gestation, these results disagreement with Bosc et al., (1982) who reported the level of placental lactogen increased progressively during gestation in sheep which had one fetus.

The present study focused on level of cortisol in different stages of pregnancy, cortisol is a glucocorticoid hormone produced by the adrenal cortex. it is released in response to stress and low level of blood glucose. Pregnancy may represent a physiological stress which increases maternal plasma cortisol especially in late pregnancy will reach to double values (Bell et al.,1991). Our results of study were reported higher significant differences of cortisol levels in late trimester of pregnancy compare with non- pregnant and first and second trimester of pregnancy agreement with the results (Bell et al.,1991). Also, this study similar with Medan et al., (2015) who reported the level of cortisol was significant higher in gestation ewes compared with the non-gestation.

The profile of maternal serum placental lactogen, cortisol, estradiol and progesterone concentration during different stages of pregnancy in the experimental ewe is presented in table 4. Statical analysis in the present study were appeared the level of estradiol is significant differences in first and second trimester of pregnancy compare with non-pregnant, these results disagreement with Ismaeel et al.(,2023) who reported the level of estrogen concentration seemed to decline since second months of pregnancy, reaching its lesser level at fourth months (62.41 ± 6.272 pg/m) , shawetha et al (1017) who recorded increased estradiol levels during late pregnancy period in ewes therefore, these different of estradiol level during different stage of pregnancy may be attributed to estradiol demand for rapid growth of mammary gland at second and late stage stage , estrogen and progesterone synchronized for increase mammary gland development during pregnancy.

Maximum serum level of progesterone in awassi ewes in the present study reached to 32.10 ± 1.08 ng/ml , high level in non-pregnant may be attributed to diestrus phase or other pathological causes such as luteal cyst or persist corpus luteum . the level of progesterone significant differences in first and second trimester compare with late trimester, this result disagreement with Ismaeel et al., (2023) who recorded low level about 16.72 ± 1.46 ng/ml in second and late gestation. While agreement with Mugerwa and Viviani, (1992) who reported higher progesterone levels during mid gestation and lowered in labor progressed these differences in progesterone levels during different stages of pregnancy may contributed to different breed or due to variation in progesterone concentration is affected by, functional efficiency of the corpus luteum and placenta.

Novelty Statement:

This study investigates the hematological and hormonal changes throughout different stages of pregnancy in Awassi sheep, highlighting their critical role in maintaining pregnancy. Disruptions in these parameters can compromise the health of both the ewe and the fetus, potentially endangering fetal survival. This study provides a novel insight into the risk of cross-reactivity between placental lactogen and structurally related hormones in non- pregnant ewes, emphasizing the need for highly specific assay selection in future research to avoid misleading interpretations.

Funding

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Ethical Approval

The Scientific Committee of the College of Veterinary Medicine at the University of Shatrah approved and supported this study. All laws and procedures concerning the handling of animals were reviewed and approved by the Ethical Committee of the College of Veterinary Medicine, University of Shatrah, Thi-Qar



Province, Iraq, and were implemented in accordance with national and institutional guidelines for the care and use of animals in scientific research. Pregnant animals were monitored based on the study results, and recommendations were provided to enhance their welfare and reduce pregnancy-related burdens during the study period.

Generative AI and AI-Assisted Technology Statement

We confirm that no AI-based technologies were used in the preparation or drafting of this manuscript, and that it was solely the result of the authors' own work.

CONFLICT OF INTEREST

The authors have declared no conflict of interest regarding the content of this manuscript

IV. References

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