



Evaluation of serum interleukin differences between pregnant and non-pregnant Iraqi ewes

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Abstract

This study examines the dynamic fluctuations in interleukin-1 α (IL-1 α) serum concentrations in pregnant versus non-pregnant Iraqi ewes during the early stages of gestation. Blood specimens were collected on Days 0, 10, 15, 25, and 30 after breeding, and IL-1 α levels were quantified using an enzyme-linked immunosorbent assay (ELISA). The results indicated a statistically significant elevation ($P < 0.05$) in IL-1 α concentrations in pregnant ewes, with peak levels observed on Day 15 (96.675 ± 2.384 pg/mL) and a subsequent gradual decline. In contrast, non-pregnant ewes exhibited stable IL-1 α levels throughout the observation period. These findings imply that IL-1 α may play a crucial role in the initiation of pregnancy, potentially facilitating embryo implantation, immune system modulation, and placental development in Iraqi ewes. On Day 15 after mating, ultrasound examination revealed that the uterine horns contained distinct, anechoic, fluid-filled structures that resembled early gestational sacs. Echogenic uterine walls encircled these anechoic vesicles, suggesting early intrauterine fluid accumulation, which is typical of conceptus formation. A well-defined anechoic gestational sac with a core hyperechoic structure suggestive of an embryo was discovered on Day 25 of gestation. The internal features of the echogenic structure were organized and reflected embryonic development. A more pronounced, well-formed gestational sac with a clear internal structure was identified by further inspection around Day 30. The observed increase in the embryo's size and echogenicity was consistent with expected trends in fetal growth. This study provides essential insights into reproductive immunology in sheep and underscores the critical role of cytokine regulation during the early gestational period. Further research is necessary to elucidate the underlying mechanisms and potential applications of these findings for enhancing fertility management in sheep populations.

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Introduction

The ewe (*Ovis aries*), an essential domesticated ruminant, is valued globally for its production of meat (lamb), milk, wool, and leather. Substantial evidence suggests sheep were among the first species domesticated by humans. In ewe lambs, puberty onset depends on the age at first estrus and the accumulation of adequate body fat and muscle mass. Consequently, the onset of puberty in ewe lambs transpires when they attain 55-70% of the body weight

characteristic of the mature ewe (1). The hormonal regulation of the estrous cycle in ewes involves multifaceted interactions among hormones and environmental factors (2). This cycle, which typically endures for 16-17 days, is predominantly governed by gonadotropin-releasing hormone (GnRH) synthesized in the hypothalamus, which in turn stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (3). Interleukins (ILs) are cytokine proteins initially characterized for mediating communication between

leukocytes (4). They are systematically organized as a gene cluster that comprises two bioactive ligands, IL-1 and its natural receptor antagonist (IL-1ra). Distinct genes encode these three biomolecules, which subsequently bind to two receptor types: type 1 (IL-1R1) and type 2 receptors (IL-1R2). A multitude of cellular entities synthesize IL-1, functioning as a paracrine or autocrine agent on target cells. They perform crucial functions in the activation and differentiation of immune cells, as well as in proliferation, maturation, migration, and cellular adhesion. Furthermore, they exhibit both pro-inflammatory and anti-inflammatory characteristics. Interleukins are integral to reproductive mechanisms in ewes, particularly with respect to ovarian function and immune response modulation. These cytokines, notably interleukin-8 (IL-8) and interleukin-1 (IL-1), are implicated in various reproductive processes, including ovulation, luteinization, and the regulation of immune responses within the reproductive tract, as well as in the governance of ovarian activities such as follicular development, ovulation, and steroidogenesis (5). Their synthesis occurs within ovarian cells, including oocytes, granulosa cells, and theca cells. The presence and functional activity of interleukins within the reproductive system of ewes underscore their critical role in promoting successful reproductive outcomes (6).

An investigation into the roles of interleukins in ewe reproduction is warranted, particularly to assess maternal serum concentrations of pro-inflammatory cytokines (interleukin-1) in both pregnant and non-pregnant ewes.

Materials and methods

Ethical approve

All protocols were in accordance with SECAE guidelines numbered UM.VET.2024.118 dated 1/9/2024.

Animals

This study was conducted at the Nineveh Sheep Experimental Farm between September 24 and November 12, 2024. The experimental animals included 24 healthy, multiparous Awassi ewes, aged 2–4 years, with body weights ranging from 45 to 55 kg. All ewes were non-pregnant and had been dried off before the experiment. The ewes were housed in partially open shade shelters, provided with roughages and a concentrated feed mixture, and given unrestricted access to fresh water. Furthermore, six fertile Awassi rams were employed for estrus detection and natural mating. These rams were isolated from the ewes for six weeks before the initiation of treatments to prevent pre-exposure effects. Details on ewe grouping and treatment protocols are provided in the subsequent section.

Blood sampling and interleukin assay

Blood samples were collected from all ewes on Days 0, 10, 15, 25, and 30 of the experimental periods. Serum was

separated and stored at -20°C until analysis. Interleukin-1 alpha (IL-1 α) concentrations were measured using a species-specific sheep ELISA kit (Shanghai Ideal Medical Technology, China), with an analytical range of 30-1200 ng/L. The enzyme-linked immunosorbent assay (ELISA) procedures were performed in the Immunology Laboratory at the College of Veterinary Medicine, University of Mosul, according to the manufacturer's instructions. IL-1 α levels at each time point (Days 0, 10, 15, 25, and 30) were analyzed statistically to assess temporal changes and differences between experimental groups. The results were expressed as mean \pm standard error (SE), and significance was determined at $P < 0.05$.

Ultrasound equipment and pregnancy diagnosis

A portable real-time B-mode ultrasound device (KAIXIN KX5100, China) was used to diagnose early pregnancy in ewes. The device was equipped with a linear-array transrectal transducer operating at 5.5–7.5 MHz. Transrectal ultrasonographic examinations were performed on Days 15–20 and 25–30 of gestation, following the procedure described by Hariom (7) for early detection of pregnancy. Pregnancy diagnosis was established by identifying echogenic areas representing fetal fluids within the uterine lumen (8). To confirm pregnancy status, a transabdominal ultrasonographic scan was conducted on Day 40 using a 3.5 MHz convex-array transducer, in accordance with protocols standardized for sheep and goats (9,10).

Statistical analysis

The chi-square test was used to compare the metrics of accuracy, sensitivity, and specificity, as well as the predicted positive and negative values generated by SPSS (IBM SPSS Statistics, Version 29.0.2.0). P values that were equal to or less than 0.05 were deemed statistically significant.

Results

Serum IL-1 α concentrations were evaluated at multiple time points (Days 0, 10, 15, 25, and 30) in pregnant Iraqi ewes. But non statistically significant differences were observed in the non-pregnant ewes (those without pregnancy). IL-1 α levels were relatively stable throughout the study, ranging from 60.930 ± 3.630 to 65.137 ± 2.285 pg/mL (Table 1). Within this group, no significant differences ($P > 0.05$) were observed across days. Conversely, when comparing data from pregnant ewes, significant differences ($P < 0.05$) were observed on Days 10, 15, 25, and 30. In contrast, IL-1 α concentrations in pregnant ewes showed notable alterations, characterised by a pronounced increase from Day 0 (62.217 ± 1.971 pg/mL) to Day 25 (96.675 ± 2.384 pg/mL), representing the peak level observed. Subsequently, levels decreased by Day 30 (73.272 ± 1.501 pg/mL). Significant differences ($P < 0.05$) were observed across days,

with Day 15 exhibiting the highest concentration. Intra-group analyses: Pregnant ewes demonstrated notable variations ($P<0.05$), whereas non-pregnant ewes exhibited stable IL-1 α concentrations. Inter-group analyses: Noteworthy differences ($P<0.05$). These findings imply that

the onset of pregnancy in Iraqi ewes induces a substantial elevation in serum IL-1 α , reaching its zenith around Day 15 before progressively declining. Conversely, non-pregnant ewes maintained uniform levels, underscoring the role of IL-1 α in regulating immune responses during early gestation.

Table 1: Serum concentration of IL-1 α in pregnant and non-pregnant ewes

	Day 0	Day 10	Day 15	Day 25	Day 30
Nonpregnant	61.137 \pm 2.218 a	60.930 \pm 3.630 a*	64.480 \pm 3.138 a*	65.137 \pm 2.285 a*	61.823 \pm 2.974 a*
Pregnant	62.217 \pm 1.971 d	78.710 \pm 2.305 c	96.675 \pm 2.384a	84.629 \pm 2.140 b	73.272 \pm 1.501 c

Different horizontal letters indicate significant differences within the same groups across days at $P<0.05$. * Mean significant differences on the same day between different groups at $P<0.05$.

Transrectal ultrasonography was conducted on ewes to ascertain early pregnancy status within the estimated gestational period of 15 days. The ultrasonographic images demonstrated distinct anechoic, fluid-filled structures within the uterine horns of several subjects. These structures exhibited consistency in shape, size, and echotexture with early gestational sacs; anechoic vesicles were well defined and encircled by echogenic uterine walls. These findings, taken together, indicate the presence of early pregnancy in the assessed ewes, characterised by the development of gestational sacs, while embryos remain undetectable at this stage (Figure 1).

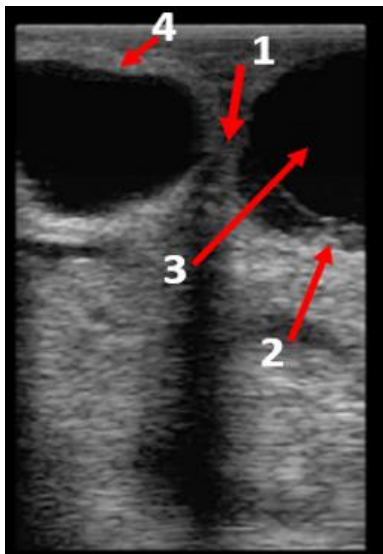


Figure 1: Ultrasonographic image demonstrating a uterine bifurcation (1), within a single early gestational sac (2) in the uterine horn at 15 days post-mating. Visible as echogenic endometrium (4) and a non-echogenic uterine fluid (3).

Subsequent transrectal ultrasonographic evaluation of an additional ewe within the analogous gestational timeframe (approximately 25 days) disclosed a distinctly defined, anechoic gestational sac encompassing a hyperechoic

formation indicative of an embryo or developing fetus. The embryo was situated centrally within the fluid-filled sac and exhibited a more organised internal structure, characterised by an echogenic appearance (Figure 2). Subsequent transrectal ultrasonography performed during the same investigation revealed a single, well-formed gestational sac containing a distinctly outlined echogenic embryonic structure. The embryo appears to increase in size and echogenicity relative to the preceding image, consistent with anticipated progressive fetal development around Day 30 of gestation. The gestational sac maintained an anechoic appearance, characterised by well-defined margins, while the embryo was situated eccentrically within the sac, a finding typical of this developmental stage. The adjacent uterine wall exhibited normal echotexture (Figure 3).



Figure 2: Ultrasonographic image demonstrating an early umbilical cord (1), within an anechoic gestational sac (2) in the uterine horn at 25 days post-mating. Visible as echogenic endometrium (4) and a non-echogenic uterine fluid (3).

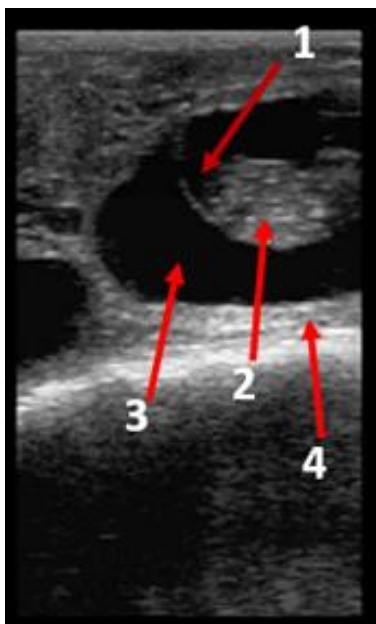


Figure 3: Ultrasonographic image demonstrating an umbilical cord (1), within an anechoic gestational sac (2) in the uterine horn at 30 days post-mating. Visible as echogenic endometrium (4) and a non-echogenic uterine fluid (3).

Discussion

The results of this investigation reveal a notable increase in serum IL-1 α concentrations in gestating Iraqi ewes compared with non-gestating counterparts, with levels peaking at Day 15 of gestation before gradually decreasing. This fluctuating trend indicates that IL-1 α is integral to early gestational processes, presumably influencing maternal immune modulation, embryo adhesion, and placental formation. This variability in the involvement of pro-inflammatory cytokines during the early stages of pregnancy suggests that IL-1 α is essential for maternal immune modulation, embryo implantation, and placental growth in ovine species (11,12). The elevation of IL-1 α by Day 15 aligns with the pivotal phase of embryo fixation and trophoblast penetration in sheep (13). IL-1 α , characterized as a pro-inflammatory cytokine, facilitates endometrial restructuring and immune tolerance through the recruitment of regulatory T cells and the modulation of uterine natural killer cell functionality (14). The subsequent reduction after Day 15 may indicate a transition toward an anti-inflammatory state, which is vital for maintaining pregnancy (15). Comparable investigations of IL-1 α have been reported in other ruminants, such as bovines and caprine, in which the early stages of gestation elicit transient inflammatory responses that facilitate conceptus maturation (16). The stable IL-1 α levels observed in non-gestating ewes indicate a baseline immune activity that is independent of reproductive mechanisms. The marked differences ($P < 0.05$)

between the gestating and non-gestating groups from Day 10 onwards support the proposition that IL-1 α is specifically upregulated in response to pregnancy (17).

Utilized via a transrectal probe during the anticipated early gestational phase (approximately Day 15). The most prominent characteristic is the existence of anechoic, rounded, fluid-filled entities within the uterine cavity, aligning with early gestational vesicles. At this juncture, the embryo is generally not yet observable, as implantation in ovine species transpires approximately between Day 15 and Day 16, with the conceptus (blastocyst) remaining free-floating within the uterine cavity before it adheres to the endometrium (18). The anechoic (black) manifestation of the vesicles is attributable to amniotic fluid, which envelops the developing embryo and precedes the sonographic identification of embryonic structures. The subsequent ultrasound image depicts a later stage of early gestation in an ewe, specifically on Day 25. In contrast to the preceding examination, this image reveals a well-defined anechoic gestational sac that contains a hyperechoic embryonic structure, likely representing a developing embryo or fetus. The recognition of an echogenic structure within the amniotic cavity signifies a transition from the pre-embryonic period, characterized solely by the presence of fluid-filled vesicles, to the embryonic phase, wherein the conceptus becomes sonographically apparent. This developmental milestone corresponds to organogenesis and the initial shaping of the fetus, rendering this stage pivotal for assessing pregnancy viability and predicting developmental progression. The embryonic structure is centrally or slightly eccentrically positioned within the gestational sac, a typical feature of ovine pregnancies. It is surrounded by clear amniotic fluid, indicative of a robust intrauterine environment. Although cardiac motion is not perceptible in a static image, it is expected to become evident in real-time ultrasonography after Day 26, as indicated by prior research (19).

The third ultrasound image depicts a more advanced stage of early gestation in an ewe, approximately on Day 30. This phase is characterized by a well-developed, anechoic gestational sac that harbors a distinctly defined echogenic embryonic structure, indicating continued embryonic maturation after implantation (20). The embryonic structure shows increased size and echogenicity relative to the preceding image, consistent with expected normal morphological progression (21). The rounded morphology, increased dimensions, and well-defined margins are indicative of a viable developing embryo approaching the transition to the fetal phase (22). While cardiac activity is not discernible in this static image, the dimensions and clarity of the embryo can be observed in real-time imaging utilizing high-frequency probes at 5-7.5 MHz (23), as is conventionally documented post day 26-28 (24). At this stage of gestation, ultrasonography achieves greater precision in assessing fetal number, viability, and gestational

age. The embryo depicted in this image is situated eccentrically within the gestational sac, a phenomenon that is characteristic of small ruminants.

Conclusions

The results of this study demonstrate notable changes in blood IL-1 α levels in Iraqi ewes during early gestation, with concentrations peaking around Day 15 before gradually decreasing. These findings indicate that IL-1 α plays an essential role in placental development, embryo implantation, and modulation of the maternal immune response during early pregnancy. In contrast, IL-1 α levels remained stable in non-pregnant ewes, supporting its specific involvement in pregnancy regulation.

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Conflict of interest

None.

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الصدى) ومحددة بوضوح داخل قرني الرحم، وهي متطابقة في الشكل والحجم والتركيب الصدوي مع الأكياس الجنينية المبكرة. وقد كانت هذه الحويصلات محاطة بجدران رحمية ذات صدى عالٍ، مما يشير إلى تراكم ميكروالسوائل داخل الرحم كعلامة على تطور المضغة. في اليوم الخامس والعشرين من الحمل، كشفت التقييمات اللاحقة عن كيس حملي محدد يحتوي على بنية شديدة الصدى (فرط صدى) تقع في مركزه، ما يُشير إلى وجود جنين. وقد أظهرت هذه البنية الداخلية تنظيمًا أكبر في تفاصيلها، مما يعكس تطورًا جنينيًا. وفي اليوم الثلاثين تقريبًا، لوحظ كيس حملي واضح المعالم يحتوي على جنين شديد الصدى وذو حجم أكبر، يقع بشكل غير مركزي داخل الكيس، وهو توزيع شائع في هذه المرحلة من التطور. كما أظهرت جدران الرحم المجاورة تركيبًا صدويًا طبيعيًا. تؤكد هذه النتائج على فاعلية التصوير بالموجات فوق الصوتية عبر المستقيم كوسيلة تشخيصية دقيقة للكشف المبكر عن الحمل ومتابعة تطور الجنين في النعاج تشير هذه النتائج إلى أن الإنترلوكين الأول الفا يلعب دورًا مهمًا في الحمل المبكر، مما قد يساهم في انغراس الجنين وتعديل المناعة وتطور المشيمة لدى النعاج العراقية. تقدم الدراسة رؤى قيمة في علم المناعة التناسلية لدى الأغنام وتسلط الضوء على أهمية تنظيم السيتوكينات خلال فترة الحمل المبكرة. هناك حاجة إلى إجراء المزيد من البحوث لاستكشاف الآليات الأساسية والتطبيقات المحتملة لتحسين إدارة خصوبة الأغنام.

تقييم الفروقات في مستويات الإنترلوكين الفا ١ في مصل الدم بين النعاج العراقية الحوامل وغير الحوامل

عمر خالد حمدون و براء دريد الوتار

فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

بحثت هذه الدراسة في التغيرات الديناميكية في مستويات الإنترلوكين- الفا في مصل النعاج العراقية الحوامل مقارنة بالنعاج غير الحوامل خلال فترة الحمل المبكرة. جُمعت عينات الدم في الأيام ٠ و ١٠ و ٢٥ و ٣٠ بعد التكاثر، وقيست تركيز الإنترلوكين الأول الفا باستخدام اختبار الاليزا. كشفت النتائج عن زيادة ملحوظة في مستوى الإنترلوكين الأول الفا لدى النعاج الحوامل، وبلغت ذروتها في اليوم ١٥ ($96,675 \pm 2,384$ بيكوغرام/مل) قبل أن تنخفض تدريجيًا، بينما حافظت النعاج غير الحوامل على مستويات ثابتة طوال فترة الدراسة. أظهرت الفحوصات بالموجات فوق الصوتية التي أجريت في اليوم الخامس عشر بعد التلقيح وجود تراكيب مملوءة بسائل شفاف (عديم