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Original Paper

Analysis of miR-29a and miR-34a Expression During Various Reproductive Stages in Iraqi Awassi Ewes and Their Correlation with Litter Size, Offspring Sex, and Blood Attributes

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This study was investigated the correlation between miR-29a and miR-34a expression in Iraqi Awassi ewes during different reproductive stages and their potential as biomarkers for assessing pregnancy health, birth type, sex as well as blood parameters. Blood samples were obtained from five Iraqi Awassi ewes (3.5–4.5 years with 45–50 kg body weight), pre-mating (0), 14, and 45 day post-mating (PM), along with 75 and 135 days of pregnancy. miRNAs (miR-29a and miR-34a) were detected by using RT-qPCR. The relative expression of miR-29a was significantly increased ($P \leq 0.05$) within different day as 45 (16.25 ±5.194), 75 (14.32 ±4.09), and 135 (13.29 ±6.52) of pregnancy compared to 0 and 14 day respectively. Additionally, a higher relative expression of miR-34a ($P \leq 0.05$) was observed on day 135 of pregnancy (2.3 ±1.05) when compared to the other reproductive periods. Both miR-29a and miR-34a showed no differences in terms of birth type and sex. Regression coefficient analysis identified a significant relationship between miR-29a expression and various metabolic and biochemical parameters, such as serum amino acids, fatty acids, and estrogen levels. The analysis indicated that miR-29a exhibited an inverse correlation with amino acids while demonstrating a direct correlation with fatty acids and estrogen. In conclusion, miR-29a and miR-34a could serve as valuable biomarkers for monitoring the health of pregnancy in Awassi ewes. This study provides new insights into the function of miRNA in reproductive physiology, paving the way for improved management of sheep reproduction.

Keywords: miR-29a, miR-34a, Iraqi Awassi ewes, biomarkers, pregnancy, blood parameters

1 Introduction

Reproductive efficiency is essential for enhancing the productivity of livestock breeding stations, as it ensures a steady supply of offspring for meat, milk, and wool production (Abdulkareem et al., 2023; Ali et al., 2024a). Blood, and semen biomarkers can indicate for both physiological and pathological conditions, which helps in effective livestock management, the improvement of reproductive, productive and animal care practices (Al-Gebouri & Eidan, 2024a,b; Ali et al., 2024b; Eidan et al.,2024; Musa & Abdulkareem, 2024a,b; Abdulkareem et al.,2024). Advances in genomic technologies have transformed molecular genetics, providing exciting opportunities to identify genes associated with desirable productive traits (Eidan & Khudhir, 2023; Seger et al., 2023).

MicroRNAs (miRNAs) are non-coding ribonucleic acids that range from 21 to 24 nucleotides in length. They are transcribed from DNA sequences into primary miRNAs (Pri-miRNAs) and processed into mature miRNAs (Ha & Kim, 2014). Extensive research on miRNAs in sheep suggests that these molecules play a crucial role in the female reproductive system and are involved in various stages of the reproductive process. For instance, they influence ovarian function (Guo et al., 2019), oocyte formation (Yang et al., 2019; Zhang et al., 2020), corpus luteum development (McBride et al., 2012; Donadeu et al., 2016), seasonal estrus regulation (Yang et al., 2018), fertility (Pokharel et al., 2018), embryo implantation (Gebremedhn et al., 2018), pregnancy development (Wu et al., 2016), and other biological processes. MicroRNAs (miRNAs) play crucial roles in the proliferation and

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differentiation of myoblasts at various embryonic stages (Zhang et al., 2021). Gene expression patterns connected to embryo implantation in the uterine lining of sheep showed that miRNAs were differentially expressed on the thirteenth day of pregnancy (Kiyma et al., 2015). Additionally, studies have characterized miRNA expression patterns in the endometrium on days 12, 16, and 22 of the pregnancy cycle (Kose et al., 2022). Recent studies indicate that microRNAs are essential for recognizing pregnancy and for the implantation of embryos in various species (Song et al., 2015; Batra et al., 2024; Paulson et al., 2022; Sadowska et al., 2024). In pregnant ewes, extracellular vesicles play a role in cell communication and are involved in miRNA pathways (Sun et al., 2021; Ono et al., 2022; Zhanmin Lv et al., 2025). According to Zhanmin Lv et al. (2025), miRNAs can be detected in the maternal bloodstream during the early stages of pregnancy (days 14–30) of ewes. Furthermore, miRNA molecules can be found in body fluids, and their stability during freezing and high temperatures makes them promising candidates for early pregnancy detection and monitoring pregnancy maintenance (Tan et al., 2020; Zhanmin Lv et al., 2025).

On day 39 of pregnancy, researchers found elevated expression of the miR-29a gene in the blood of cattle (Sánchez et al., 2021). Previous studies have indicated that the miR-29 family could serve as a potential biomarker for adverse pregnancy outcomes (Sørensen et al., 2021). Furthermore, miR-29a has been recognized as an indicator for detecting pregnancy in cows (Ono et al., 2022). It is important to note that no previous studies have reported the expression of miR-29a in pregnant ewes. Moreover, Winger et al. (2020) conducted a study involving pregnant women, which showed that the expression of miR-34a was elevated in the first trimester. This finding could assist in assessing the risk of preterm delivery during the early stages of pregnancy (Winger et al., 2020). Previous studies have indicated that plasma miRNAs in cows and goats can serve as early biomarkers for pregnancy and endometrial receptivity related to the implantation of a fertilized egg (Zang et al., 2021; Zhao et al., 2021 and Mazzarella et al., 2024). In ewes, Hitit et al. (2022) identified a connection between two specific miRNAs – oar-miR-218a and oar-miR-1185-3p – and the pre-implantation stage (12 days post-mating). However, there has been no prior data on the expression analysis of miR-29a and miR-34a during the second and third trimesters in ewes. This lack of information led us to investigate the relationship between these miRNAs and factors such as birth type, sex, and blood parameters across five reproductive stages: days 0, 14, and 45 PM, as well as days 75 and 135 of pregnancy in Iraqi Awassi ewes.

2 Material and methods

2.1 Experimental Animals

This study was conducted from November 2023 to March 2024 at the Khairat Al-Ittihad private station, located in the Shumali area of Babylon governorate and the laboratories of the Ministry of Science and Technology. Five Iraqi Awassi ewes, aged 3.5 to 4.5 years and weighing 45 to 50 kg, were in good health and under continuous veterinary supervision throughout the experimental period. Ewes were fed (2% of live body weight) a concentrate diet daily during the first three months of pregnancy. This diet included 30% barley, 12% wheat bran, 10% maize, 10% soybean meal, 1% limestone, 1% salt, and 1% vitamins and minerals. Additionally, they received 20 kg of alfalfa hay and 15 kg of wheat straw. The total crude protein percentage of this diet was 14.3%, and its gross energy content was 3416 $Mcal \cdot kg^{-1}$. In the last two months of pregnancy, the diet for the ewes was adjusted (3% of live body weight). The new mixture comprised 25% barley, 25% wheat bran, 22% maize, 10% soybean meal, 1% limestone, 1% salt, and 1% vitamins and minerals. Ewes were also provided with 10 kg of alfalfa hay and 5 kg of wheat straw. This adjusted diet increased the total crude protein percentage to 15.6% and the gross energy content to 3777 $Mcal \cdot kg^{-1}$.

2.2 Estrus Synchronization and Pregnancy Detection

Estrus was synchronized using CIDR vaginal-impregnated device. It was monitored three times a day with the help of teaser rams. The onset of estrus in cyclic ewes was designated as day 0. Three methods were used to confirm pregnancy in ewes:

1. detecting ewes that did not return to estrus (non-return rate) with the assistance of professional veterinarians on days 17 to 18 post-mating (PM);
2. using an ultrasound device on day 45 PM;
3. measuring serum progesterone concentrations on days 14 and 45 PM.

The flock's fertility, conception lambing, and twinning rates, as well as litter size and barrenness, were evaluated (Vlahek et al., 2023).

2.3 Blood Sampling and miRNAs Expression Analysis

Blood samples were obtained from each ewe at five different reproductive stages: Day 0 (pre-mating), 14 days post-mating (PM), 45 PM, and 75 and 135 days of pregnancy. We investigated the expression of miR-29a and miR-34a at each of these stages. Additionally, we assessed the effects of litter size and the sex of the fetus on these miRNAs. The samples were obtained using jugular venipuncture with vacutainer needles and tubes that did not contain the anticoagulant

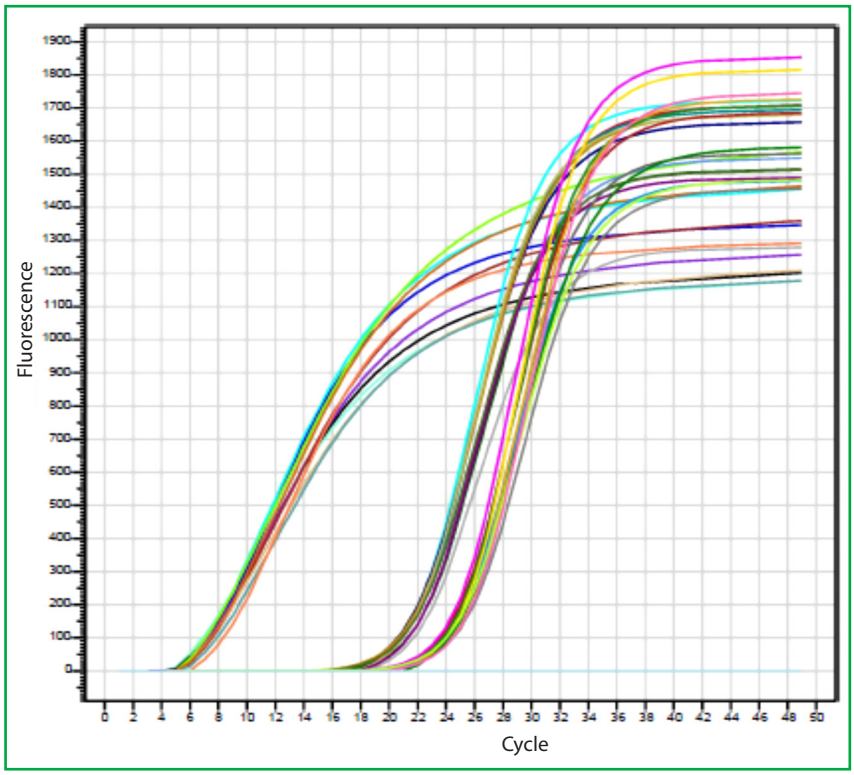

Figure 1 RNU6 houskeeping gene amplification plots by RT-PCR from serum samples for Awassi ewes

Ethylenediaminetetraacetic acid (EDTA). After collection, the blood was allowed to clot, and serum was extracted by centrifuging the samples at 3,000 rpm for 5 minutes. Isolation of RNA was done from 0.5 ml of serum using TRIzol™ reagent. This process included phase separation with chloroform, precipitation of RNA with isopropanol, washing with ethanol, and re-suspension in RNase-free water, with the final sample stored at -20 °C. For miRNA quantification using the Qubit 4.0, a selective assay is used that has a high tolerance for contaminants. Preparing of working solutions, adding standards or samples, vortexing, incubating, creating a concentration curve, and measuring the samples were also included. Similar steps are followed for cDNA quantification, albeit with different reagents. The RT-qPCR protocol involves synthesizing cDNA from miRNA 146a using specific primers and the Protoscript cDNA synthesis kit, followed by relative quantitative PCR with SYBR Green detection, utilizing U6 snRNA as a housekeeping gene (Flatz et al., 2006). The primers used in this study were sourced from Macrogen® (Korea). Their names and sequences are provided in Table (1). RNU6 Houskeeping genes account for mjority of the active genes in the genome, and their expression is obviously vital in the genome. The housekeeping gene expression levels are fine-tuned to meet the metabolic requirement in various tissues (Figure 1).

2.4 Statistical Analysis

The statistical analysis was assessed throughout using Statistical Analysis System (SAS, 2018) program with one-way classification approach (Completly Randomized Design "CRD"). The differences among means were compared by applying Duncan multiple range test. Exponential equations based on simple nonlinear regression, including the coefficient of determination (R^2) calculation, were utilized to assess the regression coefficient of blood biochemical analysis, as well as amino, fatty, and carboxylic acids, in relation to the relative expression of miR-29a and miR-34a.

Table 1 The primers of miR-29a and miR-34a expression

Primer	Sequences
miR-29a-RT	GTATGCTGCTACCTCGGACCCTGCTTAGTGCCATGCCTGCCATCGAGCAGCATACCTGAAC
miR-29a-F	GCGTGATTTCTTTTGGTGTTCAG
miR29a-R	GCAAGGATGACACGCAAATTCG
miR-34a-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGGGCA
miR-34a-F	GGGTGGCAGTGTCTTAGC
miR-34a-R	CAGTGCGTGTCGTGGAGT
U6-FP	CTCGCTTCGGCAGCACA
U6-RP	AACGCTTCACGAATTTGCGT

3 Results and Discussion

3.1 Reproductive Performances of the Experimental Ewe Flock

Fertility, conception, lambing, and twinning rates of the experimental ewe flock were 93, 100, 114, and 14.2%, respectively. Litter size and barrenness of the flock were 1.14 and 6.7% respectively.

3.2 Relative Expression of Serum miR-29a and miR-34a at Different Reproductive Stages

Dynamic changes in the relative expression of serum miR-29a and miR-34a were observed in ewes during different stages of pregnancy (Table 2). On day 0, the relative expression levels of both miR-29a and miR-34a were not detected, recorded as 0.00 ±0.00. However, by day 14 PM, there was a slight numerical increase in the relative expression of miR-29a and miR-34a, measured at 0.24 ±0.09 and 0.37 ±0.10, respectively, compared to day 0 (Table 2). At day 45 PM (early embryonic development), the relative expression of miR-29a was significantly greater ($P \leq 0.02$) compared to its levels at days 0 and 14 PM, and numerically higher than at both days 75 and 135 of pregnancy, measured at 16.25 ±5.19 (Table 2). In contrast, the relative expression of miR-34a remained unchanged (0.59 ±0.24) compared to the previous two days and day 75 of pregnancy, while being significantly lower than at day 135 of pregnancy (Table 2). At mid-pregnancy (day 75), the relative expression of miR-29a decreased numerically (14.32 ±4.09) compared to its level at day 45 PM and was higher than the level at day 135 of pregnancy, despite being statistically non-significant. At a similar stage (day 75 of pregnancy), miR-34a remained unchanged (0.43 ±0.27) compared to previous stages and was significantly lower ($P \leq 0.03$) than its expression at day 135 of pregnancy (Table 2). In the third trimester of pregnancy (day 135), the relative expression of miR-29a decreased numerically to 13.29 ±6.52 compared to its values at days 45 PM and 75 of pregnancy, but it remained higher than the values at days 0 and 14 PM. In contrast, the expression of miR-34a significantly increased ($P \leq 0.03$) compared to the other stages, with a measurement of 2.3 ±1.05 (Table 2).

3.3 Impact of Litter Size and Offspring Sex on the Expression Levels of Serum miR-29a and miR-34a

Non-significant effects of birth type and sex on both miR-29a and miR-34a were observed (Table 3). However, the expression of miR-29a was higher than that of miR-34a based on litter size. The relative expression of miR-29a in the single-birth group was 8.190 ±2.324, while in the twin group, it was 12.140 ±7.583. Furthermore, the relative expressions of miR-34a for the single and twin birth groups were 0.765 ±0.328 and 2.147 ±1.387, respectively (Table 3). In terms of the effect of offspring sex, the female group showed a higher relative expression of miR-29a (10.440 ±2.942) compared to the male group (7.744 ±3.228). On the other hand, the relative expression of miR-34a was lower in the female group (0.764 ±0.198) than in the male group (1.111 ±0.559; Table 3).

3.4 The Regression Coefficient of Amino Acids on a Rrelative Expression of miR-29a in Serum

The results indicate that the regression coefficients (RC) for the amino acids aspartic acid (0.03/ $\mu g \cdot g^{-1}$), serine (0.05/ $\mu g \cdot g^{-1}$), alanine (0.03/ $\mu g \cdot g^{-1}$), tyrosine (0.05/ $\mu g \cdot g^{-1}$), arginine (0.05/ $\mu g \cdot g^{-1}$), proline (0.02/ $\mu g \cdot g^{-1}$), glycine (0.06/ $\mu g \cdot g^{-1}$), and the total non-essential amino acids (0.46 $\mu g \cdot g^{-1}$) show a significant negative RC ($P \leq 0.05$) with the relative expression of miR-29a. The corresponding R^2 values for these amino acids are 0.16, 0.14, 0.14, 0.17, 0.19, 0.17, 0.20, and 0.14, respectively (Table 3).

Table 2 Relative expression of serum miR-29a and miR-34a in Awassi ewes during different reproductive stages (mean ±SE)

Stages / miRNAs	Day 0 ($n = 5$)	Day 14 PM ($n = 5$)	Day 45 PM ($n = 5$)	Day 75 of pregnancy ($n = 5$)	Day 135 of pregnancy ($n = 5$)	Significance level
miR-29a	0.0 ±0.0^{B}	0.24 ±0.09^{B}	16.25 ±5.19^{A}	14.32 ±4.09^{A}	13.29 ±6.52^{A}	$P \leq 0.02$
miR-34a	0.0 ±0.0^{B}	0.37 ±0.10^{B}	0.59 ±0.24^{B}	0.43 ±0.27^{B}	2.3 ±1.05^{A}	$P \leq 0.03$

Means with different superscripts within each row indicate significant differences; PM – post-mating

Table 3 Effect of litter size and offspring sex on a relative expression of miR-29a and miR-34a in serum of Awassi ewes

miRNAs	Litter size			Offspring sex		
	single (n = 26)	twin (n = 4)	significance level	male (n = 11)	female (n = 17)	significance level
miR29a	2.324 ±8.190	12.140 ±7.583	NS	7.744 ±3.228	0.440 ±2.9421	NS
miR34a	0.765 ±0.328	2.147 ±1.387	NS	1.111 ±0.559	0.764 ±0.198	NS

NS – non-significant

Table 4 The regression coefficient for amino acids on a relative expression of miR-29a in serum of Awassi ewes

Amino acids		Regression coefficient(b)	Prediction equation	Significance level	Determination coefficient (R^2)
non-essential amino acids (μg·gm^{-1})	aspartic acid	-0.03	$\bar{Y}$ = 8.33 - 0.03X	$P \leq 0.05$	0.16
	asparagine	-0.02	$\bar{Y}$ = 7.48 - 0.02X	NS	0.12
	serine	-0.05	$\bar{Y}$ = 8.90 - 0.05X	$P \leq 0.05$	0.14
	alanine	-0.03	$\bar{Y}$ = 9.43 - 0.03X	$P \leq 0.05$	0.14
	tyrosine	-0.05	$\bar{Y}$ = 10.77 - 0.05X	$P \leq 0.05$	0.17
	arginine	-0.05	$\bar{Y}$ = 8.71 - 0.05X	$P \leq 0.05$	0.19
	cysteine	-0.04	$\bar{Y}$ = 8.02 - 0.04X	NS	0.13
	proline	-0.02	$\bar{Y}$ = 5.50 - 0.02X	$P \leq 0.05$	0.17
	glycine	-0.06	$\bar{Y}$ = 7.23 - 0.06X	$P \leq 0.05$	0.20
	total	-0.46	$\bar{Y}$ = 73.39 - 0.46X	$P \leq 0.05$	0.14
essential amino acids (μg·gm^{-1})	lysine	-0.04	$\bar{Y}$ = 8.68 - 0.04X	$P \leq 0.05$	0.21
	threonine	-0.05	$\bar{Y}$ = 6.96 - 0.05X	$P \leq 0.05$	0.20
	leucine	-0.04	$\bar{Y}$ = 7.16 - 0.04X	$P \leq 0.05$	0.17
	isoleucine	0.05-	$\bar{Y}$ = 10.95 - 0.05X	$P \leq 0.05$	0.21
	valine	-0.05	$\bar{Y}$ = 16.75 - 0.05X	NS	0.12
	methionine	-0.04	$\bar{Y}$ = 7.92 - 0.04X	$P \leq 0.05$	0.18
	histidine	-0.04	$\bar{Y}$ = 6.01 - 0.04X	$P \leq 0.05$	0.17
	phenylalanine	-0.04	$\bar{Y}$ = 8.90 - 0.04X	$P \leq 0.05$	0.14
	total	-0.38	$\bar{Y}$ = 73.35 - 0.38X	$P \leq 0.05$	0.18

NS – non-significant

A significant ($P \leq 0.05$) and negative RC was observed for essential amino acids, specifically for lysine (-0.04 μg·g^{-1}), threonine (-0.05/ μg·g^{-1}), leucine (-0.04/ μg·g^{-1}), isoleucine (-0.05/ μg·g^{-1}), methionine (-0.04/ μg·g^{-1}), histidine (-0.04 μg·g^{-1}), phenylalanine (-0.04 μg·g^{-1}), and total essential amino acids (-0.38/ μg·g^{-1}). The corresponding R^2 values for these amino acids were 0.21, 0.20, 0.17, 0.21, 0.18, 0.17, 0.14, and 0.18, respectively (Table 4).

3.5 The regression Coefficient of Fatty and Carboxylic Acids on a Relative Expression of miR-29a in Serum

Fatty acids have a significant positive RC ($P \leq 0.05$) with the relative expression of miR-29a. The levels of specific fatty acids are as follows: palmitic (0.03%), stearic (0.03%), total saturated fatty acids (0.06%), oleic (0.03%), α-linolenic (0.05%), eicosapentaenoic (0.04%), and arachidonic (0.01%). The corresponding R^2 values for these fatty acids are 0.20, 0.21 0.21, 0.21, 0.21, 0.19, and 0.20, respectively (Table 5). A negative and significant ($P \leq 0.01$) RC of -0.03% was observed for volatile fatty acids, specifically acetic, butyric, and propionic acids, correlated to the relative expression of miR-29a. This finding corresponds to an R^2 value of 0.25, indicating that for every increase in the relative expression of miR-29a, the levels of carboxylic acids decreased by 0.03%. However, no significant correlation was found for the other acids concerning the relative expression of miR-29a (Table 5).

3.6 The Regression Coefficient of Biochemical Attributes on a Relative Expression of miR-29a in Serum

The results demonstrated a enunciated ($P \leq 0.01$) and positive RC for several parameters: glucose (0.12), cholesterol (0.15), total protein (0.04), albumin (0.01), triglycerides (0.67), HDL (0.04), vLDL (0.13, and AST (0.4), with R^2 was 0.33, 0.31, 0.26, 0.03, 0.36, 0.35, 0.36, and 0.38 respectively (Table 7). Similarly, globulins (0.02) and ALP (1.27) also showed a significant ($P \leq 0.05$) and positive RC with R^2 0.24 and 0.20. Non-significant RC was noticed for LDL (-0.04) on a relative expression of miR-29a.

3.7 The Regression Coefficient of Amino Acids on a Relative Expression of miR-34a in Serum

The regression coefficients for all amino acids showed no significant relationship with the relative expression of miR-34a in the serum of Awassi ewes (Table 7). Both essential and non-essential amino acids demonstrated negative RC. Specifically, the coefficients ranged from -0.006 for aspartic acid, which had an R^2 value of 0.0001,

Table 5 The regression coefficient for fatty, and carboxylic acids on a relative expression of miR-29a in serum of Awassi ewes

Attributes		Regression coefficient (b)	Prediction equation	Significance level	Determination coefficient (R^2)
Saturated fatty acids (%)	palmitic	0.03	$\bar{Y} = 4.50 + 0.03X$	$P \leq 0.05$	0.20
	stearic	0.03	$\bar{Y} = 2.48 + 0.03X$	$P \leq 0.05$	0.21
Total		0.06	$\bar{Y} = 6.99 + 0.06X$	$P \leq 0.05$	0.21
Unsaturated fatty acids (%)	oleic	0.03	$\bar{Y} = 7.36 + 0.03X$	$P \leq 0.05$	0.21
	linoleic	0.16	$\bar{Y} = 13.52 + 0.16X$	NS	0.02
	α-linolenic	0.05	$\bar{Y} = 9.01 + 0.05X$	$P \leq 0.05$	0.21
	eicosapentaenoic	0.04	$\bar{Y} = 5.59 + 0.04X$	$P \leq 0.05$	0.19
	arachidonic	0.01	$\bar{Y} = 0.70 + 0.01X$	$P \leq 0.05$	0.20
Total		0.46	$\bar{Y} = 37.17 + 0.46X$	NS	0.01
Volatile fatty acids (%)	acetic	-0.03	$\bar{Y} = 6.24 + 0.03X$	$P \leq 0.01$	0.25
	butyric	-0.03	$\bar{Y} = 3.11 + 0.03X$	$P \leq 0.01$	0.25
	propionic	-0.03	$\bar{Y} = 4.25 + 0.03X$	$P \leq 0.01$	0.25
Total		0.10	$\bar{Y} = 13.61 + 0.10X$	$P \leq 0.01$	0.26

NS – non-significant

Table 6 The regression coefficient for biochemical attributes on a relative expression of miR-29a in serum

Attributes	Regression coefficient (b)	Prediction equation	Significance level	Determination coefficient (R^2)
Glucose (mg·dl^{-1})	0.12	$\bar{Y} = 63.13 + 0.12X$	$P \leq 0.01$	0.33
Cholesterol (mg·dl^{-1})	0.15	$\bar{Y} = 61.54 + 0.15X$	$P \leq 0.01$	0.31
Total protein (g·dl^{-1})	0.04	$\bar{Y} = 6.23 + 0.04X$	$P \leq 0.01$	0.26
Albumin (g·dl^{-1})	0.01	$\bar{Y} = 3.40 + 0.01X$	$P \leq 0.01$	0.03
Globulin (g·dl^{-1})	0.02	$\bar{Y} = 2.82 + 0.02X$	$P \leq 0.05$	0.24
Triglycerides(mg·dl^{-1})	0.67	$\bar{Y} = 40.23 + 0.67X$	$P \leq 0.01$	0.36
HDL (mg·dl^{-1})	0.04	$\bar{Y} = 23.76 + 0.04X$	$P \leq 0.01$	0.35
LDL (mg·dl^{-1})	-0.04	$\bar{Y} = 30.03 - 0.04X$	NS	0.13
vLDL (mg·dl^{-1})	0.13	$\bar{Y} = 8.04 + 0.13X$	$P \leq 0.01$	0.36
AST (U·L^{-1})	0.40	$\bar{Y} = 129.84 + 0.40X$	$P \leq 0.01$	0.38
ALT (U·L^{-1})	-0.03	$\bar{Y} = 20.48 - 0.03X$	$P \leq 0.05$	0.18
ALP (U·L^{-1})	1.27	$\bar{Y} = 153.75 + 1.27X$	$P \leq 0.05$	0.20
Progesterone (ng·ml^{-1})	0.08	$\bar{Y} = 10.11 + 0.08X$	NS	0.02
Estrogen (pg·ml^{-1})	0.81	$\bar{Y} = 28.61 + 0.81X$	$P \leq 0.05$	0.21

NS – non-significant

Table 7 The regression coefficient for amino acids on a relative expression of miR-34a in serum of Awassi ewes

Attributes		Regression coefficient(b)	Prediction equation	Significance level	Determination coefficient (R^2)
Non-essential amino acids (μg·gm^{-1})	aspartic acid	-0.006	$\bar{Y} = 8.04 - 0.006X$	NS	0.0001
	asparagine	-0.04	$\bar{Y} = 7.25 - 0.04X$	NS	0.004
	serine	-0.10	$\bar{Y} = 8.53 - 0.10X$	NS	0.008
	alanine	-0.02	$\bar{Y} = 9.15 - 0.02X$	NS	0.001
	tyrosine	-0.16	$\bar{Y} = 10.45 - 0.16X$	NS	0.02
	arginine	-0.07	$\bar{Y} = 8.31 - 0.07X$	NS	0.006
	cysteine	-0.07	$\bar{Y} = 7.69 - 0.07X$	NS	0.004
	proline	-0.7	$\bar{Y} = 5.32 - 0.07X$	NS	0.0
	glycine	-0.18	$\bar{Y} = 6.79 - 0.18X$	NS	0.02
	total	-0.43	$\bar{Y} = 699.63 - 0.43X$	NS	0.001
Essential amino acids (μg·gm^{-1})	lysine	-0.07	$\bar{Y} = 8.35 - 0.07X$	NS	0.009
	threonine	-0.09	$\bar{Y} = 6.56 - 0.09X$	NS	0.008
	leucine	-0.14	$\bar{Y} = 6.90 - 0.14X$	NS	0.02
	isoleucine	-0.12	$\bar{Y} = 10.55 - 0.12X$	NS	0.01
	valine	-0.10	$\bar{Y} = 16.38 - 0.10X$	NS	0.007
	methionine	-0.03	$\bar{Y} = 7.51 - 0.03X$	NS	0.001
	histidine	-0.22	$\bar{Y} = 5.81 - 0.22X$	NS	0.07
	phenyl	-0.11	$\bar{Y} = 8.59 - 0.11X$	NS	0.01
	total	-0.91	$\bar{Y} = 70.67 - 0.91X$	NS	0.01

NS – non-significant

to -0.91 for total essential amino acids, with an R^2 value of 0.01 (Table 7).

3.8 The Regression Coefficient of Fatty and Carboxylic Acids on a Relative Expression of miR-34a in Serum

The results indicated a non-significant positive regression coefficient for saturated, unsaturated fatty acids, and volatile fatty acids (Table 8).

3.9 The Regression Coefficient of Biochemical Attributes on a Relative Expression of miR-34a in Serum

The relative expression of miR-34a demonstrated a highly significant positive RC ($P \leq 0.01$) with alkaline phosphatase (ALP) levels, indicating an increase of 14.23 U·L^{-1} for every unit increase in miR-34a expression, as reflected in an R^2 value of 0.34 (Table 9). Additionally, a significant positive RC ($P \leq 0.05$) was observed for several other parameters: glucose (0.82), total protein (0.29), albumin (0.08), globulins (0.21), high-density lipoprotein (HDL) (0.29), and aspartate aminotransferase (AST; 2.25), all with R^2 values of 0.19, 0.19, 0.21, 0.18, 0.19, and 0.16, respectively. In contrast, cholesterol (0.84/), triglycerides (3.33/), low-density lipoprotein (LDL; -0.09), very low-density lipoprotein (vLDL; 0.66), and alanine aminotransferase (ALT; 0.04) exhibited non-significant correlations, with R^2 values of 0.13, 0.12, 0.008, 0.12, and 0.003, respectively (Table 9).

This study is the first to address serum miR-29a and miR-34a expression levels across different reproductive stages as potential biomarkers, along with their relationship to litter size, offspring sex, and blood parameters in Iraqi Awassi ewes. The selection of the current miR-29a and miR-34a was conducted based on their well-documented roles in reproductive physiology, metabolic regulation, and placental development. The miR-29a is known to regulate glucose and lipid metabolism (Dalgaard et al., 2022), which are critical for supporting fetal growth and maintaining maternal energy balance during pregnancy. Its expression is particularly sensitive to metabolic disturbances, such as gestational diabetes mellitus (GDM), making it a potential biomarker for pregnancy-related metabolic changes (Dalgaard et al., 2022). Additionally, miR-29a plays a role in the remodeling of the extracellular matrix and has been implicated in the dynamics of uterine and placental tissues (Wang et al., 2023). In contrast, miR-34a is recognized for its involvement in cell cycle regulation (Achari et al., 2017), apoptosis (He et al., 2007), and stress response (Andolina

Table 8 The regression coefficient for fatty acids on a relative expression of miR-34a in serum of Awassi ewes

Attributes		Regression coefficient (b)	Prediction equation	Significance level	Determination coefficient (R^2)
Saturated Fatty acids %	palmitic	0.08	$\bar{Y} = 4.73 + 0.08X$	NS	0.01
	stearic	0.08	$\bar{Y} = 2.74 + 0.08X$	NS	0.01
Total		0.17	$\bar{Y} = 7.47 + 0.17X$	NS	0.01
Unsaturated Fatty acids %	oleic	0.03	$\bar{Y} = 7.61 + 0.03X$	NS	0.003
	linoleic	0.16	$\bar{Y} = 13.52 + 0.16X$	NS	0.02
	α-linolenic	0.11	$\bar{Y} = 9.36 + 0.11X$	NS	0.01
	eicosapentaenoic	0.10	$\bar{Y} = 5.90 + 0.10X$	NS	0.01
	arachidonic	0.04	$\bar{Y} = 0.76 + 0.04X$	NS	0.04
Total		0.46	$\bar{Y} = 37.17 + 0.46X$	NS	0.01
Volatile fatty acids %	acetic	0.03	$\bar{Y} = 6.51 + 0.03X$	NS	0.003
	butyric	0.08	$\bar{Y} = 3.39 + 0.08X$	NS	0.01
	propionic	0.10	$\bar{Y} = 4.51 + 0.10X$	NS	0.02
Total		0.21	$\bar{Y} = 14.42 + 0.21X$	NS	0.01

NS – non-significant

Table 9 The regression coefficient for biochemical attributes on a relative expression of miR-34a in serum of Awassi ewes

Attributes	Regression coefficient (b)	Prediction equation	Significance level	Determination coefficient (R^2)
Glucose (mg·dL^{-1})	0.82	$\bar{Y} = 63.65 + 0.82X$	$P \leq 0.05$	0.19
Cholesterol (mg·dL^{-1})	0.84	$\bar{Y} = 62.28 + 0.84X$	NS	0.13
Total protein (g·dL^{-1})	0.29	$\bar{Y} = 6.37 + 0.29X$	$P \leq 0.05$	0.19
Albumin (g·dL^{-1})	0.08	$\bar{Y} = 3.44 + 0.08X$	$P \leq 0.05$	0.21
Globulin (g·dL^{-1})	0.21	$\bar{Y} = 2.92 + 0.21X$	$P \leq 0.05$	0.18
Triglycerides(mg·dL^{-1})	3.33	$\bar{Y} = 43.70 + 3.33X$	NS	0.12
HDL (mg·dL^{-1})	0.29	$\bar{Y} = 23.95 + 0.29X$	$P \leq 0.05$	0.19
LDL (mg·dL^{-1})	-0.09	$\bar{Y} = 29.71 - 0.09X$	NS	0.008
vLDL (mg·dL^{-1})	0.66	$\bar{Y} = 8.74 + 0.66X$	NS	0.12
AST (U·L^{-1})	2.25	$\bar{Y} = 131.79 + 2.25X$	$P \leq 0.05$	0.16
ALT (U·L^{-1})	0.04	$\bar{Y} = 20.13 + 0.04X$	NS	0.003
ALP (U·L^{-1})	14.23	$\bar{Y} = 154.51 + 14.23X$	$P \leq 0.01$	0.34
Progesterone (ng·ml^{-1})	0.05	$\bar{Y} = 10.84 + 0.05X$	NS	0.0002
Estrogen (pg·ml^{-1})	6.9	$\bar{Y} = 30.69 + 6.9X$	$P \leq 0.05$	0.21

NS – non-significant

et al., 2017). These processes are essential during early embryonic development and placentation. Furthermore, miR-34a has been found to inhibit trophoblast invasion by targeting genes like MYC, linking it to regulating placental development and fetal-maternal exchange (Sun et al., 2015). Given their functional relevance to reproductive biology and pregnancy-associated metabolic changes, miR-29a and miR-34a were selected as target molecules for evaluating molecular responses during gestation in ewes. The significant outcomes of the flock's reproductive performance indicate that effective mating practices, along with proper feeding and healthcare management, are being implemented. These results surpassed those reported by Alkass et al. (2004) and Abdulkareem et al. (2023) for Awassi ewes in various provinces of Iraq.

Both miR-29a and miR-34a gene expression levels change dynamically during various phases of pregnancy. Undetectable miR-29a levels during the premating stage may be due to the uterine environment not requiring any major structural or physiological changes during this period. Moreover, the slight rise of miR-29a and miR-34a relative expression on day 14 of pregnancy suggested that miRNAs potentially play a role in remodeling the uterine tissue to support embryo implantation (Salilew-Wondim et al., 2020). The current findings align with those of Hitit et al. (2022), who reported that the miR-29 family was expressed on day 16 post-mating in ewes. Analyzing pathways involving miRNAs could provide valuable biomarkers for pregnancy-related changes in the uterus and signal maternal recognition of pregnancy (Cameron, 2012). The increase in miR-29a during early embryonic development (day 45) highlights its essential role in promoting physiological changes, including placental vascularization (Hayder et al., 2018). A study by Sánchez et al. (2021) found that elevated levels of miR-29a were present in the blood of cows at day 39 of gestation. This microRNA serves as a biomarker for pregnancy detection, and its regulation of the Wnt/β-catenin signaling pathway is essential for proper fetal development and adult homeostasis (Liu et al., 2022). The stability of the uterine and placental environment during the second trimester of pregnancy may lead to a decrease in the relative expression of miR-29a by day 75 of pregnancy. This drop in miR-29a levels continued through day 135, indicating that important physiological processes had taken place, which are essential for the final preparations for parturition. Simultaneously, the relative expression of miR-34a showed a slight increase, indicating potential regulation of tissue maturation or apoptosis as the body prepares for labor (He et al., 2007).

The current results indicate that the higher relative expression of miR-29a compared to miR-34a, based on litter size, suggests that these miRNAs have distinct regulatory roles in pregnancy and fetal development. miR-29a is involved in remodeling the extracellular matrix and has been implicated in different physiological and pathological processes during gestation. In contrast, miR-34a regulates the cell cycle and apoptosis, and its dysregulation has been associated with placental diseases like preeclampsia (Doridot et al., 2014). Study has indicated that serum levels of miR-29a are significantly lower in pregnant women diagnosed with GDM compared to healthy controls. This reduction is associated with adverse neonatal outcomes, including pathological jaundice. Elevated levels of miR-29a have also been found in the exosomes derived from the placenta of women with GDM, indicating a potential involvement in the altered metabolic state associated with this condition (Sørensen et al., 2021 & Deng et al., 2020). Moreover, miR-34a has been demonstrated to inhibit trophoblast cell invasion by targeting MYC, further highlighting its role in placental development and function (Sun et al., 2015 & Doridot et al., 2014). Depending on litter size, the varying expression patterns of miR-29a and miR-34a may indicate their unique roles in placental development and function. Increased levels of miR-29a may indicate normal placental function and better birth outcomes, while changes in miR-34a expression could lead to placental disorders, resulting in complications like preeclampsia (Jalivand et al., 2021).

The observed differences in the expression of miR-29a and miR-34a between sexes highlight the complex role of microRNAs in fetal development and placental function. Females show higher levels of miR-29a and lower levels of miR-34a than males. Recent studies have indicated that miRNA expression was biased toward fetal sex in the first trimester (Chavira-Suárez et al., 2023). Similarly, Tsamou et al. (2020) reported that these specific miRNAs exhibited sexual dimorphism in human newborns, with higher expression levels in females. The increased expression of miR-29a in females may correlate with improved placental function and better birth outcomes. miR-29a levels can change in response to metabolic conditions during pregnancy, such as GDM, and it may serve as a valuable biomarker for pregnancy-related complications. Moreover, miR-29a regulates reproductive processes, where its absence is associated with hormonal disruptions and reduced fertility in female models (Guo et al., 2021). In contrast, miR-34a is well-known for controlling the cell cycle and promoting apoptosis. Its elevated expression in males might indicate a sex-dependent regulatory role during the formation of the placenta. Research has demonstrated that the expression of miR-34a is modulated by maternal factors, including pre-pregnancy body mass index (BMI;

Meghan et al., 2025). A negative association was observed between the visceral adipose tissue PPARγ expression and miR-34a expression in obese cases (β = -0.594, *P* = 0.039; Zarkesh et al.2022). The current results confirmed this association by demonstrating a positive and significant regression between miRNA -34a and HDL (Table 9). HDL diminished lipid accumulation in adipocytes (Zhao et al., 2008). Elevated maternal BMI is associated with reduced placental miR-34a expression in female fetuses. These findings suggest that the regulation of miR-34a may be influenced by fetal sex and maternal health status (Varì et al., 2021).

The current findings show that the expression of miR-29a is inversely correlated with amino acid levels. This suggests that higher levels of these amino acids may lead to a decrease in the expression of this microRNA in serum. These results are consistent with those reported by Liu et al. (2018), who noted that certain amino acids can reduce gene expression by regulating transcription factors or influencing the activity of enzymes involved in the metabolism of miRNAs. In contrast to amino acids, fatty acids were found to have a positive effect on the expression of miR-29a. According to Zheng et al. (2020), fatty acids may enhance the gene expression of miRNAs that are associated with metabolic processes. On the other hand, volatile fatty acids showed a strong negative correlation with miR-29a expression. This viewpoint is supported by Smith and Macfarlane (2011), who suggested that carboxylic acids might influence gene regulation and inflammatory processes. Biochemical attributes, including glucose, cholesterol, total protein, albumin, and triglycerides, demonstrated a strong positive correlation. This may indicate the role of miR-29a in regulating energy and lipid metabolism. In skeletal muscle, overexpression of miR-29a disrupts insulin signaling pathways, resulting in decreased glucose uptake and altered lipid oxidation (Rottiers & Näär, 2012). Furthermore, elevated levels of miR-29a have been observed in animals with metabolic disorders such as type 2 diabetes mellitus (T2DM) and GDM, highlighting its role in metabolic dysregulation (Massart et al., 2017). The positive relationship between miR-29a and albumin levels may indicate its role in maintaining protein homeostasis, possibly through regulating extracellular matrix components and fibrotic processes. Increased expression of miR-29a have been associated to fibrotic changes in diabetic nephropathy, suggesting a role in renal function and protein metabolism (Liu et al., 2022). In contrast, miR-34a did not show significant correlations with most biochemical variables. However, alkaline phosphatase (ALP), glucose, total protein, albumin, and globulin significantly affected miR-34a, suggesting a potential link to liver function and various metabolic processes. Chang et al. (2019) found that miR-34a can alter glucose metabolism in liver cancer cells, affecting their growth. Increased expression of miR-34a may be linked to dyslipidemia and endothelial dysfunction specifically in metabolic diseases like T2DM (Ibrahim et al., 2020). The positive correlation between miR-34a and alkaline phosphatase (ALP), a marker of liver function, suggests that the liver may play a role in the metabolic regulation mediated by miR-34a (Liu et al., 2019).Moreover, the links to total protein, albumin, and globulin suggest that these factors may play a role in protein production and immune function – both of which are essential for sustaining maternal and fetal well-being during pregnancy. These findings are consistent with the notion that miR-29a may play a functional role in early pregnancy and contributes to embryonic development. The regression coefficient analysis can give us an estimate of the degree of association between two components. It is an absolute measure of relationship. Moreover, the prediction based on RC analysis will be more reliable and near to reality (Ali and Younas, 2021 and Musa and Abdulkareem, 2024).

4 Conclusions

The miR-29a and miR-34a microRNAs may serve as potential biomarkers for pregnancy in Iraqi Awassi ewes. miR-29a plays a crucial role in early pregnancy by supporting placental development and modulating the immune response. In contrast, miR-34a may be important for tissue maturation during late gestation. The significant correlations observed between metabolic and biochemical factors highlight their relevance in reproductive and metabolic regulation. These findings offer new insights for the early detection and monitoring of pregnancy in Iraqi Awassi ewes.

Authors Contribution

MicroRNAs expression analysis, original draft preparation and editing; Rwaida A. Ali and the corresponding author, experimental design, selection and analysis of specific microRNAs, statistical analysis, manuscript writing and comments; Dr.Talal A. Abdulkareem.

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References

Abdulkareem, T. A. et al. (2023). Effect of pre- and post-mating vitamin AD3E treatment on reproductive performance of Awassi ewes. *Iraqi Journal of Agricultural Sciences*, 54(2), 431–437. https://doi.org/10.36103/ijas.v54i2.1717

Abdulkareem, T. A. et al. (2024). Effect of sperms freezability on some semen attributes and amino acid concentrations in seminal plasma of Holstein bulls. *Iraqi Journal Agricultural Science*, 55(2), 665–674. https://doi.org/10.36103/pfgrpt.81

Achari, C. et al. (2014). Expression of miR-34c induces G2/M cell cycle arrest in breast cancer cells. *BMC cancer*, (14), 1–9.

Al-Gebouri, F. G., & Eidan, S. M. (2024b). Metabolites and semen characteristics in different bulls fertility. *Iraqi Journal of Agricultural Sciences*, 55(Special Issue), 206–216. https://doi.org/10.36103/ijas.v55iSpecial.1899

AL-Gebouri, F. G., & Eidan, S.M. (2024a). Effect of season on metabolites and semen traits of bulls. *Iraqi Journal of Agricultural Science*, 55(5), 1579–1587. https://doi.org/10.36103/193kb044

Ali, R. A. et al. (2024a). Impact of short-term of estrous synchronization on some reproductive performance characteristics in Awassi sheep. *Acta fytotechnica et zootechnica*, 27(3). https://doi.org/10.15414/afz.2024.27.03.198-202

Ali, R. A., et al. (2024b). Evaluation of semen characteristics, testicular measurements, and blood parameters for three genetic groups of goat bucks in Iraq. *Journal of Animal Health and Production*, 12(2), 233–239.

Ali, P., & Younas, A. (2021). Understanding and interpreting regression analysis. *Evidence-Based Nursing*, 24(4), 116–118.

Alkass, J. E., et al. (2004). Reproductive performance of Iraqi Awassi ewes in response to treatment with equine chorionic gonadotropin. *Journal of Agricultural Invest*, 2, 74–77.

Andolina, D. et al. (2017). MiRNA-34 and stress response. *Oncotarget*, 8(4), 5658–5659. https://doi.org/10.18632/oncotarget.13923

Batra K., et al. (2024). Identification of circulatory microRNA based biomarkers for early pregnancy diagnosis in buffalo. *Front. Cell Dev. Biol.,* 12, 1386241. https://doi.org/10.3389/fcell.2024.1386241

Cameron, A. D. (2012). *Exosomal microRNAs as a biological marker of maternal recognition of pregnancy in the mare* (Master's thesis, Colorado State University).

Chang, H. et al. (2019). miR-34a inhibits liver cancer cell growth by reprogramming glucose metabolism. *Cell Death and Disease*, 10(2), 144. https://doi.org/10.3892/mmr.2018.8399

Chavira-Suárez, E. et al. (2023). Longitudinal large-scale changes in maternal circulating microRNAs associated with gestation-related compartments, fetal sex, and growth during and post-pregnancy. *Genomics*, 115, 110628.

Dalgaard, L. T. et al. (2022). The microRNA-29 family: role in metabolism and metabolic disease. *American journal of physiology*, 323(2), 367–377. https://doi.org/10.1152/ajpcell.00051.2022

Deng, L. et al. (2020). Serum miR-29a/b expression in gestational diabetes mellitus and its influence on prognosis evaluation. *The Journal of international medical research*, 48(9). https://doi.org/10.1177/0300060520954763

Donadeu, F. X. et al. (2016). Involvement of miRNAs in ovarian follicular and luteal development. *Journal of Endocrinology*, 215, 323–334.

Doridot, L. et al. (2014). miR-34a expression, epigenetic regulation, and function in human placental diseases. *Epigenetics*, 9(1), 142–151. https://doi.org/10.4161/epi.26196

Duncan D. B. (1955). Multiple ranges and multiple F tests. *Biometrics*, 11, 1–41. https://doi.org/10.2307/3001478

Eidan, S.M. et al. (2024). Some of fatty acid and semen characteristics of Holstein bulls as influenced by different sperm freezability. *Iraqi Journal of Agricultural sciences*, 55(2), 675–682. https://doi.org/10.36103/cg6xrf46

Eidan, S. M., & Khudhir, S. A. (2023). Association between ATP1A1 gene polymorphisms with semen characteristics in Holstein bulls. *Iraqi Journal of Agricultural Sciences*, 54 (2), 330–337. https://doi.org/10.36103/ijas.v54i2.1706

Flatz, L., Bergthaler, A., de la Torre, J. C., & Pinschewer, D. D. (2006). Recovery of an arenavirus entirely from RNA polymerase I/II-driven cDNA. *Proceedings of the National Academy of Sciences*, 103(12), 4663–4668.

Gebremedhn, S. et al. (2018). Exploring maternal serum microRNAs during early pregnancy in cattle. *Theriogenology,* 121, 196–203. https://doi.org/10.1016/2018.08.020

Guo S. et al. (2021). Variation of microRNA expression in the human placenta driven by population identity and sex of the newborn. *BMC Genomics*, 22, 286.

Guo, T. Z. et al. (2019). CircINHA resists granulosa cell apoptosis by upregulating CTGF as a ceRNA of miR-10a-5p in pig ovarian follicles. *Biochimica et biophysica acta Gene regulatory mechanisms*, 1862(10), 194420. https://doi.org/10.1016/j.bbagrm.2019.194420

Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nature reviews Molecular cell biology*, 15(8), 509–524.

Hayder, H. et al. (2018). MicroRNAs: crucial regulators of placental development. *Reproduction*, 155(6), 259–271. https://doi.org/10.1530/REP-17-0603

He, X. et al. (2007). The guardian's little helper: microRNAs in the p53 tumor suppressor network. *Cancer Research*, 67, 11099–101

Hitit, M. et al. (2022). Circulating miRNAs in maternal plasma as a potential biomarkers of early pregnancy in sheep. *Front. in Genetics,* 13, 929477. https://doi:10.3389/fgene.2022.929477

Ibrahim, A. A. et al. (2020). Association of Exosomal miR-34a with Markers of Dyslipidemia and Endothelial Dysfunction in Children and Adolescents with T1DM. *Journal of clinical research in pediatric endocrinology*, 12(4), 401–409. https://doi.org/10.4274/jcrpe.galenos.2020.2020.0134

Jalilvand, T. et al. (2021). The expression of miR-17 and miR-29a in placenta-derived exosomes in LPS-induced abortion mice model: An experimental study. *Int. Journal of Reproductive Biomedicine*, 19(5), 433–440. http://doi.org/10.18502/ijrm.v19i5.9252

Kiyma, Z. et al. (2015). Preliminary investigation of microRNAs in ovine endometrium: P70. *Reproduction Domestics Animals,* 50. https://doi.org/10.1111/rda.12581

Kose, M. et al. (2022). Expression pattern of microRNAs in ovine endometrium during the peri-implantation. *Theriogenology*, 191, 35–46. https://doi.org/10.1016/07.015

Liu, H. et al. (2019). miR-34a promotes bone regeneration in irradiated bone defects by enhancing osteoblastic differentiation of mesenchymal stromal cells in rats. *Stem cell research & therapy*, 10, 1–14.

Liu, J. et al. (2022). Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduction and Targeted Therapy, 7(3). https://doi.org/10.1038/s41392-021-00762-6

Liu, Q. et al. (2022). Significance of serum miR-29a in the occurrence and progression of diabetic nephropathy: A cross-sectional study. *Journal of clinical laboratory analysis*, 36(2), e24210. https://doi.org/10.1002/jcla.24210

Liu, X. et al. (2018). The role of amino acids in regulating gene expression and cellular signaling pathways. *Nutrients*, 10(7), 915.

Massart, J. et al. (2017). Altered miR-29 Expression in Type 2 Diabetes Influences Glucose and Lipid Metabolism in Skeletal Muscle. *Diabetes*, 66(7), 1807–1818. https://doi.org/10.2337/db17-0141

Mazzarella, R. et al. (2024). Extracellular vesicles-coupled miRNAs from oviduct and uterus modulate signaling pathways related to lipid metabolism and bovine early embryo development. *Journal of Animal Science Biotechnology*, 15(51). https://doi.org/10.1186/s40104-024-01008-5

McBride, D. et al. (2012). Identification of miRNAs associated with the follicular-luteal transition in the ruminant ovary. *Reproduction*, 144, 221–233. https://doi.org/10.1530/REP-12-0025

Muse, M. E., Wang, Y., Diamond, D. G., Armstrong, D. A., Hoen, A. G., Romano, M. E., Gui, J., Palys, T. J., Kolling, F. W., Christensen, B. C., Karagas, M. R., & Howe, C. G. (2025). Maternal diet quality in pregnancy and human milk extracellular vesicle and particle microRNA. *Epigenetics Reports*, 3(1), 1–10. https://doi.org/10.1080/28361512.2025.2508883

Musa, K. S., & Abdulkareem, T. A. (2024a). Regression coefficient of semen characteristics of buffalo bulls on seminal plasma proteins. *IOP Conf. Ser.: Earth Environ. Sci.*, 1302, 012055. https://doi:10.1088/1755-1315/1302/1/012055

Musa, K. S., & Abdulkareem T. A. (2024b). Some biochemical attributes in seminal plasma of Iraqi buffalo bulls and their relation to the semen quality. *Iraqi Journal Agricultural Science*, 55(1), 402–412. https://doi.org/10.36103/nrfkex70

Ono, K. et al. (2022). Analysis of circulating microRNA during early gestation in Japanese black cattle. *Domestic Animal Endocrinology*, 79. https://doi.org/10.1016/j.domaniend.2021.106706

Pokharel, K. et al. (2018). Integrated ovarian mRNA and miRNA transcriptome profiling characterizes the genetic basis of prolificacy traits in sheep (*Ovis aries*). *BMC Genomics*, 19(104). https://doi.org/10.1186/s12864-017-4400-4

Paulson, E. E. et al. (2022). Embryonic microRNAs are essential for bovine preimplantation embryo development. *Proc Natl Acad Sci USA*, 119(45), e2212942119. https://doi.org/10.1073/pnas.2212942119

Rottiers, V., & Näär, A. (2012). MicroRNAs in metabolism and metabolic disorders. *Nature Reviews Molecular Cell Biology*, 13(4), 239–250. https://doi.org/10.1038/nrm3313

Salilew-Wondim, D. et al. (2020). The Role of MicroRNAs in Mammalian Fertility: From Gametogenesis to Embryo Implantation. *International Journal of Molecular Sciences*, 21(2), 585. https://doi.org/10.3390/ijms21020585

Sánchez, J. M. et al. (2021). MicroRNAs in amniotic fluid and maternal blood plasma associated with sex determination and early gonad differentiation in cattle. *Biology of Reproduction*, 105, 345–358. https://doi.org/093/IOAB079

Sadowska, A. et al. (2024). Bioinformatic analysis of endometrial miRNA expression profile at day 26–28 of pregnancy in the mare. *Sci. Rep.*, 14, 3900. https://doi.org/10.1038/s41598-024-53499-x

SAS. (2018). *Statistical Analysis System, User's Guide Statistical Version 91st ed SAS Inst*. Inc. Cary NC. USA

Seger, D. K. et al. (2023). Polymorphisms of somatostatin receptor (sstr-1) gene and its relationship with carcass traits in Iraqi Awassi sheep. *Advance of Animal Veterinary Science*, 11(10), 1644–1650. http://dx.doi.org/10.17582/journal.aavs/2023/11.10.1644.1650

Smith, P. M., & Macfarlane, G. T. (2011). The impact of the gut microbiota on human health. *Clinical Microbiology Reviews*, 24(3), 562–591.

Song, Y. et al. (2015). Identification and profiling of microRNAs in goat endometrium during embryo implantation. *PLoS One*, 10(4), e0122202. https://doi.org/10.1371/journal.pone.0122202

Sørensen, A. E. et al. (2021). The predictive value of miR-16,-29a and-134 for early identification of gestational diabetes: a nested analysis of the DALI cohort. *Cells*, 10(1), 170. https://doi.org/10.3390/cells10010170

Sun, D. et al. (2021). MiR-455-5p upregulation in umbilical cord mesenchymal stem cells attenuates endometrial injury and promotes repair of damaged endometrium via Janus kinase/signal transducer and activator of transcription 3 signaling. *Bioengineered*, 12(2), 12891–12904. https://doi.org/10.1080/21655979.2021.2006976

Sun, M. et al. (2015). MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC. *BMC Cell Biol.,* 16(21). https://doi.org/10.1186/s12860-015-0068-2

Tan, Q. et al. (2020). MicroRNAs in small extracellular vesicles indicate successful embryo implantation during early pregnancy. *Cells*. https://doi.org/10.3390/CELLS90306459

Tsamou, M. et al. (2020). Genome-wide microRNA expression analysis in human placenta reveals sex-specific patterns: an ENVIRONAGE birth cohort study. *Epigenetics*, 16, 373– 388.

Varì, R. et al. (2021). Significance of Sex Differences in ncRNAs Expression and Function in Pregnancy and Related Complications. *Biomedicines*, 9(11), 1509. https://doi.org/10.3390/biomedicines9111509

Vlahek, I. etal. (2023). Prediction of reproductive performance of ewes based on the early production data, ewe birth rank, dam age, and dam birth rank. *Arch. Anim. Breed.*, 66, 145–151. https://doi.org/10.5194/aab-66-145-2023

Wang, M., Huo, Z., He, X., Liu, F., Liang, J., Wu, L., & Yang, D. (2023). The role of MiR-29 in the mechanism of fibrosis. *Mini Reviews in Medicinal Chemistry*, 23(19), 1846–1858.

Winger, E. E. et al. (2020). MicroRNAs isolated from peripheral blood in the first trimester predict spontaneous preterm birth. *PLoS ONE*,15. https://doi.org/10.1371/journal.pone.0236805

Wu, W. et al. (2016). Analysis of microRNA of ovine pre-implantation embryo developed *in vitro*. *Journal of Animal Science*, (94), 69–70. https://doi.org/10.2527/jas2016.94supplement469a

Yang, H. et al. (2018). Identification and analysis of microRNAs-mRNAs pairs associated with nutritional status in seasonal sheep. *Biochemical and Biophysical Research Communications*, 499, 321–327. https://doi.org/10.1016/j.bbrc.2018.03.155

Yang, J. et al. (2019). Comparative mRNA and miRNA expression in European mouflon (*Ovis musimon*) and sheep (*Ovis aries*) provide novel insights into the genetic mechanisms for female reproductive success. *Heredity*, 12, 172–186. https://doi.org/10.1038/s41437-018-0090-1

Zang, X. et al. (2021). Differential microRNA expression involved in endometrial receptivity of goats. *Biomolecules*, 11(3), 472.

Zarkesh, M. et al. (2022). Association of miR-34a and miR-143 levels with PPARγ gene expression in adipose tissues of non-diabetic adults. *J Physiol Anthropol*., 41(1), 13. https://doi.org/10.1186/s40101-022-00286-0

Zhang, J. Q. et al. (2020). miR-181a promotes porcine granulosa cell apoptosis by targeting TGFBR1 via the activin signaling pathway. *Molecular and cellular endocrinology*, 499, 110603. https://doi.org/10.1016/j.mce.2019.110603

Zhang, G., He, M., Wu, P., Zhang, X., Zhou, K., Li, T., Zhang, T., Xie, K., Dai, G., & Wang, J. (2021). MicroRNA-27b-3p targets the myostatin gene to regulate myoblast proliferation and is involved in myoblast differentiation. *Cells*, 10(2), 423.

Zhao, S. P. et al. (2008). Effect of niacin on LXRalpha and PPARgamma expression and HDL-induced cholesterol efflux in adipocytes of hypercholesterolemic rabbits. *Int J Cardiol,* 124, 172–178. https://doi.org/S0167-5273(07)00349-X10.1016/j.ijcard.2006.12.032

Zhao, L. et al. (2021). Screening and identification of microRNAs from plasma-derived extracellular vesicles (EVs) of Dazu black goat (*Capra hircus*) in early pregnant stages. *Gene*, 790,145706.

Zhanmin, L. V. et al. (2025). Differences in microRNA within plasma extracellular vesicles at various time points during early pregnancy in sheep revealed by high-throughput sequencing data. *Animal Reproductive Science*, 107833. https://doi.org/10.1016/j.anireprosci.2025.107833

Zheng, J. et al. (2020). The role of fatty acids in microRNA regulation and implications for metabolic diseases. *Journal of Molecular Medicine*, 98(5), 655–666.