



Histological Development and Irisin Expression in the Goat Placenta During Pregnancy

Merve PEKİNCE ÖZÖNER^{1, a}

¹ Siirt University,
Faculty of Veterinary
Medicine,
Department of Histology,
Siirt, TÜRKİYE

^a ORCID: 0000-0002-5378-2726

In recent studies, it has been suggested that irisin, a myokine associated with exercise, plays a role not only in energy metabolism, but also in regulating the differentiation and specialisation of placental trophoblast cells. The aim of this study was to investigate the histological development of the interplacentomal region and the placentomal in Siirt Hair goats, and to determine the immunohistochemical expression and localisation of irisin at different stages of pregnancy. For the study, placental tissue was obtained from 18 fetuses collected from private slaughterhouses and determined to be at various stages of gestation. After routine tissue processing, haematoxylin and eosin (H&E) staining was used to examine the morphological structure and immunohistochemistry was used to evaluate irisin expression. Histological analysis shows that the placenta consists of a maternal caruncle and a fetal cotyledon. The primary villi were initially separated from the chorionic plate and transformed into secondary and tertiary villi in later stages of gestation. Irisin was detected in both maternal and fetal placental tissues, as well as in the interplacentomal region of goats. The strongest expression was observed in the maternal stroma during the early stages of pregnancy ($p < 0.05$). While most differences between gestational stages were not statistically significant, these data suggest that irisin may be a regulator of early placental development and maternal-fetal relations.

Key Words: *Irisin, goat, hair goat, placenta*

Keçi Plasentasında Gebelik Süresince Histolojik Gelişim ve Irisin Ekspresyonunun İncelenmesi

Son çalışmalarda, egzersizle ilişkili bir miyokin olan irisin'in sadece enerji metabolizmasında değil, aynı zamanda plasental trofoblast hücrelerinin farklılaşması ve özelleşmesinin düzenlenmesinde de rol oynadığı öne sürülmüştür. Bu çalışmanın amacı, Siirt Kıl keçilerinden elde edilen interplasentomal ve plasentomal bölgenin histolojik gelişimini araştırmak ve gebeliğin farklı aşamalarında irisin'in immünohistokimyasal ekspresyonunu ve lokalizasyonunu belirlemektir. Çalışma için, özel kesimhanelerden toplanan ve gebeliğin çeşitli aşamalarında olduğu belirlenen 18 fetüsten plasental doku elde edildi. Rutin doku işlemlerinden sonra, morfolojik yapıyı incelemek için hematoksilen ve eozin (H&E) boyaması ve irisin ekspresyonunu değerlendirmek için immünohistokimya tekniği kullanılmıştır. Histolojik analiz plasentanın maternal bir karunkül ve fetal bir kotiledondan oluştuğunu göstermektedir. Koryonik plakdan ayrılan primer villuslar, gebeliğin ilerleyen aşamalarında sekonder ve tersiyer villuslara dönüştüğü gözlemlendi. Irisin hem maternal hem de fetal plasental dokularda ve keçilerin interplasental bölgesinde tespit edilmiş, en güçlü ekspresyon erken gebelik döneminde maternal stromada gözlenmiştir ($p < 0.05$). Gebelik evreleri arasındaki farkların çoğu istatistiksel olarak anlamlı olmasa da, bu bulgular irisin'in erken plasental gelişim ve maternal-fetal gelişimin potansiyel bir düzenleyicisi olduğunu vurgulamaktadır.

Anahtar Kelimeler: *Irisin, keçi, kıl keçisi, plasenta*

Introduction

Goat farming is highly valued for its short breeding intervals, high twinning rates, short growth periods, and ease of rearing (1). One of the most important aspects of breeding is the healthy offspring of healthy mothers resulting from a healthy pregnancy and the continuation of the offspring. The reproductive performance of farm animals such as goats, sheep, cattle, and pigs is closely linked to genetic and environmental factors, as well as endocrine factors (2).

The placenta is composed of two distinct regions, the placentomal and interplacentomal areas, which develop and differentiate as pregnancy progresses. The interplacentomal area has been found to be simply located between the fetal membranes and the uterine epithelium. Placentomies, on the other hand, function as functional units of the placenta, forming the connection between the caruncular uterine and fetal cotyledons in later pregnancies (3). The placenta, formed from the uterine mucosa and the offspring's chorion at the beginning of pregnancy, plays a role in the fetal development and protection. Healthy placentation not only provides nutrition, development, excretion, and respiration for the embryo, but also provides a healthy connection between the mother and the developing fetus by secreting various hormones throughout embryonic development (4). Maternal endocrine glands are paracrine,

Correspondence

Merve PEKİNCE ÖZÖNER
Siirt University,
Faculty of Veterinary
Medicine,
Department of Histology
Siirt – TÜRKİYE

mervepknc2323@gmail.com

autocrine and endocrine secretory organs that synthesize a wide variety of steroids and peptide hormones, primarily progesterone, estrogen, relaxin, hCG, and placental hormones, which support fetal development by providing hormonal support to the fetus via the fetoplacental unit and change maternal physiology in the process (5).

Irisin is an adipokine and myokine that increases with pregnancy and regularly functions to maintain energy homeostasis throughout pregnancy (4, 6). Irisin, first identified in 2012 as a myokine polypeptide released from muscle tissue in response to exercise. It results from the proteolytic degradation of FNDC5, regulates glucose and lipid metabolism in adipose tissue (7). Recently, it has been demonstrated that irisin is not only involved in energy storage and secretion, but also plays a role in meeting energy needs related to reproductive function and fetal growth, and in balancing pregnancy-related metabolic changes (8).

Serum irisin levels were found to be higher in pregnant women than in non-pregnant women during pregnancy (9, 10). Furthermore, the expression of the fibronectin type III domain-containing protein 5 (FNDC5) protein was determined in the placenta of pregnant women (9). Irisin has been shown to have cytoprotective properties in human preeclamptic placentas and in placental disease models (10, 11). Recently, it has been demonstrated that irisin may play a role in the placenta by regulating trophoblast differentiation through AMPK activation (12).

Studies on irisin hormone in twelve different species (including humans) have revealed the presence of irisin protein sequences (4). It has been reported that irisin hormone is expressed in smaller amounts in the testes, liver, pancreas, brain, spleen, heart, and stomach, in addition to skeletal muscle and adipose tissue (13–15). Expression has also been observed in the ovaries, placental tissues, and umbilical cord serum of newborns (9). In human placental tissue, it is localized in cytotrophoblasts and syncytiotrophoblast cells in the placental decidua (9). A study specific to ruminants showed that irisin hormone is expressed in the placenta during pregnancy in Merino sheep and that serum levels are higher throughout pregnancy (16). In recent years, studies have focused on the possible endocrine functions of adipose tissue and the adipokines it secretes on the reproductive system. Investigating the functions of adipokines in the reproductive system is essential for understanding energy homeostasis. Studies exist on farm animals as well, but they are insufficient.

In consideration of the aforementioned background, the objective of this study is to elucidate the histological alterations that occur in the placentomal and interplacentomal region in goats during the early, middle and late stages of pregnancy. Furthermore, the study seeks to ascertain the presence of irisin and its subsequent localization within the tissue using immunohistochemical methods.

Materials and Methods

Research and Publication Ethics: The procedures used in our study were approved by the Ethics Committee of Siirt University Experimental Animal Application and Research Center dated 30/01/2025 and numbered 2025/01/01.

Sample Collection and Gestational Age Determination: The study was conducted using fetal samples obtained from private slaughterhouses in Siirt province. After slaughter, an incision was made in the uterine horn, where the pregnancy is located, wide enough to allow for the removal of the fetus. After the fetal membranes and fluids were removed, the crown-rump length of each resulting fetus was measured. Gestational age was determined using the following formula: $X = 2.1(Y + 17)$ (X = gestational age (days), Y = crown-anus length (17, 18)). Singleton pregnancies were considered in the study. Eighteen pieces of uterine tissue from different periods of pregnancy including first trimester (0-66 days), second trimester (67-99 days) and third trimester (100-140 days) were taken together with the placenta and uteroplacental tissue was used (19).

Tissue Processing and Hematoxylin-Eosin: Tissue samples were taken from the placentome and interplacentome regions in three groups formed in the study and were fixed in 10% neutral formalin solution for 24 hours. Tissues were dehydrated with alcohol series and cleared with xylene in accordance with standard histological tissue processing procedures. The tissues were then subjected to routine tissue processing in xylene (2 x xylene, 1.5 hours each step) for clarification. The paraffin-embedded tissues were then blocked. 5 μ m-thick sections were cut from the paraffin blocks using a rotary microtome (Thermo Scientific, USA), and the sections were placed on slides. The slides were dried in a 37°C oven and prepared for staining. The slides were stained with hematoxylin and eosin for histopathological analysis. After staining was completed, the slides were dehydrated by passing through a series of alcohols of increasing concentrations, made transparent with xylene, and coverslipped with entellan (20).

Irisin Immunohistochemical Staining: The immunoperoxidase method was used for the immunohistochemical analyses performed in the study (21). First, 5 μ m-thick sections were cut from paraffin blocks of placentome and interplacentome region tissues. After deparaffinization with xylene, they were rehydrated by passing through a graded alcohol series. To inactivate endogenous enzymes, the tissues were held in 3% H₂O₂ for 10 minutes. Washing was carried out with phosphate buffered saline (PBS). Then, they were treated with Ultra V Block solution for 10 minutes and incubated with primary antibodies (Irisin, 1/100 dilution, 201r-0335, China) at +4°C overnight. For the negative control, phosphate-buffered saline (PBS) was applied in place of the primary antibody. The slides were incubated with a secondary antibody, followed by streptavidin-peroxidase, in a humid environment at 37 °C for 30 minutes. AEC (3-amino-9-ethylcarbazole substrate + AEC chromogen, LabVision Corporation,

USA) was applied as a chromogen, and the sections were stained with Mayer's haematoxylin for nuclear staining. They were then mounted with CC/mount Aqueous Mounting Medium and examined under a light microscope. Histopathological examination and immunostaining evaluation were performed blindly by two independent researchers.

Immunohistochemical histoscores were performed according to the extent and intensity of immunoreactivity as previously described (22). (Histoscore = extent (0.1: <25%, 0.4: 26%–50%, 0.6: 51%–75%, 0.9: 76%–100%) × intensity (0: absent, +0.5: very slight, +1 slight, +2: moderate, +3: severe). Irisin immunostaining in placentome and interplacentome tissue samples were examined microscopically at 10x, 20x, and 40x objective magnifications. For evaluation purposes, 4 random fields were selected from each section of placentome and interplacentome of each animal. A single value was determined for each animal by averaging the results obtained (23). The luminal epithelium, glandular epithelium, stromal tissue, and muscle cells of the interplacentomal region, and the maternal epithelium, fetal single and dual-nucleated trophoblast cells, and stromal cells of the placental region were evaluated.

Statistical Analysis: The data obtained were expressed as mean ± standard deviation, and statistical analyses were performed using the SPSS 22 program (IBM Corp., Armonk, NY, USA). When data showed a normal distribution, one-way ANOVA and Tukey's post hoc test were applied for comparisons of more than two groups. If the data did not meet the normality assumptions, the Kruskal-Wallis test was used, and pairwise comparisons were made using the Mann-Whitney U test. A p -value < 0.05 was calculated to determine whether the results were statistically significant. For the sample size analysis, the power of the test was determined to be 80%, and the type-1 error of 5% (G*Power statistics program, ver.3.1.9.4).

Results

Histological Findings of Interplacentomal and Placentome at different gestational ages: When the uterus is examined histologically, it consists of three layers: endometrium, myometrium, and perimetrium (Figure 1a–1c). Numerous glandular epithelia were found within the uterine stroma (Figure 1c-1g). Blood vessels were also observed among the glandular structures (Figure 1c-1l). The myometrium was determined to consist of neatly arranged smooth muscle cells (Figure 1e). In early pregnancy samples, it was observed that the uterine surface was in regular and compatible contact with trophoblast cells. During the first trimester, no shedding was observed in the uterine epithelium. As pregnancy progressed, occasional shedding in the uterine epithelium was observed, and cotyledons and caruncles became more prominent (Figure 1b).

Following implantation, the maternal caruncular structures and fetal cotyledons unite to form placental regions. Trophoblastic cells arising after embryonic

development play a role in this connection between the maternal endometrium and the fetal embryo (Figure 1d).

Samples taken from the placental region showed mononuclear trophoblast cells (MTC) and binucleated trophoblast cells (BTC) in the early period. As pregnancy progressed, the presence of large binucleated trophoblast cells became noticeable (Figure 1h). Primary villi from the chorionic plate of the placental region branched to form secondary and tertiary villi (Figure 1b). An increase in the number of secondary and tertiary villi was observed in the later stages of pregnancy compared to the early stages (Figure 1j). These chorionic villi contained nested fetal and maternal tissues (Figure 1j). Within the fetal stroma of the placental region, fetal mesenchyme composed of fibrocytes and fibroblasts was present (Figure 1f).

When the embryo began to attach to the uterine caruncular regions, the columnar epithelial cells of the uterus transformed into syncytial plaques (Figure 1h). The placental barrier between maternal and fetal tissues consisted, on the fetal side, of fetal mesenchyme, fetal vascular endothelium, and fetal trophoblastic epithelium, while on the maternal side it consisted of maternal connective tissue, maternal uterine epithelium, and maternal vascular endothelium. During the second and third trimesters, an increase in the number of mononuclear and binucleated trophoblast cells embedded in the trophoblastic epithelium was observed. Mononuclear trophoblast cells had round, euchromatic stained nuclei and columnar or cuboidal cells. Binucleated trophoblast cells were round or oval in shape with two nuclei (Figure 1h).

Immunohistochemical Localization of Irisin in Interplacentomal and Placentome Region: In the interplacentomal region of uterine samples obtained from early, mid, and late pregnancy, irisin was detected in the cytoplasmic and nuclear compartments of luminal and glandular epithelial cells, as well as in stromal and smooth muscle cells (Figure 2). When the immunoreactivity of irisin in these cells was evaluated according to histoscores, no statistically significant difference was observed among the three distinct gestational periods ($p < 0.05$, Table 1).

Analysis of irisin positivity in the cellular components of the interplacentomal region revealed particularly strong staining around the glandular structures (Figure 2c-2g-2k). Furthermore, an increase in reactivity was noted in the later stages of pregnancy; however, this increase was not statistically significant ($p > 0.05$, Table 1). Irisin immunoreactivity was detected in the luminal epithelium of the uterus throughout all pregnancy stages, and similar histoscores were observed across different gestational periods. Irisin also exhibited positive staining in the cytoplasm of smooth muscle cells located in the interplacentomal region (Figure 2a-2e).

In the fetal portion of the placenta, irisin demonstrated both cytoplasmic and nuclear immunoreactivity in fetal stromal cells as well as in mononuclear and binuclear trophoblasts (Figure 2d-2l).

This immunoreactivity was higher in the first trimester and decreased in later stages; however, this change was not statistically significant ($p>0.05$, Table 1).

Evaluation of the maternal compartment of the placenta showed that maternal stroma was more intense

in the first trimester (Figure 2d). A statistically significant difference was detected between groups ($p<0.05$, Table 1). Irisin reactivity was generally absent in the maternal epithelium. No positivity was observed in the negative control preparations.

Table 1. Distribution of irisin protein expression in the placenta and uterus of hair goats during early (0–66 days), mid (67–100 days), and late (100–150 days) pregnancy based on immunostaining intensity scores (IS). All values are presented as mean \pm standard deviation

		Endometrium				Placenta maternalis		Placenta fetalis		
		Luminal Epithelium	Glandular Epithelium	Stroma	Smooth Muscle	Maternal Epithelium	Maternal Stroma	MTC	BTC	Fötal Stroma
		IS	IS	IS	IS		IS	IS	IS	IS
Irisin IS	I. Period	0.83 \pm 0.6 ^a	1.2 \pm 0.8 ^a	0.8 \pm 0.3 ^a	0.14 \pm 0.11 ^a	-	1.25 \pm 1.11 ^a	0.73 \pm 0.05 ^a	0.5 \pm 0.51 ^a	0.53 \pm 0.41 ^a
	II. Period	0.85 \pm 0.4 ^a	1.5 \pm 0.3 ^a	0.8 \pm 0.6 ^a	0.05 \pm 0.03 ^a	-	0.04 \pm 0.02 ^b	0.20 \pm 0.02 ^a	0.2 \pm 0.21 ^a	0.18 \pm 0.16 ^a
	III. Period	0.87 \pm 0.7 ^a	1.8 \pm 0.9 ^a	0.8 \pm 0.6 ^a	0.07 \pm 0.06 ^a	-	0.6 \pm 0.06 ^b	0.07 \pm 0.02 ^a	0.1 \pm 0.07 ^a	0.19 \pm 0.34 ^a

A statistically significant difference is observed between values that do not share the same letter ($p<0.05$).

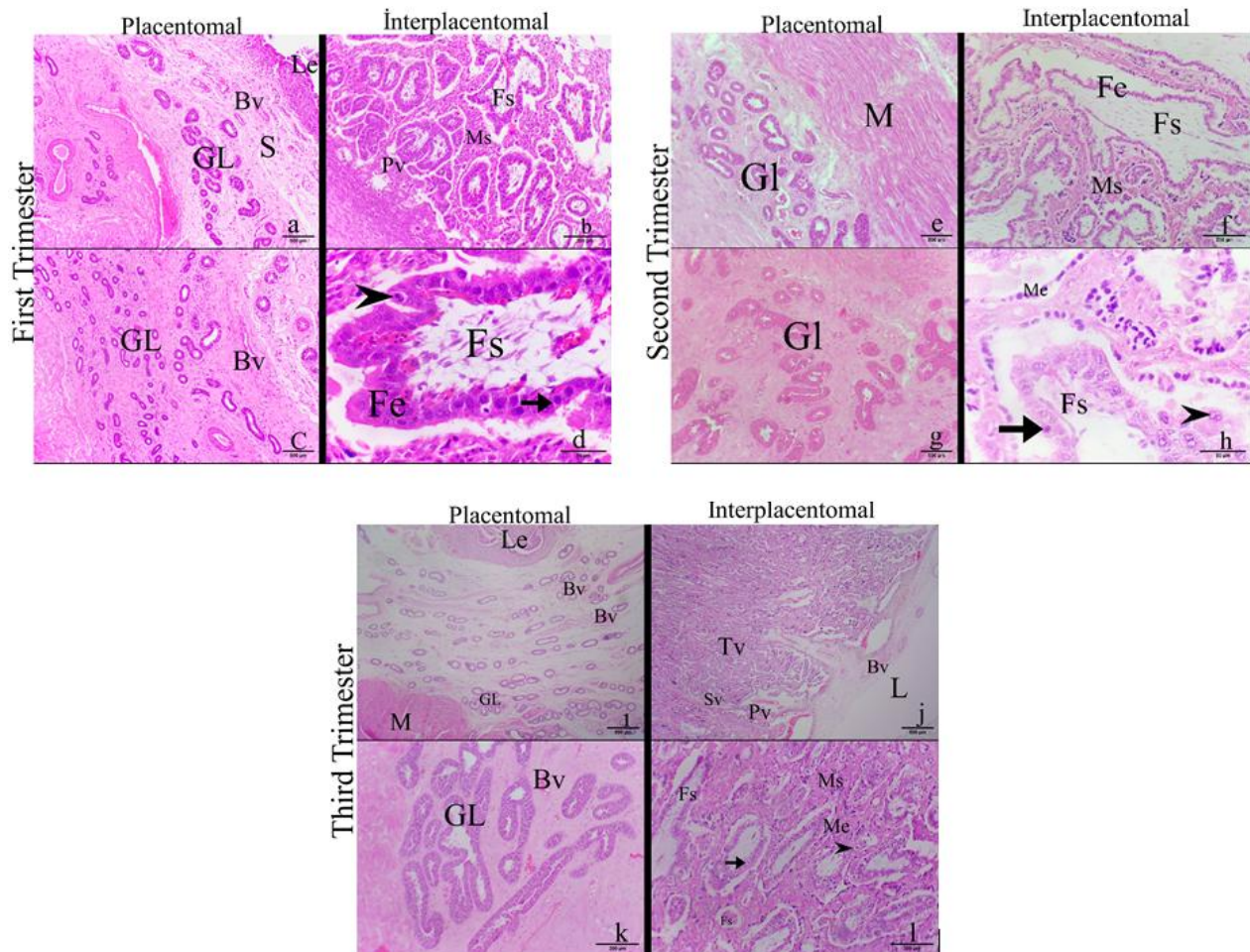


Figure 1. Histological images of the goat placenta and interplacentomal region during early (0–66 days) (a–d), mid (67–100 days) (e–h), and late (100–150 days) (i–l) stages of gestation. Gestation. Fe: Fetal Epithelium; FS: Fetal Stroma; Bv: Blood Vessel; Me: Maternal Epithelium; Ms: Maternal Stroma; Le: Luminal Epithelium; S: Stroma; Gl: Gland Epithelium; M: Myometrium; Pv: primary chorionic villi; Sv: secondary chorionic villi; Ts: tertiary chorionic villi. Scale bars: 500 μ m (i, j); 200 μ m (a, b, e, f, k, l); 50 μ m (c, d, g, h). Staining: H&E.

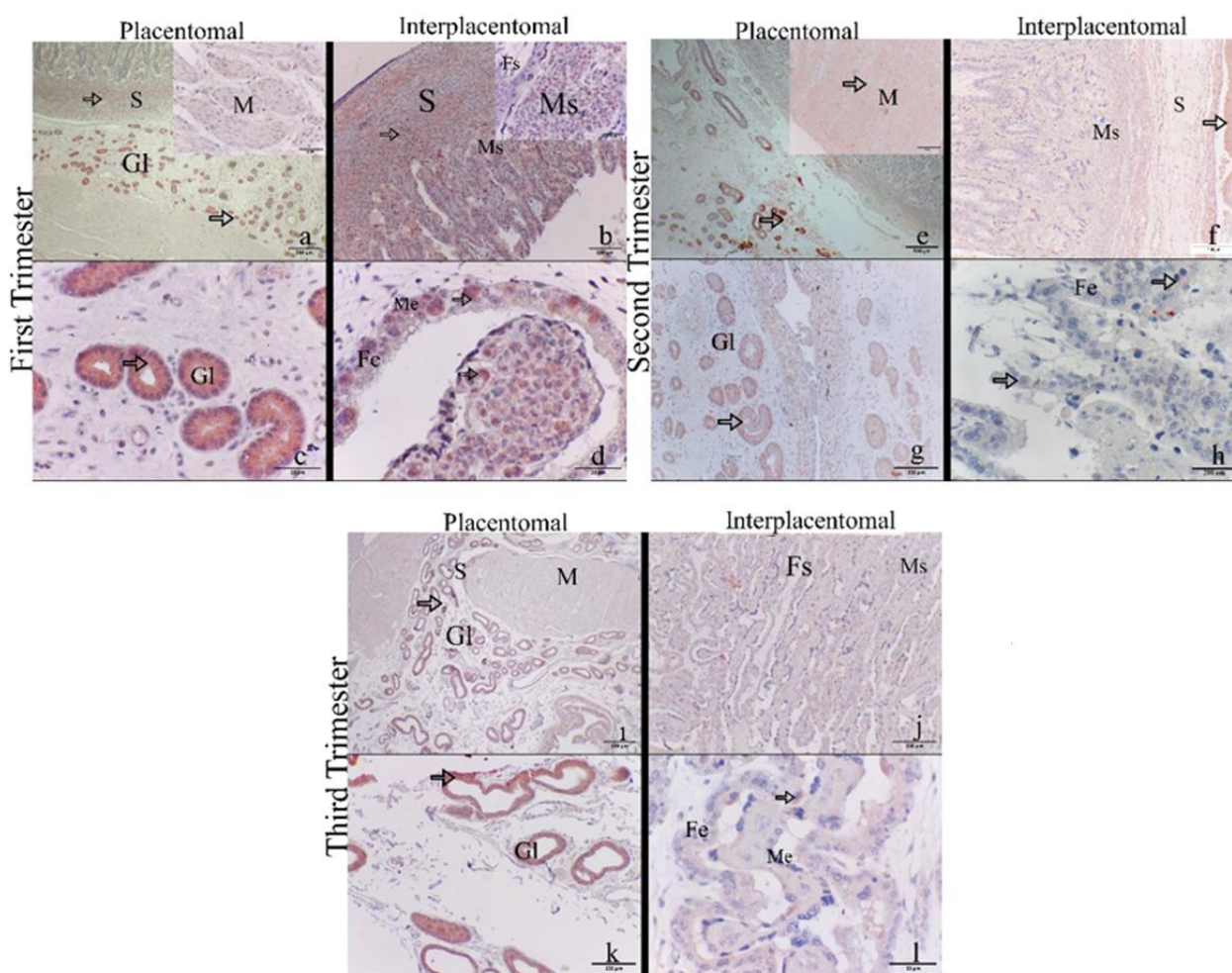


Figure 2. Immunolocalisation of irisin in goat placental and interplacentomal part, in the first period of pregnancy early (0–66 days) (a–d), mid (67–100 days) (e–h), and late (100–150 days) (i–l) stages of gestation Fe: Fetal Epithelial, FS: Fetal Stroma, ME: Maternal Epithelium, MS: Maternal Stroma, LE: Luminal Epithelium, S: Stroma, Gl: Gland Epithelium, M: Miyometrium, hollow arrow(⇄): positive cell. Scale bars: 200 µm (a, b, e, f, g, i, j, k); 50 µm (c, d, h, l). Counterstain: Mayer's hematoxylin.

Discussion

The aim of this study is to evaluate the histological development of the interplacentomal region of the uterus and the maternal and fetal components of the placenta in Siirt Hair goats at different stages of pregnancy, and to determine the expression and localization of irisin in these structures immunohistochemically. Specifically, the study aimed to identify which cellular structures express irisin throughout pregnancy, compare whether there are differences in immunoreactivity levels depending on the gestational stage, and elucidate the possible role of irisin in the developmental processes of placental-uterine tissues.

The placentome is the structure formed by the combination of the caruncular structure of the mother and the cotyledonary structure of the offspring shaped by glandless endometrium areas (24). In goats (17, 25) and sheep (26, 27) cotyledon and caruncular tissues form in the placentome and are intertwined with each other. Consistent with the literature, our study showed

the formation of fetal cotyledon tissues consisting of the chorionic plate and the maternal caruncular structure consisting of the basal plate. In the early stages of pregnancy, the cotyledons were not yet well defined; as gestation progressed, they fused with the caruncles to form the placentomes.

After uterine implantation, the trophoblast cells that form as a result of divisions first become mononuclear cells (single-nucleated trophoblast cells), and then become specialized into binuclear cells (double-nucleated trophoblast cells) (24). Mononucleate trophoblast cells and binucleate trophoblast cells were observed in the fetal trophoblastic epithelium, as in West African Dwarf goats (24) and Yankasa and Balami sheep (26). In early pregnancy, mononuclear trophoblasts predominate, whereas binuclear trophoblasts increase in number as gestation progresses. This increase and fetomaternal communication are important for placental development and hormone production

Meeting the increased nutrient and energy needs of mothers and offspring during pregnancy is important for the healthy development of both mother and offspring. Irisin was first discovered by Boström et al. (7) and is known to be associated with energy metabolism by activating numerous pathways in muscle and fat cells (7, 16, 28-30). In addition to its energy-regulating effects, irisin also supports the blood-placental barrier by promoting the proliferation and development of trophoblast cells and supporting vascular structure through its regulating properties of endothelial cells (12, 31). Irisin is expressed in the ovary, placenta (9), and neonatal cord serum (32) and plays a role in embryonic development that occurs during pregnancy (33).

The precursor of irisin, FNDC5, is expressed in the placenta of pregnant women, and one study reported that maternal serum irisin levels were higher in pregnant women than in non-pregnant women throughout pregnancy (9, 10). In the study by Yuksel et al. (34), maternal serum irisin levels were found to be significantly lower in women with gestational diabetes, while umbilical cord blood irisin levels did not differ between the GDM and control groups.

This is the first study to describe irisin expression in the interplacentomal and placentomal regions of ruminant goats. The present study investigated the immunoeexpression and tissue distribution of irisin at different stages of pregnancy in goats. The detection of irisin immunoeexpression in the interplacentomal and placentomal regions suggests a potential association with metabolic regulation, energy homeostasis, or trophoblast functions.

Irisin expression was observed to increase throughout pregnancy in the luminal and glandular epithelium interplacentomal region. The increased number of irisin-positive cells in the glandular epithelial region of the interplacentomal zone, in relation to meeting energy needs during pregnancy, suggested in previous studies that increased trophoblast activity via AKT-AMPK (11) signaling may contribute to elevated irisin levels. This finding supports the presence of an increasing immune response during pregnancy, consistent with previous reports in the literature (9).

Reference

1. Navarrete-Molina C, Meza-Herrera CA, De Santiago-Miramontes A, et al. Dairy goat production: Socioeconomic, environmental, and cultural importance across time (1970–2022) and possible scenarios (2050). *Resources* 2024; 13: 177.
2. Magnusson U. Environmental endocrine disruptors in farm animal reproduction: Research and reality. *Reprod Domestic Anim* 2012; 47: 333-337.
3. Bayram B, Topaloğlu U, Sağsöz H. Localisation of leptin, ghrelin, their receptors, obestatin and GPR39 in the cow placenta during pregnancy. *Harran Üniversitesi Veteriner Fakültesi Dergisi* 2025; 14: 187-197.
4. Lai E, Unniappan S. Irisin in domestic animals. *Domest Anim Endocrinol* 2023; 83: 106787.
5. Gootwine E. Placental hormones and fetal-placental development. *Anim Reprod Sci* 2004; 82-83: 551-566.
6. Armistead B, Johnson E, Vander Kamp R, et al. Placental regulation of energy homeostasis during human pregnancy. *Endocrinology* 2020; 161: bqaa076.
7. Boström P, Wu J, Jedrychowski MP, et al. A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; 481: 463-468.
8. Ebert T, Stepan H, Schrey S, et al. Serum levels of irisin in gestational diabetes mellitus during pregnancy and after delivery. *Cytokine* 2014; 65: 153-158.
9. Garcés MF, Peralta JJ, Ruiz-Linares CE, et al. Irisin levels during pregnancy and changes associated with the

As for the irisin expression in the placentomal region, higher irisin expression was determined in the maternal and fetal portions of the placenta during the early stages of pregnancy. This suggests that irisin may contribute to placental development and implantation. The high irisin expression observed in the maternal stroma during early pregnancy ($p < 0.05$), and its subsequent decrease as pregnancy progresses, further suggests that irisin may play a role in the interactions between trophoblast and endometrial tissues during implantation in ruminant animals. Increased oxidative stress in the early stages of pregnancy, increased inflammatory signals, and the high energy requirements of the fetus for placentation, despite its small size, may have increased irisin expression. This increase may be an adaptive mechanism to support placental development. In the second and third trimesters of pregnancy, the largely formed placenta, resulting in decreased trophoblastic cell invasion, and subsequent more balanced hormones may have led to a more stable irisin expression.

This study presents the first immunohistochemical evidence of irisin expression in the interplacentomal and placentomal regions of ruminant goats. Irisin manifested as increased expression in the glandular, luminal, stromal, and muscle components of the interplacentomal region throughout pregnancy. The higher expression observed in early pregnancy and the increase in maternal stromal tissues suggest that irisin may play a role in implantation and the interaction between trophoblast and endometrial tissues. Furthermore, the persistence of irisin expression in later stages may indicate a potential role in supporting placental function and contributing to metabolic and immunological processes, but further studies are needed to confirm these relationships. Overall, these findings highlight irisin as a possible modulator of placental development and maternal-fetal communication in goats, however, the study was conducted with a limited number of slaughterhouse materials and samples; this can be considered a limitation of the study. However, this forms a basis for future studies with larger sample sizes that will investigate their functional roles in the reproduction of ruminant animals.

- development of preeclampsia. *J Clin Endocrinol Metab* 2014; 99: 2113-2119.
10. Zhang LJ, Xie Q, Tang CS, Zhang AH. Expressions of irisin and urotensin II and their relationships with blood pressure in patients with preeclampsia. *Clinical and Experimental Hypertension* 2017; 39: 460-467.
 11. Kohan-Ghadr HR, Armistead B, Berg M, et al. Irisin protects the human placenta from oxidative stress and apoptosis via activation of the Akt signaling pathway. *Int J Mol Sci* 2021; 22: 11229.
 12. Drewlo S, Johnson E, Kilburn BA, Kadam L, Armistead B, Kohan-Ghadr H. Irisin induces trophoblast differentiation via AMPK activation in the human placenta. *J Cell Physiol* 2020; 235: 7146-7158.
 13. Aydin S, Kuloglu T, Aydin S, et al. A comprehensive immunohistochemical examination of the distribution of the fat-burning protein irisin in biological tissues. *Peptides* 2014; 61: 130-136.
 14. Martinez Munoz IY, Camarillo Romero EDS, Garduno Garcia JDJ. Irisin: A novel metabolic biomarker: present knowledge and future directions. *Int J Endocrinol* 2018; 2018: 1-8.
 15. Pekince-Özöner M, Timurkaan S, Gür FM, et al. Effect of sea buckthorn (*Hippophae rhamnoides*) on kidney and testicular damage, sperm quality and expression of irisin and asprosin in streptozotocin-induced diabetic rats. *Rev Cient FCV-LUZ* 2025; 35: 10.
 16. Bayraktar B, Tekce E. Anadolu merinoslarında irisin hormon yanıtı üzerine bazı fizyolojik parametrelerin etkisi. *Etilik Vet Mikrobiyol Derg* 2021; 32: 145-150.
 17. İşbilir F, Kandil B, İşbilir İ, Koca D, Güzel BC. Evaluation of placentome morphology in the last two periods of pregnancy in hair goats (*Capra aegagrus hircus*). *Reprod Domestic Anim* 2024; 59: e14731.
 18. Singh LK, Singh U, Singh P. Fetal dystocia and estimation of fetal age in sheep. *Int J Anim Res* 2023; 44: 104-106.
 19. Noakes DE, Parkinson TJ, England GCW. *Arthur's Veterinary Reproduction and Obstetrics*. 8th ed. Philadelphia: WB Saunders; 2001: 138.
 20. Gür FM, Aktaş İ, Bilgiç S, Pekince M. Misoprostol alleviates paclitaxel-induced liver damage through its antioxidant and anti-apoptotic effects. *Mol Cell Toxicol* 2022; 18: 393-400.
 21. Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. *Am J Clin Pathol* 1981; 75: 816-821.
 22. Erdem Güzel E, Kaya Tektemur N. Hesperetin may alleviate the development of doxorubicin-induced pulmonary toxicity by decreasing oxidative stress and apoptosis in male rats. *Tissue Cell* 2021; 73: 101667.
 23. Topaloğlu U, Aydın Ketani M. The distribution of some homeobox proteins in the bovine placenta during gestation. *Theriogenology* 2021; 166: 71-82.
 24. Igwebuike UM. A review of uterine structural modifications that influence conceptus implantation and development in sheep and goats. *Anim Reprod Sci* 2009; 112: 1-7.
 25. Stephen J, Adeyeye A, Wiam I, et al. Changes in foetal fluid volume, weight of gravid uterus, and histology of the uterus and placenta in Sahel goats during different trimesters of pregnancy. *Veterinaria* 2022; 71: 335-344.
 26. Gazali Y, Gambo B, Zakariah M, et al. Morphological characteristics of the placenta of Balami and Yankasa ewes at different stages of gestation in Maiduguri, Nigeria. *Sokoto J Vet Sci* 2023; 21: 11-20.
 27. Kandil B, Turgut AO, Koca D, et al. Comprehensive evaluation of changes in placentomes in the second and third trimesters of pregnancy in cross-bred Hamdani sheep. *Vet Med Sci* 2025; 11: e70208.
 28. Shimba Y, Togawa H, Senoo N, et al. Skeletal muscle-specific PGC-1 α overexpression suppresses atherosclerosis in apolipoprotein E-knockout mice. *Sci Rep* 2019; 9: 4077.
 29. Timurkaan S, Gür FM, Gençer Tarakçı B, Yalçın MH, Girgin M. Identification of irisin immunoreactivity in porcupine (*Hystrix cristata*) adrenal glands and kidneys. *Anat Histol Embryol* 2018; 47: 405-409.
 30. Gür F, Timurkaan S, Yalçın M, Girgin A, Gençer Tarakçı B. Immunohistochemical localization of irisin in mole rats (*Spalax leucodon*). *Biotechnic Histochemistry* 2017; 92: 245-251.
 31. Wu F, Song H, Zhang Y, et al. Irisin induces angiogenesis in human umbilical vein endothelial cells in vitro and in zebrafish embryos in vivo via activation of the ERK signaling pathway. *PLoS One* 2015; 10: e0134662.
 32. Piya MK, Harte AL, Sivakumar K, et al. The identification of irisin in human cerebrospinal fluid: Influence of adiposity, metabolic markers, and gestational diabetes. *Am J Physiol Endocrinol Metab* 2014; 306: E512-18.
 33. Salem H, Yatchenko Y, Anosov M, et al. Maternal and neonatal irisin precursor gene FNDC5 polymorphism is associated with preterm birth. *Gene* 2018; 649: 58-62.
 34. Yuksel MA, Oncul M, Tuten A, et al. Maternal serum and fetal cord blood irisin levels in gestational diabetes mellitus. *Diabetes Res Clin Pract* 2014; 104: 171-175.