Effect of Peri-Uterine Fat Deposition on Placental Efficiency and Pregnancy Indicators at Different Gestational Stages in Arabi Ewes Using Ultrasonography

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Abstract

This study was conducted to evaluate the effect of age and gestational stage on reproductive adipose tissue deposition and its relationship with placental efficiency and some pregnancy indicators in Arabi ewes, as well as to assess the feasibility of using transabdominal ultrasonography as a non-invasive method for estimating reproductive adipose tissue. The study was carried out on 27 Arabi ewes distributed across three age groups (1.5, 2.5, and >3.5 years) during the three gestational stages. The results revealed a significant increase ($P \leq 0.05$) in uterine weight, reproductive adipose tissue weight (peri-uterine and peri-ovarian), placental weight, and fetal weight with advancing age and gestational stage, with the highest values recorded in the oldest ewes during the third gestational stage. Despite the significant decrease in cotyledonary number with advancing age and gestational stage, the total placental surface area increased significantly, indicating a functional compensatory mechanism that contributes to maintaining maternal-fetal exchange efficiency. The results also showed that placental efficiency increased with advancing gestational stage, while it decreased with increasing ewe age, with the highest values recorded in the 1.5-year-old age group. The interaction between age and gestational stage had a significant effect on reproductive adipose tissue deposition, with percentages ranging from 0.76% to 1.12% of carcass weight, corresponding to 140.7–247.4 grams. Ewes older than 3.5 years during the third gestational stage achieved normal pregnancy with normal fetal weights (3633.7 g), without any signs of ovarian cysts or hormonal disorders. Transabdominal ultrasonography demonstrated the feasibility of evaluating reproductive adipose tissue area surrounding the uterus and ovaries during early and mid-pregnancy, with clear variation according to ewe age and gestational stage. Post-slaughter anatomical measurements were consistent with ultrasonographic estimates, supporting the potential use of ultrasonography as a non-invasive tool for assessing reproductive adipose tissue deposition in Arabi ewes. The evaluation did not include the late gestational stage due to the fetus occupying most of the uterine cavity, which limited the clarity of the surrounding reproductive tissues valuation during late gestation was not feasible because the fetus occupied most of the uterine cavity, limiting visualization of the surrounding reproductive tissu These findings i valuation during late gestation was not feasible because the fetus occupied most of the uterine cavity, limiting visualization of the surrounding reproductive tissu ndicate the pivotal role of reproductive adipose tissue as a structural and metabolic support for the uterus, contributing to placental development (placentogenesis) and fetal growth. Furthermore, ultrasonographic assessment of reproductive adipose tissue represents a promising biomarker for monitoring pregnancy competence and placental development in Arabi ewes. This parameter may therefore be incorporated into reproductive management programs to improve reproductive efficiency and flock productivity in sheep.

Keywords: Arabi ewes, placental efficiency, reproductive adipose tissue, ultrasonography, gestational stage.

1- INTRODUCTION

Sheep are considered one of the most important sources of animal protein, and local breeds such as Arabi ewes (Ovis aries) are distinguished by their remarkable adaptability to harsh environmental conditions, making them a cornerstone of food security (Kalds *et al*., 2022 Barłowska *et al*., 2025). However, the reproductive efficiency of these breeds, measured by the number of lambs born, faces multiple challenges that are not only related to genetic factors but are also subject to complex internal physiological influences, foremost among which are adipose tissue distribution and hormonal balance (Xiao & Zhang, 2002). The Arabi ewes, as a fat-tailed breed, hold particular importance in this context, as fat deposition is concentrated in the visceral adipose tissues surrounding the internal organs, a pattern that may exert distinctive effects on the uterine environment and fetal growth (Yagoubi *et al*., 2024; Mastrangelo *et al*., 2019).

The concept of adipose tissue has shifted from being merely an energy store to an active endocrine organ, secreting adipokines (such as leptin) that influence metabolic and reproductive processes through regulation of the hypothalamic-pituitary-ovarian axis (Xu *et al*., 2023; Al-Thuwaini *et al*., 2024). The adipose tissue deposited in the pelvic region, known as peri-uterine white adipose tissue, holds particular significance; it acts as a regulator of uterine blood circulation and hormonal balance, thereby preparing a favorable physiological environment for pregnancy (Osikoya *et al*., 2024). Studies indicate that pregnancy induces dynamic changes in this tissue, with visceral fat stores decreasing in late gestation as a rapid energy source, reflecting a direct relationship with placental function (Marie *et al*., 2010). Ewe age is also a pivotal factor in this interaction; younger ewes are still in the growth phase, creating competition with pregnancy demands, while mature ewes tend to store greater visceral fat to support pregnancy and lactation (Nageye *et al*., 2024; Alonso-García *et al*., 2025).

The placenta emerges as a central organ linking the mother and the fetus, as its efficiency (weight and cotyledonary number) reflects its capacity to transfer oxygen and nutrients, and is positively correlated with lamb birth weight (Şen, 2021). Recent studies have shown that a balance in the amount of visceral adipose tissue surrounding the uterus affects pregnancy success, with ewes having moderate fat levels and high uterine tension recording higher pregnancy rates (Spanner *et al*., 2024). Transabdominal ultrasonography is a precise and non-invasive technique for assessing adipose tissue deposition, in addition to being a pivotal tool for pregnancy diagnosis and measuring placental blood flow indices, making it an ideal method for linking maternal metabolic status with placental efficiency (Theron *et al*., 2023; Brzozowska *et al*., 2022).

Despite advances in understanding adipose tissue functions, studies addressing the direct relationship between fat deposition specifically in the reproductive organs (surrounding the uterus and ovaries) and placental parameters (efficiency and weight) in local fat-tailed sheep breeds (Arabi ewes), while considering the age factor, remain scarce. This gap limits the understanding of the precise mechanisms linking maternal metabolic status with placental function, and restricts the development of practical indicators for improving fertility in these valuable breeds.

This study aims to investigate the relationship between reproductive adipose tissue deposition and some pregnancy parameters in Arabi ewes at different age stages, through the following:

1.Evaluating the effect of reproductive adipose tissue deposition on placental characteristics (weight, efficiency, cotyledonary number) during different gestational stages.

2.Determining the role of ewe age in modulating the relationship between reproductive adipose tissue deposition and pregnancy parameters.

3.Exploring the potential use of ultrasonographically measured reproductive adipose tissue indices as predictive tools for placental efficiency and fetal growth.

2- MATERIALS AND METHODS

Animals and Experimental Design

This study was conducted at the Animal Farm of the College of Agriculture, University of Basrah, during the period from October 7, 2024, to April 10, 2025, with a 14-day adaptation period. A total of 27 Arabi ewes were used in the experiment and divided into three equal groups (9 ewes per group) according to age as follows:

Group 1: 1.5 years old, with a mean body weight of 31 ± 2 kg.

Group 2: 2.5 years old, with a mean body weight of 39 ± 2 kg.

Group 3: >3.5 years old, with a mean body weight of 43 ± 2 kg.

Ages were estimated using the dentition (tooth eruption) method according to Payne (1987) and Deal (2023). The ewes were housed in semi-open pens (4 × 4 m per group) and identified with plastic numbered neck tags. Three fertile rams aged 2–3 years were used for natural mating and were kept in individual pens.

Feeding

All groups were fed a uniform concentrated diet at a rate of 3% of body weight, starting two weeks before estrus synchronization and continuing for three weeks thereafter. The diet was offered in two equal meals daily (morning and evening), and the amount was adjusted weekly according to changes in body weight. The diet contained 14–16% crude protein and 2500–2700 kcal/kg metabolizable energy, formulated according to NRC (2007). Hay, mineral salt blocks, and fresh water were provided *ad libitum* throughout the experimental period. A preventive and therapeutic health program was applied under veterinary supervision.

Estrus Synchronization and Natural Mating

A hormonal protocol was applied for estrus synchronization according to Hajibemani *et al*., (2022) and Duan *et al*., (2025), as follows:

1.Day 0: Intravaginal sponges (Ovachron®) containing 40 mg of fluorogestone acetate (FGA) were inserted for 12 days.

2.48 hours before sponge removal: Each ewe was injected with cloprostenol (Alfaglandin C®) at a dose of 0.5–1 ml per ewe, depending on body weight.

3.Day 12: Each ewe received an intramuscular injection of pregnant mare serum gonadotropin (PMSG; Foligon®) at a dose of 400–750 IU per ewe.

Estrus was detected within 48–72 hours using teaser rams, and the detection rate was 100%. Natural mating was then performed (one ram per 9 ewes for one week). The rams were supplemented with vitamin E (400–800 IU/day) 48 hours before mating. Estrus was re-checked 21 days later to confirm that it had not returned.

Pregnancy Diagnosis and Monitoring

Pregnancy diagnosis and monitoring were performed using an ultrasound device (F6 Vet Ultrasound) equipped with a convex probe at a frequency of 3.5 MHz to obtain clear images for pregnancy diagnosis and adipose tissue layers, which appeared as dark (hypoechoic) areas. Examinations were conducted at three stages:

Day 40 of pregnancy

Day 75–80 of pregnancy

Day 135–140 of pregnancy

Ultrasound images were used to estimate reproductive adipose tissue deposition according to Ramos-Ramos *et al*. (2024) and Cuesta *et al*. (2025), following these steps:

1. Ultrasound images were saved on USB flash drives after completion of the pregnancy examination.
2. Images were transferred to a computer for digital analysis.
3. Images were opened using ImageJ software, specialized for medical image analysis.
4. The region of interest (ROI) representing adipose tissue visible within the broad ligament of the uterus was defined using the selection tool.
5. Thresholding and masking techniques were applied to distinguish adipose tissue areas and separate them from surrounding tissues.
6. Adipose tissue area was calculated based on the number of pixels within the defined region.
7. Fat area was estimated as a percentage of the total examined area.

Percentage of reproductive adipose tissue (%) $=\frac{Adipose\ tissue\ area\ 2\ \times 100}{Visible\ uterine\ area\ 1}$

Slaughtering, Carcass, and Organ Measurements

After pregnancy confirmation, three ewes from each group were randomly selected and slaughtered at the first stage of pregnancy (day 40). The remaining ewes continued their pregnancies, and then three ewes from each group were randomly selected at the second stage (day 75–80). The remaining ewes were slaughtered at the third stage (day 135–140).

Feed was withheld for 12 hours before slaughter, while water remained available until the time of slaughter. Slaughtering was performed according to Islamic rites (Halal method) by cutting the four jugular vessels with a sharp knife. Immediately after complete exsanguination, carcasses were weighed.

A total of 3 ewes were randomly slaughtered from each age group at each gestational stage (3 ages × 3 stages × 3 animals). Food was withheld for 12 hours before slaughter while water was continuously provided. Slaughtering was performed according to Islamic law, and organs were dissected after complete exsanguination.

Visceral adipose tissues were manually separated, and the weights of reproductive adipose tissues were recorded as follows:

Peri-uterine adipose tissue: Visceral adipose tissue surrounding the uterus in the pelvic cavity region, located along the broad ligament of the uterus and surrounding the ovaries, was weighed according to Al-Mujammai, (2011) and Knupp *et al.* (2021).

Calculation of Adipose Tissue Percentages

Visceral fat percentages were calculated according to the method described by Al-Sherwany & Alkass (2021) as follows:

Percentage of peri-uterine fat = (Weight of peri-uterine fat ÷ Carcass weight) × 100

Weights of Reproductive Organ Components and Fetal Measurements

The reproductive organs were weighed using a sensitive balance accurate to two decimal places, and the measurements included the following:

1.Uterine weight: A midline longitudinal abdominal incision was made along the linea alba, extending from the region near the pubic bone up to the diaphragm, to extract the uterus, placenta, and fetus. After separating the uterus at the level of the cervix, the total uterine weight was recorded.

2.Amniotic fluid weight: A longitudinal incision was made along the uterine horn containing the fetus, after which the fetus was removed. The fetal fluids (amniotic fluid) were collected in a sterile container and weighed separately using the sensitive balance.

3.Fetal measurements: Fetal measurements, including weight and length, were recorded. Weight was measured using a digital scale, while length was measured as the crown–rump length (CRL), according to Stieger-Vanegas and McKenzie (2021)

MEASUREMENT OF PLACENTAL PARAMETERS

Cotyledonary Number

The number of placental cotyledons was counted after manual separation from the chorioallantoic membrane according to the method described by Erichsen *et al.* (2024).

Cotyledonary Surface Area

The dimensions of each cotyledon (length, CL and width CW,) were measured using a digital vernier caliper according to the method described by Şen (2021). The following formula was applied: Cotyledon surface area (cm^2)

$ACSA = \pi \times \left(\frac{CL}{2}\right) \times \left(\frac{CW}{2}\right)$

Mean Cotyledonary Area

The mean cotyledonary area per placenta was estimated by identifying the cotyledon with the largest surface area and the one with the smallest surface area, then calculating the arithmetic mean between them according to Özyürek, & Türkyilmaz, (2020) using the following equation:

Mean cotyledonary area = (Area of largest cotyledon + Area of smallest cotyledon) ÷ 2

$ACSA = \frac{ACSAmax + ACSA\ min}{2}$

Total Placental Surface Area

Total placental (cotyledonary) surface area was estimated by multiplying the total number of cotyledons by the mean cotyledonary surface area, according to the following equation:

TCSA = ACSA × TCN

where TCSA is the total placental surface area, ACSA is the average cotyledonary surface area, and TCN is the total number of cotyledons.

Total placental surface area was considered an indicator of placental functional efficiency.

Placental Functional Efficiency

Placental efficiency was estimated using the equation according to Redifer et al. (2021) as follows:

Placental efficiency (g/cm^2) = Fetal weight (g) ÷ Total cotyledonary surface area (cm^2)

$Placental\ Efficiency = \frac{Fetal\ Weight}{Placenta\ surface\ area}$

In this study, placental efficiency was defined as fetal weight per unit of total cotyledonary surface area (g/cm^2), according to Redifer et al. (2021). This parameter reflects the functional capacity of the cotyledonary placenta to support fetal growth per unit of exchange surface area.

Statistical Analysis

Data were analyzed using the statistical software package SPSS (Version 19). Due to the factorial nature of the experimental design, a two-way analysis of variance (Two-way ANOVA) was adopted to study the effects of age, physiological stage, and their interaction (Age × Physiological Stage) on the studied traits.

Prior to analysis, data were checked for ANOVA assumptions, including: Normality using the Shapiro–Wilk test..1

2.Homogeneity of variances using Levene's test.

Differences were considered significant at a probability level of $P \leq 0.05$. When significant differences were detected among groups, the Least Significant Difference (LSD) test was used for post-hoc comparisons to determine the specific differences between factor levels. Results were presented as Mean ± Standard Error (SE). Factorial effects were presented in ANOVA tables.

The mathematical model used for data analysis was as follows:

$$Y_{ijk} = \mu + A_i + P_j + (AP)_{ij} + e_{ijk}$$

Y_{ijk} = the observed value of the trait for the kth animal within the ith age group and the jth physiological stage.

μ = the overall mean.

A_i = the effect of age i = (1.5, 2.5, and ≥ 3.5 years).

P_j = the effect of physiological stage (j = before estrus, after estrus, first stage of pregnancy, second stage of pregnancy, and third stage of pregnancy.

$(AP)_{ij}$ = the interaction effect between age and physiological stage.

e_{ijk} = the random error.

3- RESULTS

The effects of ewe age, gestational stage, and their interaction on uterine weight are presented in Arabi Ewes, in Table (1). Uterine weight was significantly influenced ($P \leq 0.05$) by all studied factors. A progressive and significant increase in uterine weight was observed with advancing gestational stage, with mean values of 595 ± 63.97, 2232 ± 156.14, and 6557 ± 160.06 g for the first, second, and third stages, respectively. Similarly, maternal age had a significant effect, as older ewes (>3.5 years) exhibited the highest uterine weight (3549.7 ± 937.16 g) compared with younger ewes (1.5 years), which showed the lowest value (2747.2 ± 874.18 g). Furthermore, a significant interaction between age and gestational stage was detected; the greatest uterine weight was recorded in the oldest ewes at the third gestational stage (7119.7 ± 69.29 g), while the lowest value was observed in the youngest ewes during the first stage (369.7 ± 13.29 g).

Table (1). Effect of ewe age and pregnancy stage on uterine weight (g) (Mean ± SE)

pregnancy stage	uterine weight (g) Age of ewes(years)			Mean Effect of pregnancy Stage
	1.5	2.5	>3.5	
Early pregnancy	369.7 ± 13.29	629.0 ± 52.04	788.7 ± 38.87	595.0 ± 63.97^{c}
Mid pregnancy	1723.7 ± 69.29	2233.0 ± 124.66	2741.0 ± 116.20	2232.0 ± 156.14^{b}
Late pregnancy	6148.3 ± 96.90	6404.3 ± 115.34	7119.7 ± 69.29	6557.0 ± 160.06^{a}
Mean Effect of Age	2747.2 ± 874.18^{c}	3088.7 ± 861.95^{b}	3549.7 ± 937.16^{a}	**LSD = 508**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters (a–c) within the same row or column are significantly different at $P \leq 0.05$. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 508

Table (2) presents the effects of ewe age, gestational stage, and their interaction on visceral reproductive adipose tissue weight (peri-uterine and peri-ovarian fat) in Arabi Ewes

Effect of gestational stage: Adipose tissue weight increased significantly ($P \leq 0.05$) with advancing pregnancy. The lowest mean value was recorded at the first stage (198.7 ± 16.21 g), while significant increases were observed at the second (214.1 ± 16.13 g) and third (247.4 ± 4.53 g) stages, with no significant difference between the second and third stages.

Effect of ewe age: Adipose tissue weight increased significantly ($P \leq 0.05$) with increasing maternal age.

The youngest ewes (1.5 years) had the lowest mean value (177.3 ± 16.35 g), while the oldest ewes (>3.5 years) showed the highest value (253.0 ± 4.46 g). Ewes aged 2.5 years exhibited intermediate values (229.9 ± 5.98 g)

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest adipose tissue weight was recorded in the oldest ewes during the third gestational stage (262.0 ± 3.93 g), whereas the lowest value was observed in the youngest ewes during the first stage (140.7 ± 18.22 g). Notably, older ewes maintained relatively higher adipose tissue deposits across all gestational stages compared with younger ewes.

Table (2). Effect of ewe age and pregnancy stage on the weight of peri-uterine and peri-ovarian fat (g) (Mean ± SE)

pregnancy stage	peri-uterine fat(g)			Mean Effect of pregnancy Stage
	Age of ewes(years)			
	1.5	2.5	>3.5	
Early pregnancy	140.7 ± 18.22	212.3 ± 3.75	243.0 ± 7.00	198.7 ± 16.21[b]
Mid pregnancy	153.3 ± 8.66	235.3 ± 11.05	253.6 ± 8.81	214.1 ± 16.13[a]
Late pregnancy	238.0 ± 1.15	241.7 ± 7.26	262.0 ± 3.93	247.4 ± 4.53[a]
Mean Effect of Age	177.3 ± 16.35[c]	229.9 ± 5.98[b]	253.0 ± 4.46[a]	**LSD = 50.33**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters (a–c) within the same row or column are significantly different at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 50.33

Table (3) presents the effects of ewe age, gestational stage, and their interaction on placental weight in Arabi Ewes. Effect of gestational stage: Placental weight was significantly (P≤0.05) affected by gestational stage, showing a progressive and significant increase with advancing pregnancy. The lowest mean value was recorded at the first stage (38.5 ± 3.50 g), followed by a sharp increase at the second stage (445.7 ± 70.79 g), and reaching the highest value at the third stage (574.4 ± 29.65 g).) Effect of ewe age: Placental weight increased significantly (P≤0.05) with increasing maternal age. The youngest ewes (1.5 years) had the lowest mean placental weight (263.9 ± 2.87 g), while the oldest ewes (>3.5 years) showed the highest value (405.4 ± 10.42 g). Ewes aged 2.5 years exhibited intermediate values (389.0 ± 9.21 g), which were close to those of the oldest group.

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest placental weight was recorded in the oldest ewes during the third gestational stage (642.0 ± 23.09 g), whereas the lowest value was observed in the youngest ewes during the first stage (24.7 ± 1.39 g). Notably, the differences in placental weight among age groups became more pronounced during the second and third stages compared with the first stage.

Table (3). Effect of ewe age and pregnancy stage on placental weight (g) (Mean ± SE)

pregnancy stage	placental weight (g)			Mean Effect of pregnancy Stage
	Age of ewes(years)			
	1.5	2.5	>3.5	
Early pregnancy	24.7 ± 1.39	44.1 ± 1.12	46.67 ± 0.88	38.5 ± 3.50[c]
Mid pregnancy	304.6 ± 1.76	504.7 ± 5.85	527.6 ± 7.31	445.7 ± 70.79[b]
Late pregnancy	462.3 ± 5.48	619.0 ± 20.64	642.0 ± 23.09	574.4 ± 29.65[a]
Mean Effect of Age	263.9 ± 2.87[c]	389.0 ± 9.21[b]	405.4 ± 10.42[a]	**LSD = 65.33**

Table note: Values are expressed as Mean ± Standard Error (SE). Means followed by different superscript letters (a–c) within the same row or column are significantly different at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 65.33

Table (4) presents the effects of ewe age, gestational stage, and their interaction on fetal weight in in Arabi Ewes. Effect of gestational stage: Fetal weight was significantly (P≤0.05) affected by gestational stage, showing a progressive and dramatic increase with advancing pregnancy. The lowest mean value was recorded at the first stage (13.3 ± 2.08 g), followed by a substantial increase at the second stage (268.7 ± 23.97 g), and reaching the highest value at the third stage (3378.7 ± 157.30 g). The greatest increment occurred between the second and third stages

Effect of ewe age: Fetal weight increased significantly (P≤0.05) with increasing maternal age. The youngest ewes (1.5 years) had the lowest mean fetal weight (1107.9 ± 486.23 g), while the oldest ewes (>3.5 years) showed the highest value (1318.3 ± 580.71 g). Ewes aged 2.5 years exhibited intermediate values (1233.7 ± 547.64 g) and did not differ significantly from either group.

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest fetal weight was recorded in the oldest ewes during the third gestational stage (3633.7 ± 70.14 g), whereas the lowest value was observed in the youngest ewes during the first stage (10.3 ± 1.18 g). Notably, differences in fetal weight among age groups became more pronounced during the third stage compared with the first and second stages.

Table (4). Effect of ewe age and pregnancy stage on fetal weight (g) (Mean ± SE)

pregnancy stage	**fetal weight (g)**			**Mean Effect of pregnancy Stage**
	Age of ewes(years)			
	1.5	**2.5**	**>3.5**	
Early pregnancy	10.3 ± 1.18	12.3 ± 0.88	17.3 ± 0.67	13.3 ± 2.08c
Mid pregnancy	222.7 ± 0.88	277.3 ± 24.18	304.0 ± 25.58	268.7 ± 23.97b
Late pregnancy	3091.0 ± 135.27	3411.6 ± 153.26	3633.7 ± 70.14	3378.7 ± 157.30a
Mean Effect of Age	1107.9 ± 486.23^{b}	1233.7 ± 547.64^{ab}	1318.3 ± 580.71^{a}	**LSD = 542.66**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters (a, b, ab) within the same row or column differ significantly at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 542.66

Table (5) presents the effects of ewe age, gestational stage, and their interaction on the number of placental cotyledons in Arabi ewes. Effect of gestational stage: Cotyledonary number was significantly (P≤0.05) affected by gestational stage, showing a progressive decrease with advancing pregnancy. The highest number of cotyledons was recorded at the first stage (58.9 ± 2.68 cotyledons), followed by a significant decrease at the second stage (45.3 ± 5.29 cotyledons), and reaching the lowest value at the third stage (41.3 ± 6.61 cotyledons).

Effect of ewe age: Cotyledonary number was significantly (P≤0.05) influenced by maternal age. The youngest ewes (1.5 years) had the highest number of cotyledons (54.9 ± 3.55 cotyledons), while the oldest ewes (>3.5 years) showed the lowest number (43.1 ± 2.94 cotyledons). Ewes aged 2.5 years exhibited intermediate values (47.5 ± 3.42 cotyledons) and did not differ significantly from the oldest group.

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest number of cotyledons was recorded in the youngest ewes during the first gestational stage (66.7 ± 5.93 cotyledons), whereas the lowest number was observed in the oldest ewes during the third stage (36.0 ± 1.33 cotyledons). Notably, the differences in cotyledonary number among age groups were more pronounced during the first stage compared with the second and third stages.

Table (5). Effect of ewe age and pregnancy stage on cotyledonary number (Mean ± SE)

pregnancy stage	**cotyledonary number**			**Mean Effect of pregnancy Stage**
	Age of ewes(years)			
	1.5	**2.5**	**>3.5**	

Early pregnancy	66.7 ± 5.93	57.3 ± 0.66	52.7 ± 0.67	58.9 ± 2.68[c]
Mid pregnancy	52.0 ± 1.16	43.3 ± 0.67	40.7 ± 0.66	45.3 ± 5.29[b]
Late pregnancy	46.0 ± 1.15	42.0 ± 1.15	36.0 ± 1.33	41.3 ± 6.61[a]
Mean Effect of Age	54.9 ± 3.55[b]	47.5 ± 3.42[a]	43.1 ± 2.94[a]	**LSD = 11.33**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters within the same row or column differ significantly at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 11.33

Table (6) presents the effects of ewe age, gestational stage, and their interaction on the total placental surface area (cm²) in Arabi ewes. Effect of gestational stage: Total placental surface area was significantly (P≤0.05) affected by gestational stage, showing a progressive increase with advancing pregnancy. The lowest mean value was recorded at the first stage (221.61 ± 19.8 cm²), followed by a significant increase at the second stage (283.33 ± 12.2 cm²), and reaching the highest value at the third stage (423.93 ± 10.26 cm²)

Effect of ewe age: Total placental surface area increased significantly (P≤0.05) with increasing maternal age. The youngest ewes (1.5 years) had the lowest mean surface area (268.66 ± 36.6 cm²), while the oldest ewes (>3.5 years) showed the highest value (350.65 ± 26.07 cm²). Ewes aged 2.5 years exhibited intermediate values (309.24 ± 29.6 cm²))

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest total placental surface area was recorded in the oldest ewes during the third gestational stage (447.59 ± 18.24 cm²), whereas the lowest value was observed in the youngest ewes during the first stage (161.88 ± 13.90 cm²). Notably, the differences in placental surface area among age groups became more pronounced during the second and third stages compared with the first stage.

Table (6). Effect of ewe age and pregnancy stage on placentome surface area (cm²) (Mean ± SE)

pregnancy stage	**Placental surface area (cm²)**			**Mean Effect of pregnancy Stage**
	Age of ewes(years)			
	1.5	**2.5**	**>3.5**	
Early pregnancy	161.88 ± 13.90	216.92 ± 3.24	286.04 ± 22.40	221.61 ± 19.80[c]
Mid pregnancy	237.84 ± 8.31	293.85 ± 2.22	318.31 ± 3.83	283.33 ± 12.20[b]
Late pregnancy	406.26 ± 14.30	417.95 ± 16.60	447.59 ± 18.24	423.93 ± 10.26[a]
Mean Effect of Age	268.66 ± 36.60[c]	309.24 ± 29.60[b]	350.65 ± 26.07[a]	**LSD = 21.01**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters within the same row or column differ significantly at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 21.01

Table (7) presents the effects of ewe age, gestational stage, and their interaction on placental efficiency (g/cm²) in Arabi ewes.

Effect of gestational stage: Placental efficiency was significantly (P≤0.05) affected by gestational stage, showing a slight non-significant increase between the first (0.059 ± 0.002 g/cm²) and second (1.178 ± 0.105 g/cm²) stages, followed by a dramatic and significant increase at the third stage (10.263 ± 0.871 g/cm²). This substantial increase reflects the exponential fetal growth during late pregnancy relative to the moderate increase in placental surface area.

Effect of ewe age: Placental efficiency was significantly (P≤0.05) influenced by maternal age. The youngest ewes (1.5 years) exhibited the highest placental efficiency (4.771 ± 2.39 g/cm²), while the oldest ewes (>3.5 years) showed the lowest efficiency (3.654 ± 1.78 g/cm²). Ewes aged 2.5 years demonstrated intermediate values (3.075 ± 1.53 g/cm²). This indicates that younger ewes have a more efficient placenta per unit of surface area compared with older ewes.

Interaction effect: A significant interaction (P≤0.05) between age and gestational stage was detected. The highest placental efficiency was recorded in the youngest ewes during the third gestational stage (13.266 ± 0.64 g/cm^2), whereas the lowest value was observed in the oldest ewes during the first stage (0.054 ± 0.002 g/cm^2). This suggests that younger ewes outperform older ewes in placental efficiency during late pregnancy

Table (7). Effect of ewe age and pregnancy stage on placental efficiency (Mean ± SE)

pregnancy stage	placental efficiency Age of ewes(years) 1.5	2.5	>3.5	Mean Effect of pregnancy Stage
Early pregnancy	0.065 ± 0.01	0.057 ± 0.002	0.054 ± 0.002	0.059 ± 0.002^c
Mid pregnancy	0.981 ± 0.04	1.004 ± 0.08	1.550 ± 0.274	1.178 ± 0.105^b
Late pregnancy	13.266 ± 0.64	8.163 ± 0.543	9.359 ± 0.339	10.263 ± 0.871^a
Mean Effect of Age	4.771 ± 2.39^b	3.075 ± 1.53^a	3.654 ± 1.78^a	**LSD = 0.447**

Table note: Values are presented as Mean ± Standard Error (SE). Means followed by different superscript letters within the same row or column differ significantly at P ≤ 0.05. The least significant difference for the interaction (Pregnancy Stage × Age of Ewes) was LSD = 0.447.

Table (8) presents the effects of ewe age, gestational stage, and their interaction on carcass weight (kg) and the percentage of reproductive adipose tissue (% of carcass weight) in Arabi ewes.

Carcass weight:

Carcass weight increased significantly (P≤0.05) with increasing maternal age. The oldest ewes (>3.5 years) had the highest carcass weights across all gestational stages, while the youngest ewes (1.5 years) had the lowest values.

Gestational stage did not show a clear significant effect on carcass weight within each age group, as carcass weights remained relatively similar across different stages for the same age.

The highest carcass weight was recorded in the oldest ewes during the first stage (24.00 ± 0.44 kg), while the lowest value was observed in the youngest ewes during the first stage (17.33 ± 0.67 kg).

Percentage of reproductive adipose tissue:

The percentage of reproductive adipose tissue increased significantly (P≤0.05) with increasing maternal age. The oldest ewes (>3.5 years) showed the highest percentages across all gestational stages (ranging from 1.02% to 1.12%), while the youngest ewes (1.5 years) showed the lowest percentages (ranging from 0.76% to 1.03%))

A slight increase in the percentage of reproductive adipose tissue was observed with advancing gestational stage within each age group, with higher percentages recorded at the third stage compared with the first and second stages; however, this increase was not significant in most groups.

A significant interaction (P≤0.05) between age and gestational stage was detected. The highest percentage of reproductive adipose tissue was recorded in the oldest ewes during the third gestational stage (1.12%), while the lowest value was observed in the youngest ewes during the first stage (0.76%). Overall, older ewes consistently maintained higher reproductive adipose tissue percentages across all gestational stages compared with younger ewes.

Table (8). Effect of ewe age and pregnancy stage on the percentage of reproductive fat in the carcass (Mean ± SE)

Age Group and Pregnancy Stage	Carcass Weight (kg)	Reproductive Organ Fat (%)

1.5 years – First pregnancy	17.33 ± 0.67^{c}	0.76 ± 0.10^{b}
2.5 years – First pregnancy	20.33 ± 0.30^{b}	1.00 ± 0.03^{b}
>3.5 years – First pregnancy	24.00 ± 0.44^{a}	1.02 ± 0.05^{a}
1.5 years – Second pregnancy	18.30 ± 0.56^{b}	0.82 ± 0.04^{b}
2.5 years – Second pregnancy	22.33 ± 0.30^{a}	1.08 ± 0.02^{a}
>3.5 years – Second pregnancy	23.00 ± 0.58^{a}	1.09 ± 0.05^{a}
1.5 years – Third pregnancy	18.67 ± 0.67^{b}	1.03 ± 0.01^{b}
2.5 years – Third pregnancy	21.50 ± 0.58^{a}	1.10 ± 0.01^{a}
>3.5 years – Third pregnancy	22.67 ± 0.30^{a}	1.12 ± 0.01^{a}

Note: Values are presented as **Mean ± Standard Error (SE)**. Means bearing different superscript letters within the same column or row differ significantly at **$P \leq 0.05$**.

DISCUSSION

Tables 1 and 2 show that ewe age, gestational stage. The present study demonstrated a significant effect ($P \leq 0.05$) of ewe age, gestational stage, and their interaction on uterine weight and reproductive adipose tissue. Uterine weight increased progressively with advancing pregnancy and with increasing maternal age, which is consistent with the findings of Younis & Aboud (2023) and Sadiq (2025), who reported that uterine enlargement during pregnancy results from myometrial hypertrophy and increased blood perfusion(Angiogenesis) under the influence of estrogen and progesterone, which stimulate angiogenesis and enhance uteroplacental blood flow to meet the increasing fetal demands.

Regarding reproductive adipose tissue, the results showed a notable increase with both age and gestational stage, which aligns with Shao *et al.* (2024) and Liu *et al*. (2024), who indicated that visceral adipose tissue in fat-tailed breeds is significantly influenced by age, with both hyperplasia and hypertrophy of adipocytes occurring during maturation, leading to increased reproductive fat deposition around the uterus and ovaries.

These observations are further supported by the carcass reproductive adipose tissue data presented in Table 8 was observed with advancing pregnancy across all age groups, suggesting a role for these fat depots as an energy reserve to support the fetus during late pregnancy, which is consistent with Knupp et al. (2021), who reported that visceral fat around the uterus serves as a strategic energy store that can be mobilized during periods of high nutritional demand.

Tables 3 and 4 show that both placental weight and fetal weight increased significantly ($P \leq 0.05$) with advancing gestational stage and increasing maternal age, with a strong positive correlation between them. These findings are consistent with Purohit (2020) and Toschi & Baratta (2021), who confirmed that the placenta grows dynamically to meet the increasing nutrient demands of the growing fetus, and that the relationship between placental and fetal weight is strongly positive, reflecting the efficiency of nutrient exchange across the placenta. Similarly, Şen (2021) and Seo *et al*. (2024) reported that the increase in placental weight during pregnancy is associated with increases in both the number and size of chorionic villi, which enhance the surface area available for maternal-fetal exchange.

Older ewes produced heavier fetuses than younger ewes, particularly during the third gestational stage, which may be attributed to their greater uterine capacity, sustained uterine growth, and improved blood perfusion with advancing age. This is in agreement with Redifer *et al*. (2021), who reported that older dams in ruminants have a greater capacity to support fetal growth during late pregnancy due to enhanced uterine vascularization and increased placental blood flow, which positively affects birth weight.

The marked increase in placental weight between the first and second gestational stages reflects rapid placental growth to establish a rich vascular network that supports the expanding fetus, while the continued but slower increase during the third stage may indicate that the placenta has reached its maximum functional capacity, a phenomenon previously described in ruminants by Wilson & Ford

(2001) and Barbato et al. (2024).

Tables 5, 6, and 7 show that ewe age, gestational stage, and their interaction had significant effects ($P \leq 0.05$) on placental morphology, including cotyledonary number, placental surface area, and placental efficiency.

The results demonstrated a progressive decrease in cotyledonary number with advancing pregnancy (from 58.9 at the first stage to 41.3 at the third stage), while total placental surface area (from 221.6 cm^2 to 423.9 cm^2) and placental efficiency (from 0.059 to 10.26 g/cm^2) increased in the same direction. These changes indicate a compensatory hypertrophy of the remaining cotyledons, where the surface area of each cotyledon increases to compensate for the reduction in number, thereby maintaining the total surface area required for nutrient exchange. This is consistent with Wang *et al*. (2025) and kumar & Iyue (2023), who described the placenta as a dynamic tissue that functionally reorganizes itself with advancing pregnancy by increasing the size of individual cotyledons without increasing their number.

Regarding the effect of age, older ewes (>3.5 years) exhibited a lower cotyledonary number (43.1) compared with younger ewes (54.9), alongside an increase in total placental surface area (350.7 cm^2 vs. 268.7 cm^2). This agrees with Gotu *et al*. (2025) and Ciornei *et al*. (2024), who reported that repeated reproductive cycles lead to histological changes in the endometrium (caruncular degeneration) with advancing age, which reduces the number of caruncles and consequently the number of cotyledons; however, compensation occurs through hypertrophy of the remaining cotyledons, as noted by Dagdelen & Esenbuga (2025).

The results also showed that placental efficiency (defined according to Redifer et al., 2021 as fetal weight per unit of cotyledonary surface area) reached its highest value during the third gestational stage (10.26 g/cm^2), reflecting the functional maturation of the placenta in the last third of pregnancy, where its capacity to transfer nutrients and oxygen per unit area increases. These findings are consistent with Wilson & Ford (2001) and Barbato *et al*. (2024) regarding the capacity versus efficiency trade-off, where a larger placenta (as in older ewes) may be less efficient in nutrient transfer per unit area compared with a smaller placenta (as in younger ewes), which shows higher efficiency as a compensatory mechanism for its smaller size.

Table 8 shows that ewe age, gestational stage, and their interaction had significant effects ($P \leq 0.05$) on carcass weight and the percentage of reproductive adipose tissue. Mean carcass weight increased with advancing age, with the oldest ewes (>3.5 years) recording significantly higher values than the younger age groups (2.5 and 1.5 years) during the first gestational stage, with carcass weights of 24.00, 20.33, and 17.33 kg, respectively. This is consistent with Nageye & Koyuncu (2024), who reported that carcass weight increases with age due to the completion of skeletal and muscular growth and the accumulation of fat mass.

Furthermore, the percentage of reproductive adipose tissue in the carcass increased significantly with advancing age and gestational stage, with the oldest ewes (>3.5 years) recording the highest percentages across the three gestational stages (1.02%, 1.09%, and 1.12%, respectively), while the lowest percentage was observed in the youngest ewes during the first stage (0.76%). This increasing trend in reproductive fat with age and pregnancy is consistent with Yousuf *et al.* (2023), who confirmed that fat deposition around the reproductive organs is influenced by a complex network of interactions among age, physiological status, and reproductive hormones.

A slight increase or relative stability in the percentage of reproductive adipose tissue was observed with advancing pregnancy across all age groups, suggesting that these fat depots serve as an energy reserve during advanced pregnancy to support fetal growth, with older ewes retaining a higher capacity for fat storage due to slower metabolism and cumulative fat deposition with age, which aligns with Knupp *et al*. (2021) and Mescher (2018) regarding the role of the broad ligament as a major visceral fat depot in females.

Ultrasonographic Evaluation of Reproductive Adipose Tissue Area

Transabdominal ultrasonography was performed on day 40 of pregnancy using a convex probe at 3.5 MHz to confirm pregnancy and evaluate the area of reproductive adipose tissue around the uterus and ovaries. Ultrasound images revealed a single gestational sac within the uterine horn, surrounded by cotyledonary plaques appearing as hyperechoic circular structures, which is consistent with Brzozowska *et al*. (2022) and Younis & Hatif (2023), who confirmed that pregnancy diagnosis in sheep can be accurately achieved using ultrasonography from day 30–40 of gestation.

From day 45–60 of gestation, fetal skeletal structures (head, spine, and limbs) became distinguishable as hyperechoic structures due to the increased density of bone tissue and its ability to reflect ultrasound waves compared with surrounding soft tissues, in agreement with Stieger-Vanegas *et al*. (2021) and Fenu *et al*. (2022). During mid-gestation (day 75–85), fetal details became more distinct as reported by Succu *et al*. (2023); however, late gestational stages were not included in the present evaluation due to the fetus occupying most of the uterine cavity, which limits the clarity of surrounding reproductive tissues, consistent with Abdul-Rahaman & Mahmood (2025), who stated that ultrasonography is an accurate and rapid tool for pregnancy diagnosis and monitoring in ewes.

Ultrasound images showed marked variation in the area of reproductive adipose tissue around the uterus and ovaries according to ewe age and gestational stage. During the first gestational stage, the percentage of reproductive adipose tissue (estimated using ImageJ software) ranged 59.62% in group 1 ewes (1.5 years) as shown in Figures (1), while it recorded 51.77% in group 2 (2.5 years) as in Figure (2), and reached 52.21% in group 3 ewes (>3.5 years) as in Figures (3).

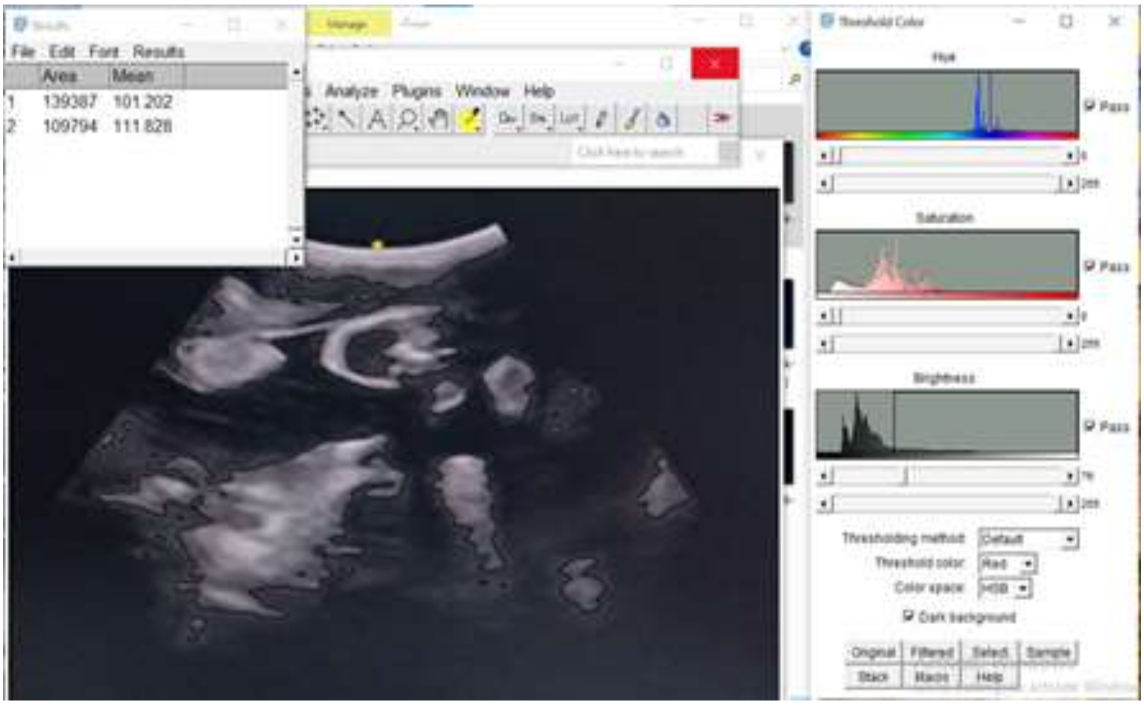

Figure (1): Abdominal ultrasound image of the uterus of a pregnant ewe, aged 1.5 years, during the first stage of gestation, using a convex transducer with a frequency of 3.5 MHz. The image shows fatty deposits (in the marked area) in the broad ligament suspending the uterus and ovaries, with a heterogeneous echotexture. The estimated fat percentage was 59.56%

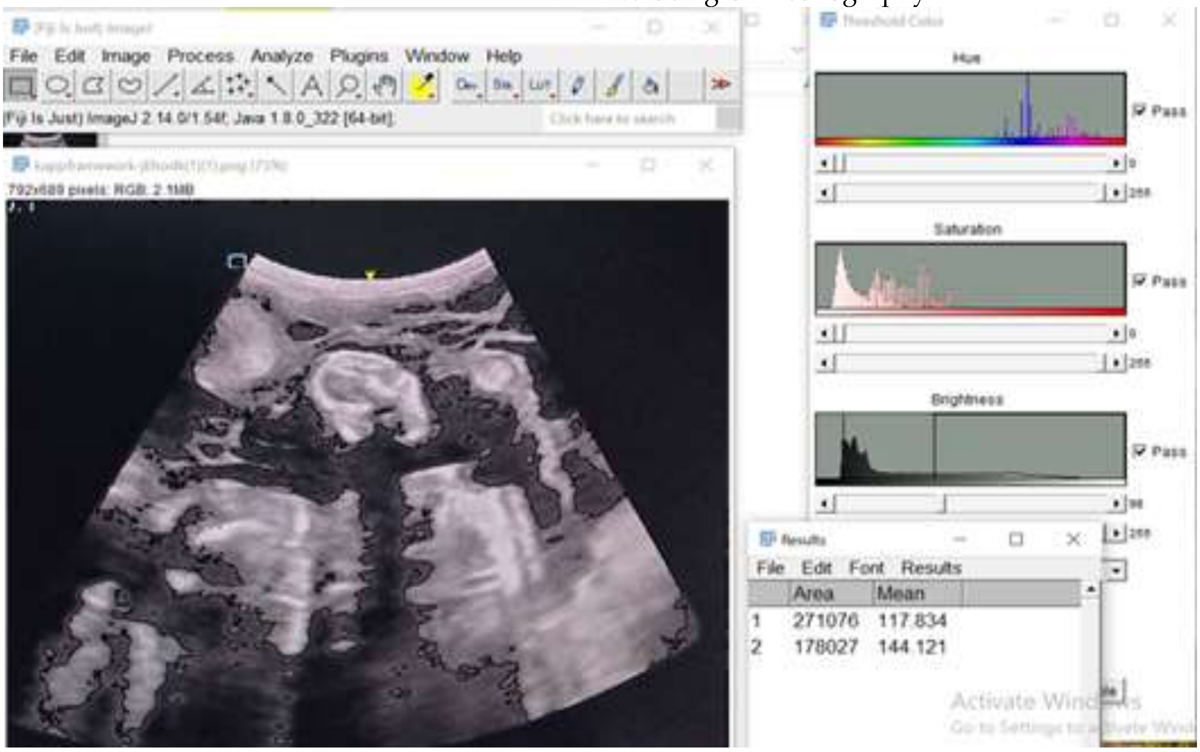

Figure (2): Abdominal ultrasound image of the uterus of a pregnant ewe, aged 2.5 years, during the first stage of gestation, using a convex transducer with a frequency of 3.5 MHz. The image shows accumulation of fatty deposits (in the marked area) in the region of the broad ligament suspending the uterus. Their percentage relative to the uterine area was 51.77%.

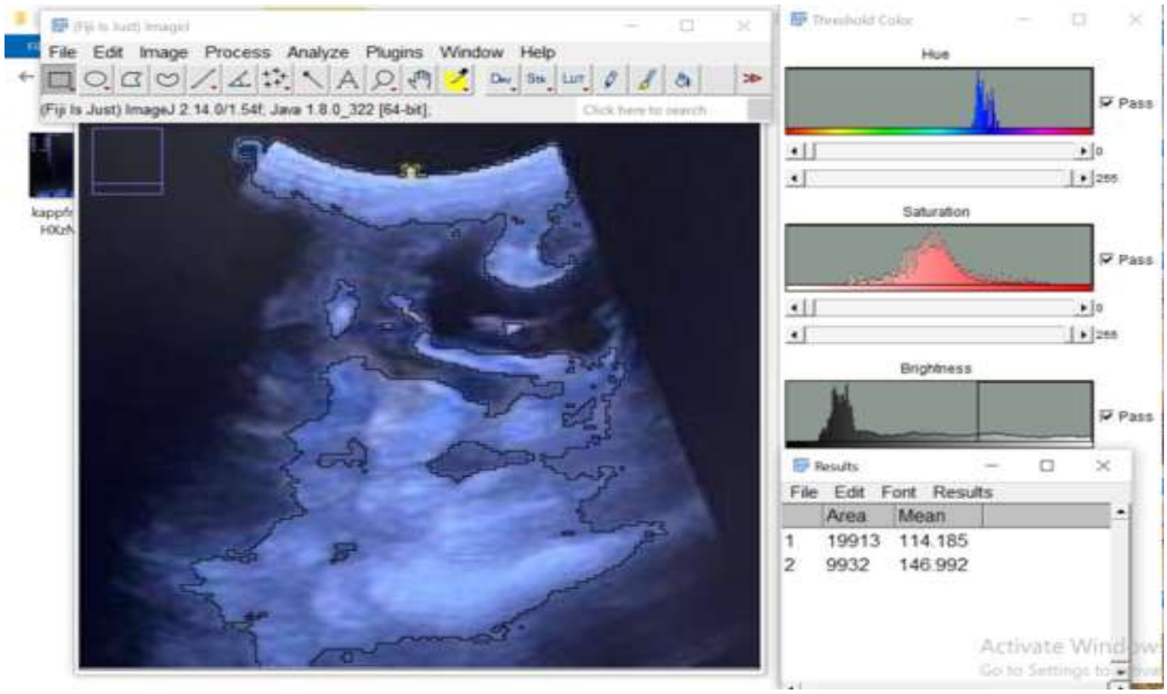

Figure (3): Abdominal sonar image of a pregnant sheep's uterus at age < 3.5 years, during the first stage, using a convex probe with a frequency of 3.5 MHz. It shows the presence of fatty deposits (in the marked area) in the broad ligament suspending the uterus, with their percentage relative to the uterine area reaching 49.9%

During the second gestational stage, a wide variation in recorded percentages was observed: the percentage increased 78.77% in group 1 ewes as in Figure (4), while it reached 65.67% in group 2 ewes as in Figures (5), and recorded 44.73% in group 3 ewes as in Figures (6).

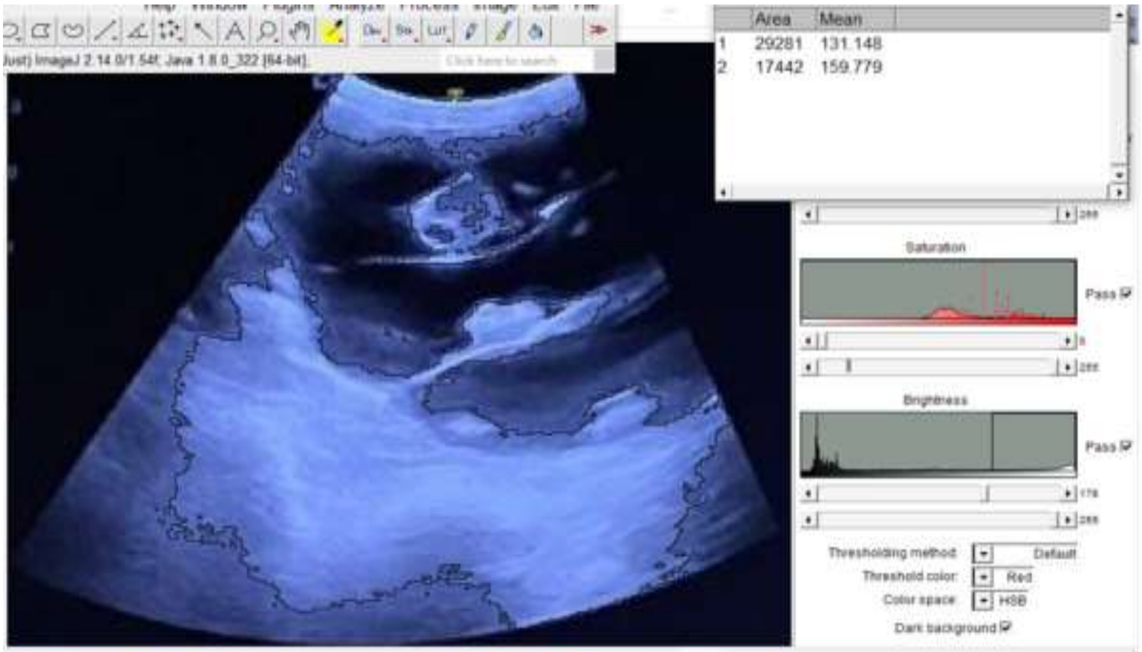

Figure (4): Abdominal ultrasound image of the uterus of a pregnant ewe, aged 1.5 years, during the second stage of gestation, using a convex transducer with a frequency of 3.5 MHz. The image shows fatty deposits (in the marked area) within the broad ligament suspending the uterus and ovaries. Their percentage relative to the uterine area was 78.77%.

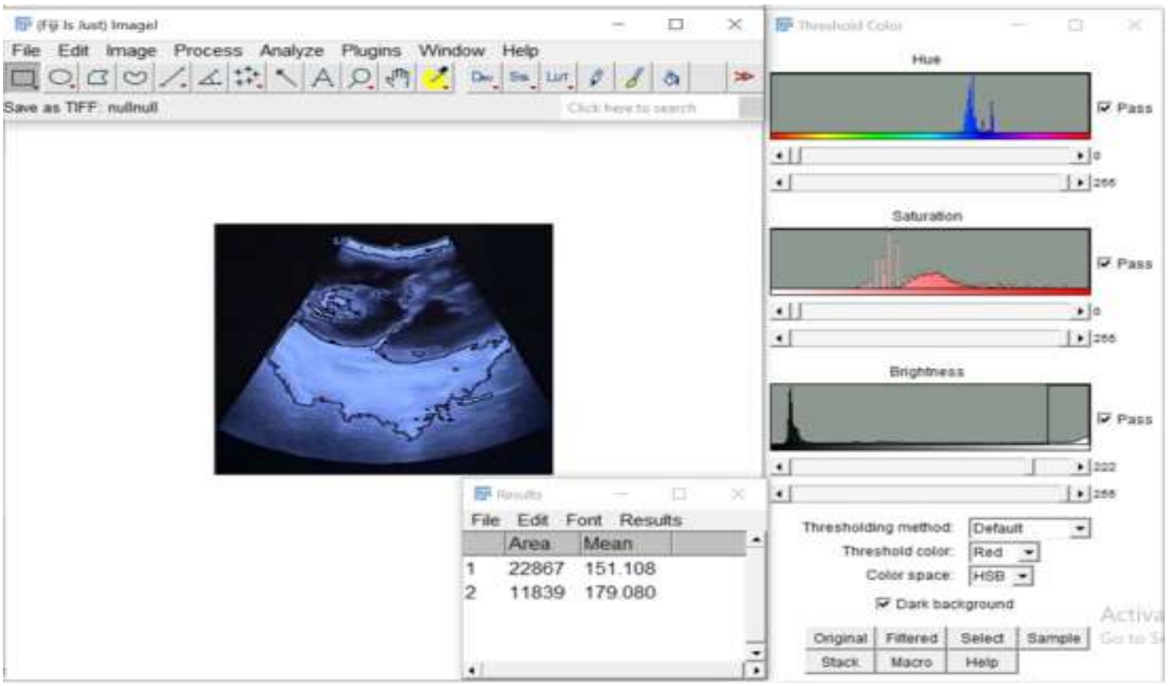

Figure (5): Abdominal sonar image of a sheep's uterus at 2.5 years of age, during the second stage of pregnancy, using a convex probe with a frequency of 3.5 MHz. An increase in fatty deposits (in the marked area) is observed in the broad ligament, with the percentage of fat relative to the uterine area reaching 65.67%.

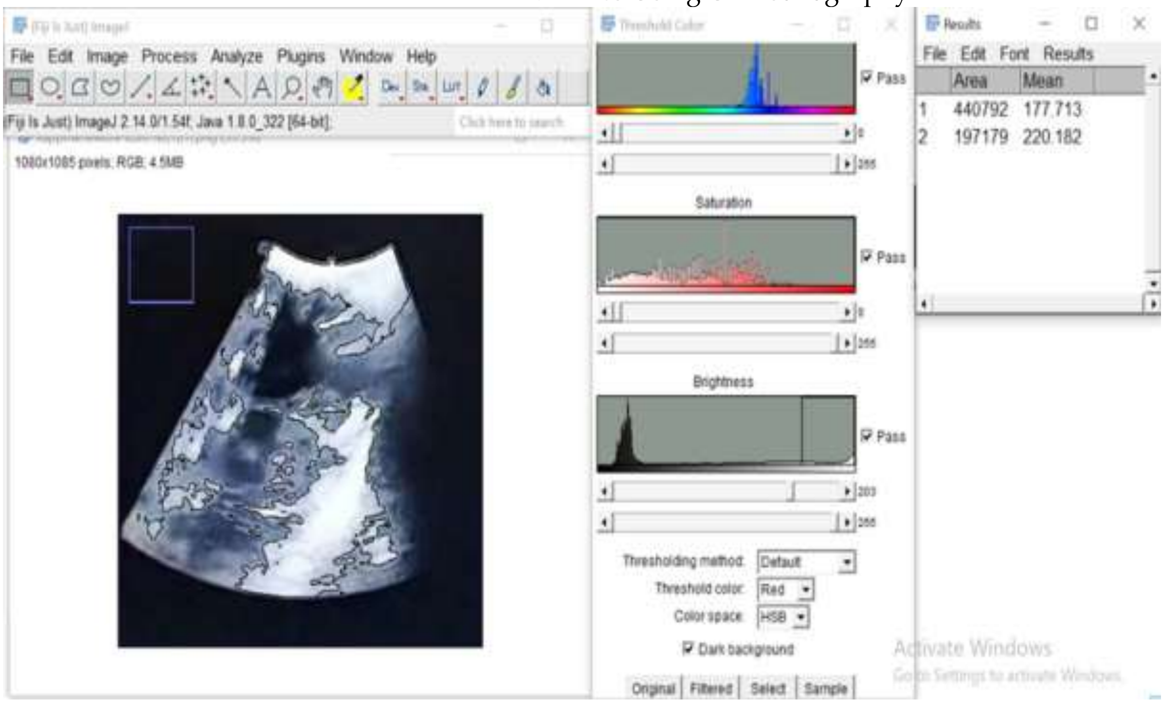

Figure (6): Abdominal sonar image of a pregnant sheep's uterus at age > 3.5 years, during the second stage, using a convex probe with a frequency of 3.5 MHz. It shows fatty deposits (in the marked area) in the broad ligament suspending the uterus and the ovaries, which gives a heterogeneous appearance to the area surrounding the uterus. Their percentage relative to the uterine area reached 44.73%

This wide variation may be attributed to the mechanical effect of uterine expansion with fetal growth during mid-pregnancy, which may lead to retraction or compression of the adipose tissue surrounding the uterus at the level of the ultrasonographic cross-section. Tissues of older animals may be less elastic, increasing the degree of retraction and resulting in a sharp decrease in the apparent percentage without reflecting an actual reduction in fat mass, as confirmed by the results of Table (2) (weight of dissected reproductive adipose tissue after slaughter). Individual variations in physiological responses to pregnancy and age may also contribute to explaining this variation in recorded percentages.

These findings suggest that the pattern of fat deposition in the broad ligament region is influenced by physiological status and hormonal changes accompanying pregnancy, which is consistent with Jeong *et al*. (2024) and Szyrzisko & Grzesiak (2024), who reported that reproductive adipose tissue around the ovaries and uterus is a major visceral fat depot associated with the female reproductive tract and reflects metabolic changes during pregnancy.

The adipose tissue surrounding the uterus and ovaries also exhibited a heterogeneous echo texture on ultrasound images, which may be attributed to variations in adipocyte density and their intermingling with blood vessels and connective tissue during pregnancy, leading to variable reflection of ultrasound waves within the adipose tissue, as described by Steiner *et al*. (2022).

This variation is related to the levels of pregnancy-associated hormones, as estrogen contributes to the regulation of lipoprotein lipase gene expression and increases the sensitivity of adipocytes to insulin, as reported by Kuryłowicz (2023), which may affect fat storage and mobilization mechanisms according to physiological status and energy requirements during pregnancy (Jeong *et al*., 2024; Hope *et al*., 2025). Similarly, progesterone may stimulate lipogenesis and increase plasma lipid levels through activation of progesterone receptors (PR-B), leading to the stimulation of lipogenic enzymes (Jeong *et al*., 2024). The fat within the broad ligament represents one of the major visceral fat stores, in addition to its role as a supporting tissue for reproductive structures, as noted by Mescher (2018).

Recent studies have confirmed the feasibility of using ultrasound images to estimate fat mass around the reproductive organs, as Theron *et al*. (2023) found a close correlation between sonographically estimated values and actual fat mass after slaughter, while Ramos-Ramos *et al*. (2024) reported agreement between ultrasound measurements and digital image analysis using ImageJ software in evaluating fat deposits and measuring their thickness. The high accuracy of digital image analysis techniques has enhanced the reliability of estimating these changes, as studies have shown that converting ultrasound images into quantitative data reduces visual bias and increases measurement accuracy compared with traditional methods Rodgers & Sferruzzi-Perri, 2021).)

Furthermore, Theron *et al*. (2023) confirmed using ultrasonography that females show superior age- and weight-related fat deposition, reflecting faster maturation and a higher capacity for fat storage, indicating an effect of age, gestational stage, and anatomical changes associated with uterine expansion, which was reflected in the apparent area of fat imaged by ultrasound in the present study.

CONCLUSIONS

The present study concludes that reproductive adipose tissue is not merely a structural tissue surrounding the reproductive organs, but rather a functional component that supports pregnancy by serving as an important energy reserve. Its deposition increases with advancing maternal age and gestational stage to meet the increasing metabolic demands of fetal development. Meanwhile, the placenta exhibits compensatory adaptations that maintain the efficiency of maternal–fetal nutrient exchange. These findings suggest that successful pregnancy depends on the integration between maternal energy reserves and the functional adaptations of the placenta.

ETHICS

All procedures involving animals were carried out in accordance with the international guidelines for the care and use of animals in research (National Research Council, 2011).

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