doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia https://veterinariamexico.fmvz.unam.mx/

Expression profile of interferon-stimulated gene 15 in leukocytes during early pregnancy in *Capra hircus*

Neha Purey[1]
0009-0000-4848-3433
Manoj Kumar Awasthi[2]
0009-0006-7489-0845
Asit Jain[3*]
0000-0002-1888-0507
Tripti Jain[4]
0000-0002-6533-3565
Girish Kumar Mishra[5]
0000-0002-4921-6596
Sanjay Shakya[2]
0000-0002-5386-6372
Javed Ur Rahman Khan[2]
0009-0004-9891-7235
Sachchidanand Sarkar[1]
0009-0004-3968-3707

[1] Government of Chhattisgarh. Department of Animal Husbandry. Chhattisgarh, India.

[2] Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya. College of Veterinary Science and Animal Husbandry. Anjora, Durg, Chhattisgarh, India.

[3] Nanaji Deshmukh Veterinary Science University. College of Veterinary Science and Animal Husbandry. Jabalpur, Madhya Pradesh, India.

[4] Nanaji Deshmukh Veterinary Science University. Animal Biotechnology Centre. Jabalpur, Madhya Pradesh, India.

[5] Nanaji Deshmukh Veterinary Science University. College of Veterinary Science and Animal Husbandry, Rewa. Jabalpur, Madhya Pradesh, India.

***Corresponding author**
Email address:
vetasit@gmail.com

Abstract

Interferon-stimulated gene 15 (ISG15) is induced by conceptus-derived interferon tau (IFNT) in the endometrium during early pregnancy in ruminants, including goats. The expression of ISG15 in extra-uterine tissues, such as peripheral blood leukocytes (PBLs), suggests its potential as a novel pregnancy biomarker. The onset and length of the breeding season in goats are influenced by various factors such as breed, latitude, climate, photoperiod, etc. The Osmanabadi, an Indian goat breed, known for its early maturity, prolificacy, and good dressing percentage, was the focus of the current study. The present investigation was designed to study the messenger ribonucleic acid (mRNA) profile of Caprine ISG15 (cpISG15) in PBLs using real-time reverse transcription PCR (qPCR) on days 0, 13, 17, 21, 25 and 30 post-service in this breed. Pregnancies were confirmed by measuring plasma progesterone (P4) concentration and conducting trans-abdominal ultrasound scanning. The study revealed that the expression of cpISG15 mRNA was 5 to 8-fold higher ($P < 0.05$) during early pregnancy on days 17, 21, and 25 compared to day 0. There was no significant difference in the expression of cpISG15 mRNA between days 0 and 30 post-service. Progesterone concentration was higher on days 17, 21, and 25 in pregnant does compared to day 0. The presence of fetal parts was observed by ultrasound between 45 and 90 d of pregnancy. It is concluded that the detection of elevated ISG15 expression in PBLs during the early stages of pregnancy may be used as a marker for pregnancy detection in does.

Keywords: Goat; Blood; ISG15; Pregnancy diagnosis marker; PBLs; Osmanabadi goats.

Submitted: 2023-09-18
Accepted: 2024-08-14
Published: 2025-01-30

Additional information and declarations can be found on page 10

Cite this as:

Purey N, Awasthi MK, Jain A, Jain T, Mishra GK, Shakya S, Khan JUR, Sarkar S. Expression profile of interferon-stimulated gene 15 in leukocytes during early pregnancy in *Capra hircus*. Veterinaria Mexico OA. 2025;12. doi: 10.22201/fmvz.24486760e.2025.1267.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

Study contribution

Goats act as a source of supplementary income along with nutritional security for poor and marginal farmers. Goat reproduction is seasonal in temperate regions, while continuous breeders are in tropical areas. Early embryonic mortality is one of the major reproductive problems in ruminants. Several genes are responsible for endometrial remodeling and conceptus development in ruminants, and interferon-stimulated gene 15 is one of them. However, the temporal expression profile of the ISG15 gene in peripheral blood leukocytes is not available during pregnancy in the Osmanabadi breed of goats. The current study reports the higher expression of ISG15 mRNA during early pregnancy as compared to late pregnancy and non-pregnant does. Confirmation of pregnancy was done by progesterone hormone estimation and ultrasound scanning. Expression of ISG15 in PBLs would be helpful for research communities to look for its exact function and mechanism, and also for novel pregnancy biomarker molecules.

Introduction

Trophectoderm of a preimplant embryo synthesizes and secretes interferon tau (IFNT) between 11 to 24 d of pregnancy, which serves as a maternal recognition of pregnancy signal in different ruminant species.[1–3] IFNT mediates anti-luteolytic activity through multiple mechanisms after binding to the Type-1 IFN receptor (IFNR) on luminal endometrial cells. During early pregnancy, IFNT plays a significant part in the recognition and establishment of the pregnancy by up-regulating and stimulating the expression of numerous genes in the uterus of various ruminant species, including caprine.[4–6] Interferon-stimulated gene ISG15, is one of such genes.[7–11]

Recent studies have shown that ISG15 is also expressed in some other extra-uterine tissues like peripheral blood leukocytes during early pregnancy (14–25 d) of cows,[12–14] buffalo,[15, 16] and sheep[17, 18]. These findings provide a possibility to diagnose pregnancy before the next cycle begins or within three weeks after artificial insemination (AI) or natural mating. Thus, early and accurate diagnosis of early pregnancy would provide a valuable management tool for enhancing the efficiency of goat production, allowing for a reduction in expenses related to feeding and vaccination. Such studies are unavailable in the Indian tropical or subtropical.

The goat is a versatile animal and has been associated with man since the dawn of agriculture and the domestication of animals. Owing to their ability to adapt to various environmental and climatic circumstances, goats are found in diverse parts of the globe.[19] Osmanabadi goats, one of the Indian goat breeds, are suited to all types of rearing systems, the most ideal being the semi-intensive system (grazing and closed enclosure). The breed is mainly reared for meat and milk purposes. Known for its adaptability and resilience in various environments, the Osmanabadi breed also exhibits early maturity, prolificacy, disease resistance, and a good dressing percentage.[20]

The estrous cycle is a series of hormonal events that alters the female reproductive system's morphology and prepares an animal for conception.[21] Early embryonic mortality can be prevented by detecting pregnancy before the beginning of the next estrous cycle, which could benefit the goat industry. Given the significance

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

and crucial role of ISG15 in reproduction, the current study was designed to generate a temporal expression profile of ISG15 in peripheral blood leukocytes (PBLs) to detect early pregnancy in Osmanabadi. Additionally, pregnancy confirmation was achieved through P4 hormone estimation and ultrasound scanning.

Materials and methods

Ethical statement

All experimental processes were permitted by the Institutional (College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh, India) Animal Ethics Committee (Proposal number: VGO-PG-1/2020/06, dated 17/11/2022; Registration No: 445/GO/ReBi/S/01/CPCSEA). Chemicals were procured from Thermo Scientific (USA), Sigma Aldrich (USA), and Macherey-Nagel unless otherwise indicated. Plastic wares used were from Eppendorf, Genaxy Scientific Pvt. Ltd., and Tarson's Product Pvt. Ltd. (India).

Location, climatic conditions, experimental animals, and design

The experimental goats were maintained as per the standard practices followed at Osmanabadi goat seed Centre, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya (DSVCKV), Anjora, Durg, Chhattisgarh, India. This farm is located at an of altitude 317 m above sea level on 20—22° 23—02' N latitude and 80—81° 46—58' E longitudes. Temperature ranges from 32—42 °C but can be as high as 45 °C in the summer and down to 12 °C in winter. Average rainfall ranges from 1 071—1 400 mm. Does, 1—1.5 years old, were selected. Goats were reared in a semi-intensive system All the animals were fed according to the feeding standards followed at DSVCKV, Anjora, Durg.

The animals were fed high-quality fodder and roughages along with concentrates, as per the recommendation of 12—13 % digestible crude protein and 60—65 % total digestible nutrients. The concentrate mixture consisted of 10 % groundnut cake, 10 % mustard oil cake, 25 % maize, 28 % wheat bran, 24 % deoiled-rice-bran, 2 % mineral mixture, and 1 % common salt. Clean fresh tap water was provided to all the goats throughout the day.

All the animals were healthy and free from any defect/disease. To ensure the health of all animals, prophylactic, veterinary support, and hygienic measures were taken care of throughout the experiment as and when required. The total period of experiments was eight months. Does in estrus were continuously watched and isolated. Bucks were introduced to these does and mating data were recorded till kidding. Day '0' was considered a day of mating. Confirmation of pregnancy was done by progesterone estimation and ultrasound.

Twenty Osmanabadi (10 pregnant and 10 non-pregnant) were selected for the present study. Blood samples (approximately 5 mL) were collected from jugular vein on days 0, 13, 17, 21, 25, and 30 post-mating and non-pregnant animals in EDTA-coated vacutainer tubes for expression profile of cpISG15 mRNA and estimation of plasma P4.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

RNA extraction and cDNA synthesis

For the expression analysis of cpISG15 mRNA, total RNA was extracted from 200 µL of blood using an RNA isolation kit (Macherey-Nagel) following the manufacturer's protocol. The purity and concentration of the extracted RNA were determined through 260/280 nm absorbance measures using the Nanodrop DS-11 Spectrophotometer (DeNovix). Further, cDNA was synthesized using 200 ng of total RNA with the Revert Aid first strand cDNA reverse transcription system (Thermo Scientific, USA) according to the manufacturer's instructions. After completing cDNA synthesis, each cDNA sample was diluted fourfold with nuclease-free water and stored at -20 °C until further use. Synthesized cDNA was checked by amplification with a housekeeping gene, Beta-actin (ACTB), in a thermal cycler (T-100, Bio-Rad). The PCR reaction mixture contained 10 pM of each gene-specific primer, 5 µL of diluted cDNA template, 2 × PCR master mix (Sigma, USA), and nuclease-free water to make the total reaction volume of 25 µL. The cycling conditions of PCR were initial denaturation for 3 min at 94 °C, followed by 35 cycles of denaturation for 30 s at 94 °C; annealing for 30 s at 57 °C; extension for 1 min at 72 °C and final extension for 72 °C for 5 min. The PCR products were visualized in 1.5 % agarose gel electrophoresis.

Expression profile of cpISG15 mRNA in PBLs

For the qPCR, published primers for cpISG15 (ISG15F: 5′-TGATGGTATCYGAGCTGAAG-3′; ISG15R: 5′-CTTGAGCACAGCCACAGTCT-3′) and for Beta-actin (ACTB F: 5′- TCGGCAATGAGCGGTTCC -3′; ACTBR: 5′-ACYGTGTTGGCGTAGAGGTC -3′) were used.[11] The qPCR was carried out in a CFX96 Real-Time PCR System (Bio-Rad, USA). Primer concentration was optimized using the primer matrix experiments for valid transcript quantification. All qPCR reactions were performed in duplicates in total volume of 20 µL. The reaction mixture contained 10 pM of each gene-specific primer, 2 µL of cDNA template, 1 × SYBR Green PCR master mix (Sigma, USA), and nuclease-free water to make the total reaction volume of 20 µL. Cycling conditions of PCR were initial denaturation for 4 min at 94 °C, followed by 40 cycles of denaturation for 30 s at 94 °C; annealing for 30 s at 60 °C; extension for 1 min at 72 °C and final extension for 72 °C for 5 min.

A negative control was included for the qPCR assay, in which, cDNA was not added. For each sample, a dissociation curve was generated after the completion of amplification and analyzed in comparison to negative control, to determine the specificity of PCR reaction. The cpISG15 mRNA expression was calculated by relative quantitation using the comparative CT method described by Livak and Schmittgen.[22] Mean threshold cycle values (C_T) for genes under study were calculated from duplicate samples. The ΔC_T value is determined by subtracting the average ACTB C_T value from the average cpISG15 C_T values. The calculation of $\Delta\Delta C_T$ involves subtraction by the C_T calibrator value. Here, day '0' was taken as a calibrator. The relative quantification was determined by the formula, $2^{-\Delta\Delta C_T}$.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

Progesterone (P4) assay

Blood samples were collected from the jugular vein of does as previously described. Plasma was separated by centrifugation at 1600 rpm for 30 min at room temperature, then transferred into 1.5 mL Eppendorf tubes and stored at -20 °C until assayed for P4. The concentrations of plasma P4 were estimated using a radioimmunoassay kit (Immunotech, France) following the procedure described by the authors.[23]

Ultrasound scanning for pregnancy diagnosis

Ultrasound scanning of mated does was conducted between 45 and 90 d after mating, utilizing a real-time, B-mode, diagnostic scanner equipped with a trans-abdominal 3.5 MHz linear array transducer (Aloka, Prosound 2 Japan).

Statistical analysis

Statistically significant differences ($P < 0.05$) in relative cpISG15 expression levels in pregnant does and plasma P4 concentrations were determined using the one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) post hoc test by using SPSS software (version 2015). Shapiro-Wilk and Levene's tests were applied to test normality and homogeneity of variances, respectively. cpISG15 expression levels (day 21) and P4 concentration (days 0, 21) between pregnant and non-pregnant were assessed by t-test. The results were reported as mean ± standard error of the mean, and differences between means were assessed for significance at the 5 % level ($P < 0.05$).

Results

Expression profile of cpISG15 mRNA

The ratio of RNA (260/280: 1.9 or above; 260/230 above 2.0) was used as a criterion for cDNA synthesis. Amplification of synthesized cDNA by ACTB revealed a specific amplicon of 144 bp (Figure S1). Constitutive expression of cpISG15 mRNA was detected in pregnant and non-pregnant PBLs of does. Expression of cpISG15 mRNA was significantly higher on day 17 post-service and remained high until day 21. The mean fold change in cpISG15 mRNA was 5 to 8.5-fold greater in blood during 17–21 d of pregnancy compared to day 0 (Figure 1). A decrease in expression began on day 25, and by day 30, the level of cpISG15 expression had decreased to match that of days 13 and 0. There was no significant difference in cpISG15 mRNA expression between day 30 and day 0 of pregnancy (Figure 1).

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

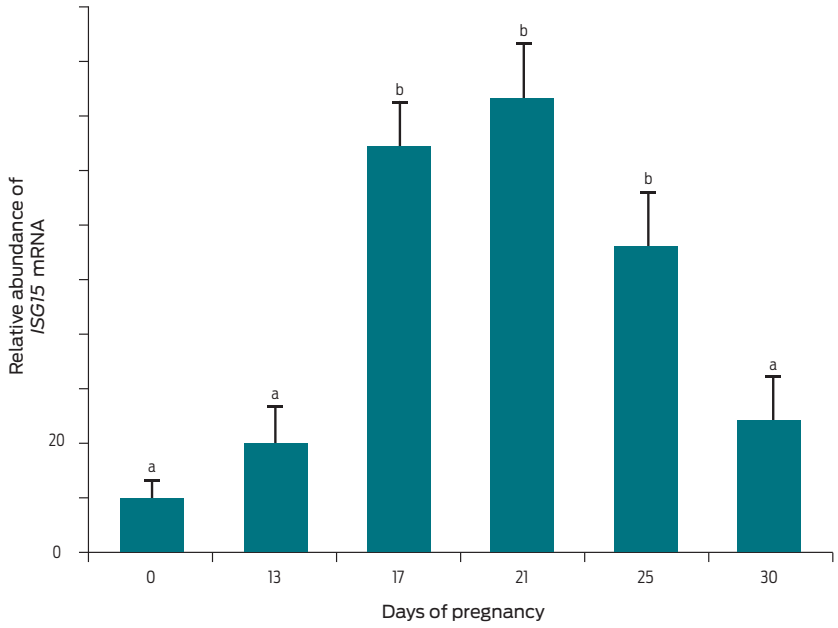

Figure 1. Relative quantitative PCR analysis of cpISG15 mRNA during different days of early pregnancy of Osmanabadi does. Bars indicate mean ± SEM values (n = 10, each day). Different letters indicate a significant difference ($P < 0.05$).

The expression level of cpISG15 mRNA was significantly higher on day 21 in pregnant does compared to non-pregnant does, with an almost 9-fold higher increase (Figure 2). Both cpISG15 and ACTB showed a single peak in the dissociation curve, indicating the specificity of the qPCR reaction and confirming that the primer was highly specific to the target with no primer dimer formation (Figure S2).

Progesterone assay

Plasma P4 concentration was significantly higher ($P < 0.05$) in non-pregnant does compared to pregnant does on day 0, though both groups had plasma progesterone concentrations below 1 ng/mL (Table 1). The plasma progesterone concentration was significantly lower on day 0 compared to days 17, 21 and 25 in pregnant does. However, no significant difference in plasma progesterone levels was observed among days 17, 21, and 25 in pregnant (Table 2).

Trans-abdominal ultrasonography

Trans-abdominal ultrasonography was performed between days 45 and 90 post-service, with pregnancy confirmed by the identification of the amniotic sac, embryo, fetal head, and cotyledons (Figure S3).

Discussion

It has already been established that expression of ISG15 increased in the endometrium during early pregnancy and plays an important role in pregnancy recognition, uterine receptivity, and embryo growth in various species of ruminants including

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

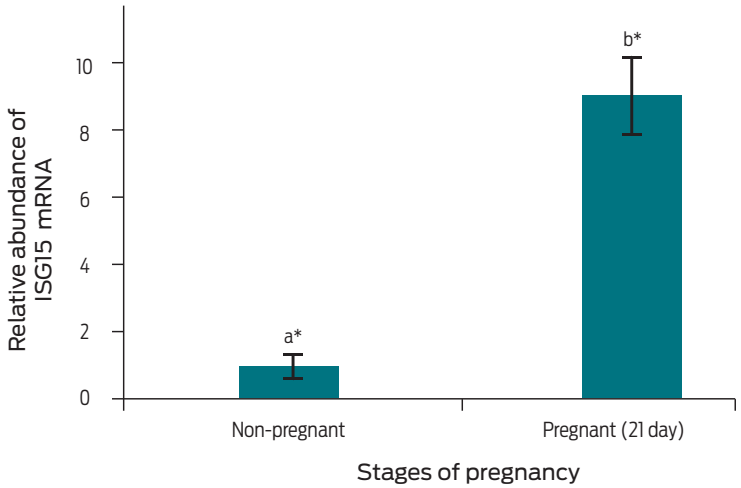

Figure 2. Relative quantitative PCR analysis of cpISG15 mRNA in pregnant (n = 10; on day 21) and non-pregnant (n = 10) Osmanabadi does. Bars indicate mean ± SEM values. Different letters indicate a significant difference ($P < 0.05$).

Table 1. Plasma progesterone concentration (ng/mL) in pregnant and non-pregnant Osmanabadi does

Days	Pregnant (n = 10)	Non-pregnant (n = 10)	P-value
0	0.45 ± 0.17^{a}	0.79 ± 0.35^{a}	0.09071
17	9.04 ± 0.38^{b}	-	
21	9.58 ± 0.39^{b}	0.47 ± 0.21^{a}	0.00014
25	10.0 ± 0.41^{b}	-	

This means that a column having different superscripts differs significantly ($P < 0.05$); the (-) sign indicates: not available data.

Table 2. Comparison of plasma progesterone concentration during different days of early pregnancy in Osmanabadi does

Days	0 and 17	17 and 21	21 and 25
Pregnant does (ng/mL) (n = 10)	22.6^{a}	0.87^{b}	0.53^{b}
P value	0.0241		

This means that rows having different superscripts differ significantly ($P < 0.05$).

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

caprine.[11, 24] The presence of ISG15 in extra-uterine tissues like corpus luteum (CL), uterine artery and vein,[17] uterine-placental interface,[25] and peripheral blood mononuclear cells (PBMCs) has presented new research opportunities to explore its precise mechanism and role, as well as its potential as a novel pregnancy biomarker. It has been demonstrated that IFNT is released into the uterine vein during early pregnancy, where it acts in an endocrine manner to induce ISGs in the CL.[17, 26]

The expression of ISG15 within the CL serves as a valuable marker for assessing embryonic mortality as well as distinguishing pregnancy losses caused by the death of embryos from a failure of fertilization in cattle, sheep and goat.[17, 27] Additionally, ISG15 at the uteroplacental junction plays a crucial role in the sequential process of concepts development, implantation, and placentation.[25] The expression of ISG15 in PBMC might serve as a peripheral and easily monitorable surrogate marker for early pregnancy diagnosis in ruminants.[12] To the best of the authors' knowledge, the current study is the first report revealing the expression profile of ISG15 in the PBLs during early pregnancy in the Osmanabadi breed of goat (*Capra hircus*), except one study conducted on Aardi goats in Saudi Arabia.[28] The onset and length of the breeding season in goats rely on various factors, notably photoperiod, as well as latitude, climate, breed, physiological stage, and the presence of a male. Goat reproduction is seasonal in temperate and polar regions, with the breeding season occurring in the fall and winter. Nonetheless, goats are regarded as continuous breeders in tropical and equatorial areas, where there are variations in seasonality across breeds and locations.[21]

In the present study, the constitutive expression of cpISG15 mRNA in PBLs of non-pregnant does is congruent with other studies that also found ISG15 expression in the endometrium, corpus luteum, and PBLs as free and its conjugated forms in various species.[11, 27, 29] In contrast to the current findings, some studies[8, 30] did not identify the ISG15 in the endometrium of non-gestational cows and ewes. This might be the northern hybridization method they used to find ISG15 mRNA. Numerous studies indicate that, compared to the northern hybridization technique used in other studies, qPCR is more accurate and sensitive, capable of detecting as few as 1–100 copies of a particular mRNA.[31, 32] The detection of ISG15 mRNA in non-pregnants does is likely due to the use of a more sensitive qPCR technique.

In the current study, the expression of cpISG15 mRNA in the PBLs during early pregnancy, which peaked between 17 to 21 days, is congruent with various studies.[3, 7, 11] Previous research has shown that the expression of ISG15 mRNA is higher during early pregnancy (< 24 d) compared to both non-pregnant and later stages of pregnancy (> 24 d) in the endometrium and CL of does[11, 27] as well as other species.[7, 33] Several workers have reported that ISG15 mRNA/protein level increased during early pregnancy (after day 16), peaked in between 18–20 d and then declined by day 30 following AI in the blood of pregnant cows or heifers when compared with non-pregnant cows or the day of insemination. The observed changes may result from the secretion of INFT by conceptus, which acts on PBMCs to upregulate ISG15 in pregnant cows. Various studies have concluded that this molecule could serve as a reliable marker for early pregnancy diagnosis.[12, 34-38]

A positive correlation has been reported between the ISGs expression in PBMCs and intrauterine administration of the IFNT quantity.[39] Sheikh et al. conducted a study on cows and, based on gene expression, P4 assay, ultrasonography, and per-rectal palpation, found that the degree of expression of ISGs was higher in pregnant and late embryonic mortality cows than in early embryonic mortality cows. The experiment also revealed that time-dependent changes in ISGs expression in blood neutrophils coupled with proinflammatory cytokine profile could be useful biomarkers for bovine gestation.[40]

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

In buffalo, numerous researchers have reported the expression dynamics of various ISGs including ISG15 in PBLs. ISG15 was observed to be upregulated between days 14 and 20, peaking by day 20, and decreasing between days 25 and 30, corresponding to on or before day 14 of pregnancy. ISG15 was observed to be upregulated between days 14 to 20, peaking by day 20, and decreasing between days 25–30, which corresponds to on or before day 14 of pregnancy. ISG15 has been identified as a suitable candidate biomarker for accurate pregnancy diagnosis within 18 d post-AI in buffalo.[10, 16, 41]

It has been reported that the expression of various ISGs including ISG15 mRNA was significantly higher ($P < 0.05$) in blood, endometrium, corpus luteum, and jugular blood on day 15 in pregnant ewes as compared to non-pregnant ewes.[17] The expression of cpISG15 mRNA parallels conceptus elongation and IFNT secretion during early pregnancy, occurring between days 16 and 21 in does, and between days 11 and 21 in ewes and cows.[3, 42, 43] Some studies have also found that intrauterine infusion of IFNT in non-pregnant ewes and cows, and *in-vitro* culture of different types of endometrial cells supplementation with IFNT, increases the expression or activities of the other interferon-stimulated genes.[44, 45] The hypothesis suggests that the conceptus secretes IFNT, which acts on PBLs, leading to the upregulation of ISG15 expression.[17, 38]

In the present study, early pregnancy detection based on ISG15 mRNA was further supported by estimating plasma P4 concentration. The present findings are consistent with other studies[46–48] that found a significant difference in the serum progesterone profile between pregnant and non-pregnant does ($P < 0.05$) on days 17 and 22. Previous studies suggested that a higher concentration of P4 is needed for maintaining pregnancy. The regression of the corpus luteum (luteolysis), induced by prostaglandin F2α ($PGF_{2\alpha}$), occurs if an embryo is not present in the uterus, leading to a rapid decline in plasma P4 in non-pregnant goats. Conversely, when an embryo is present in the uterus, $PGF_{2\alpha}$ secretion is inhibited.[49]

The results from ultrasound scanning were used to identify and confirm early pregnancy (17–25 d) by demonstrating the expression of ISG15 mRNA in circulating peripheral blood leukocytes of does, which is associated with high plasma P4 concentration. The findings of the present study, along with previous studies, suggest that the upregulation of the ISG15 in PBLs during early pregnancy provides the possibility for developing a new, reliable, and accurate method for early pregnancy. This biomarker may be particular useful for detecting early pregnancy in goats, especially within 21 days after AI or natural mating.

Conclusions

The expression of ISG15 mRNA was higher during the early days of pregnancy compared to both the day of mating and the later days. Confirmation of pregnancy was achieved through measurements of plasma P4 concentration and trans-abdominal ultrasound scanning. ISG15 expression may be a marker for early pregnancy detection between 17–25 d postbreeding. Nevertheless, further research is necessary to locate the ISG15 in PBLs and to examine its expression profile at the protein level.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

Data availability

All relevant data are within the manuscript and its supporting information files.

Acknowledgments

I am thankful to Dean, College of Veterinary Science and AH; Head, Department of Animal Genetics and Breeding; Director of Research, Anjora, Durg, Chhattisgarh for their support and providing facilities.

Funding statement

This research was funded by Science and Engineering Research Board (SERB), Department of Science and Technology (DST) funded project (SB / YS / LS-27 / 2014), Government of India, New Delhi, India, to Dr. A Jain. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

The authors have no conflict of interest to declare concerning this publication.

Author contributions

Conceptualization: A Jain, T Jain, MK Awasthi, GK Mishra.
Data curation: A Jain, N Purey, T Jain.
Formal analysis: A Jain, T Jain, GK Mishra.
Funding acquisition: A Jain.
Investigation: N Purey, A Jain, S Sarkar.
Methodology: N Purey, A Jain, T Jain, JR Khan, S Sarkar.
Project administration: A Jain, T Jain, MK Awasthi.
Resources: N Purey, A Jain, S Shakya.
Software: A Jain, N Purey, GK Mishra, T Jain, JR Khan.
Supervision: A Jain, T Jain, MK Awasthi, S Shakya.
Visualization: A Jain, T Jain, MK Awasthi, S Shakya, JR Khan.
Writing–original draft: N Purey, A Jain and T Jain.
Writing–review and editing: MK Awasthi, GK Mishra, S Shakya, JR Khan.

References

1. Bazer FW, Song G, Thatcher WW. Roles of conceptus secretory proteins in establishment and maintenance of pregnancy in ruminants. Asian-Australasian Journal Animal Sciences. 2012;25(1):1–16. doi: 10.5713/ajas.2011.r.08.
2. Vallet JL, Barker PJ, Lamming GE, Skinner N, Huskinsson, NS. A low molecular weight endometrial secretory protein which is increased by ovine trophoblast protein-1 is a β2- microglobulin like protein. Journal of Endocrinology.1991;130(2):R1–R4. doi: 10.1677/joe.0.130R001.
3. Bazer FW, Spencer TE, Johnson GA. Burghardt RC. Uterine receptivity to implantation of blastocyst in mammals. Frontiers in Bioscience. 2011;3(2):745–767. doi: 10.2741/s184.
4. Mirando MA, Short EC, Jr Geisert RD, Vallet JL, Bazer FW. Stimulation of 2′,5′-oligoadenylate synthetase activity in sheep endometrium during pregnancy, by intrauterine infusion of ovine trophoblast protein-1, and by intramuscular

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

administration of recombinant bovine interferon-alpha1. Journal Reproduction Fertility. 1991;93(2):599–607. doi: 10.1530/jrf.0.0930599.

5. Rueda BR, Naivar KA, George EM, Austin KJ, Francis H, Hansen TR. Recombinant interferon-tau regulates secretion of two bovine endometrial proteins. Journal of Interferon Research. 1993;13(4):303–309. doi: 10.1089/jir.1993.13.303.
6. Naivar KA, Ward SK, Austin KJ, Moore DW, Hansen TR. Secretion of bovine uterine proteins in response to type I interferons. Biology of Reproduction. 1995;52(4):848–854. doi: 10.1095/biolreprod52.4.848.
7. Johnson GA, Austin KJ, Collins AM, Murdoch WJ, Hansen TR. Endometrial ISG17 mRNA and a related mRNA are induced by interferon tau and localized to glandular epithelial and stromal cells from pregnant cows. Endocrinology. 1999;10:243–252. doi: 10.1007/BF02738623.
8. Johnson GA, Austin KJ, Kirk AV, Spencer TE, Burghardt RC, Hansen TR, *et al.* Expression of the Interferon tau inducible ubiquitin cross-reactive protein in the ovine uterus. Biology Reproduction. 1999;61(1):312–318. doi: 10.1095/biolreprod61.1.312.
9. Joyce M, Hansen T, Johnson G. Interferon-stimulated gene 17 is expressed in the porcine uterus and may be critical to placental development across species. In: Society for the Study of Reproduction: Annual Meeting. Baltimore, Maryland. US; 2002.
10. Jain A, Baviskar PS, Kandasamy S, Kumar R, Singh R, Kumar S, *et al.* Interferon stimulated gene 15 (ISG15): Molecular characterization and expression profile in endometrium of buffalo. Animal Reproduction Science. 2012;133(3–4):159–168. doi: 10.1016/j.anireprosci.2012.06.023.
11. Chandrakar K, Jain A, Khan JR, Jain T, Singh M, Mishra OP. Molecular characterization and expression profile of interferon stimulated gene 15 (ISG15) in the endometrium of goat (*Capra hircus*). Theriogenology. 2020;142:348–354. doi: 10.1016/j.theriogenology.2019.10.013.
12. Han H, Austin KJ, Rempel LA, Hansen TR. Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. Journal of Endocrinology. 2006;191(2): 505–512. doi: 10.1677/joe.1.07015.
13. Takino T, Taku O, Tatsuya A, Katsuro H. Change in the responsiveness of interferon stimulated genes during early pregnancy in cows with Borna virus-1 infection. BMC Veterinary Research. 2016;12:253. doi: 10.1186/s12917-016-0883-5.
14. Cheng L, Xiang M, Hu X, Yu J, Xia Y, Tao B, *et al.* Duplex quantitative polymerase chain reaction of ISG15 and RSAD2 increases accuracy of early pregnancy diagnosis in dairy cows. Annals of Animal Science. 2019;19(2):383–401. doi: 10.2478/aoas-2019-0001.
15. Buragohain L, Kumar R, Nanda T, Phulia SK, Mohanty AK, Kumar S, *et al.* Serum MX2 protein as candidate biomarker for early pregnancy diagnosis in buffalo. Reproduction in Domestic Animal. 2016;51(4):453–460. doi: 10.1111/rda.12700.
16. Thakur N, Singh G, Paul A, Bharati J, Rajesh G, Vidyalakshmi GM, *et al.* Expression and molecular cloning of interferon stimulated genes in buffalo (*Bubalus bubalis*). Theriogenology. 2017;100:50–58. doi: 10.1016/j.theriogenology.2017.05.027.
17. Oliveira JF, Henkes LE, Ashley RL, Purcell SH, Smirnova NP, Veeramachaneni, DN, *et al.* Expression of interferon (IFN)-stimulated genes in extrauterine tissues

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

during early pregnancy in sheep is the consequence of endocrine IFN-τ release from the uterine vein. Endocrinology. 2008;149(3):1252–1259. doi: 10.1210/en.2007-0863.

18. Kose M, Kaya MS, AydileK N, Kucukaslan I, Bayril T, Bademkiran S, *et al.* Expression profile of interferon tau-stimulated genes in ovine peripheral blood leukocytes during embryonic death. Theriogenology. 2016;85(6):1161–1166. doi: 10.1016/j.theriogenology.2015.11.032.
19. Aziz MA. Present status of the world goat populations and their productivity. Lohman Information. 2010;45:42.
20. Rathod P, Dixit S. Effect of improved management practices on productive and reproductive performance of Osmanabadi goats under semi-intensive rearing systems. The Indian Journal of Animal Sciences. 2021;91(6): 499–504. doi: 10.56093/ijans.v91i6.115455.
21. Fatet A, Pellicer-Rubio MT, Leboeuf B. Reproductive cycle of goats. Animal Reproduction Science. 2011;124(3–4):211–219. doi: 10.1016/j.anireprosci.2010.08.029.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ method. Methods. 2001;25(4):402–408. doi: 10.1006/meth.2001.1262.
23. Kubasic NP, Hallauer GD, Brodows RG. Evaluation of direct solid – phase RIA for progesterone, useful for monitoring luteal function. Clinical Chemistry. 1984;30(2):284–286. doi: 10.1093/clinchem/30.2.284.
24. Brooks K, Burns G, Spencer TE. Conceptus elongation in ruminants: roles of progesterone, prostaglandin, interferon tau and cortisol. Journal of Animal Science and Biotechnology. 2014;5:53. doi: 10.1186/2049-1891-5-53.
25. Joyce MM, White FJ, Burghardt RC, Muniz JJ, Spencer TE, Bazer FW, *et al.* Interferon stimulated gene 15 conjugates to endometrial cytosolic proteins and is expressed at the uterine-placental interface throughout pregnancy in sheep. Endocrinology. 2005;146(2):675–684. doi: 10.1210/en.2004-1224.
26. Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, *et al.* Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. Biology of Reproduction. 2010;82(4):725–735. doi: 10.1095/biolreprod.109.079467.
27. Jain A, Jain T, Chandrakar K, Shakya S, Mukherjee K. Interferon-stimulated gene 15 (ISG15): Expression profile and role in the corpus luteum of goat (*Capra hircus*). The Indian Journal of Animal Sciences. 2022;92(8):957–960. doi: 10.56093/ijans.v92i8.109419.
28. Al-Samawi K, Al-Hassan M, Migdadi H, Ammar M, Alghamdi S. Interferon-stimulated gene 15 and interferon-1 stimulated gene 17 messenger RNA-based detection of early pregnancy in Aardi goats in Saudi Arabia. Pakistan Journal of Zoology. 2021;53(3):1005–1013. doi: 10.17582/journal.pjz/20180329180335.
29. Austin KJ, Carr AL, Pru JK, Hearne CE, George EL, Belden EL, *et al.* Localization of ISG15 and conjugated proteins in bovine endometrium using immunohistochemistry and electron microscopy. Endocrinology. 2004;145(2):967–975. doi: 10.1210/en.2003-1087.
30. Hansen TR, Austin KJ, Johnson GA. Transient ubiquitin cross-reactive protein gene expression in the bovine endometrium. Endocrinology. 1997;138(11):5079–5082. doi: 10.1210/endo.138.11.5655.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

31. Boerboom D, Brown KA, Vaillancourt D, Poitras P, Goff AK, Watanabe K, *et al.* Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. Biology of Reproduction. 2004;70(2):391–399. doi: 10.1095/biolreprod.103.020800.
32. Frade JP, Warnock DW, Arthington-Skaggs BA. Rapid quantification of drug resistance gene expression in *Candida albicans* by reverse transcriptase LightCycler PCR and fluorescent probe hybridization. Journal of Clinical Microbiology. 2004;42(5):2085–2093. doi: 10.1128/JCM.42.5.2085-2093.2004.
33. Hida S, Ogasawara K, Sato K, Abe M, Takayanagi H, Yokochi, T, *et al.* $CD8^+$ T cell mediated skin disease in mice lacking IRF-2, the transcriptional attenuator of interferon-α/β signaling. Immunity. 2000;13(5):643–655. doi: 10.1016/s1074-7613(00)00064-9.
34. Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, *et al.* Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, non-pregnant dairy cows. Journal of Dairy Science. 2007;90(1):274–280. doi: 10.3168/jds.S0022-0302(07)72628-0.
35. Carvalho PD, Consentini CC, Weaver SR, Barleta RV, Hernandez LL, Fricke PM. Temporarily decreasing progesterone after timed artificial insemination decreased expression of interferon-tau stimulated gene 15 (ISG15) in blood leukocytes, serum pregnancy-specific protein B concentrations, and embryo size in lactating Holstein cows. Journal of Dairy Science. 2017;100(4):3233–3242. doi: 10.3168/jds.2016-11996.
36. Green JC, Okamura CS, Poock SE, Lucy MC. Measurement of interferon-tau (IFN-$_T$) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18–20 d after insemination in dairy cattle. Animal Reproduction Science. 2010;121(1-2):24–33. doi: 10.1016/j.anireprosci.2010.05.010.
37. Soumya NP, Das DN, Jeyakumar S, Mondal S, Mor A, Mundhe UT. Differential expression of ISG 15 mRNA in peripheral blood mononuclear cells of nulliparous and multiparous pregnant versus non-pregnant *Bos indicus* cattle. Reproduction of Domestic Animals. 2017;52(1):97–106. doi: 10.1111/rda.12815.
38. Haq IU, Han Y, Ali T, Wang Y, Gao H, Lin L, *et al.* Expression of interferon-stimulated gene ISG15 and ubiquitination enzymes is upregulated in peripheral blood monocyte during early pregnancy in dairy cattle. Reproduction Biology. 2016;16(4):255–260. doi: 10.1016/j.repbio.2016.10.001.
39. Matsuyama S, Kojima T, Kato S, Kimura K. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. Reproductive Biology and Endocrinology. 2012;10:21. doi: 10.1186/1477-7827-10-21.
40. Sheikh AA, Om KH, Ajay KD. Interferon tau stimulated gene expression and pro-inflammatory cytokine profile relative to insemination in dairy cows. Biological Rhythm Research. 2019;50(3):335–345. doi: 10.1080/09291016.2018.1440777.
41. Nag BSP, Arunmozhi N, Sarath T, Asokan SA, Vijayarani K, Monica G, *et al.* Interferon stimulated gene 15 and Myxovirus resistance 2 gene are Upregulated during early pregnancy in buffaloes. International Journal of Current Microbiology and Applied Sciences. 2018;7(12):1755–1762. doi:10.20546/ijcmas.2018.712.204.

doi: 10.22201/fmvz.24486760e.2025.1267
Vol. 12 | 2025

42. Bazer FW, Spencer TE, Johnson GA. Interferons and uterine receptivity. Seminars in Reproductive Medicine. 2009;27(1):90–102. doi: 10.1055/s-0028-1108013.
43. Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy, CN. Expression of trophoblastic interferon genes in sheep and cattle. Biology of Reproduction.1990;43(2):210–218. doi: 10.1095/biolreprod43.2.210.
44. Schmitt RA, Geisert RD, Zavy MT, Short EC, Blair RM. Uterine cellular changes in 2′,5′- oligoadenylate synthetase during the bovine estrous cycle and early pregnancy. Biology of Reproduction. 1993;48(3):460–466. doi: 10.1095/biolreprod48.3.460.
45. Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, *et al.* Effects of the estrous cycle, pregnancy, and interferon tau on 2′,5′- oligoadenylate synthetase expression in the ovine uterus. Biology Reproduction. 2001;64(5):1392–1399. doi: 10.1095/biolreprod64.5.1392.
46. Panicker SS, Kanakkaparambil R, Kanjirakuzhiyil P, Koodathil R. Progesterone profile of goats subjected to oestrus synchronization and its relevance in predicting pregnancy outcome. Advances in Animal and Veterinary Sciences. 2015;3(4):207–210. doi: 10.14737/journal.aavs/2015/3.4.207.210.
47. Boscos CM, Samartzi FC, Lymberopoulos AG, Stefanakis A, Belibasaki S. Assessment of progesterone concentration using enzyme immunoassay, for early pregnancy diagnosis in sheep and goats. Reproduction in Domestic Animal. 2003;38(3):170–174. doi: 10.1046/j.1439-0531.2003.00407.x.
48. Devalal K, Joseph M, Kurien MO, Aravind A, Ajitkumar S. Serum progesterone and oestradiol profile in pseudopregnant and pregnant goats below 3 months post-service. Journal of Veterinary and Animal Sciences. 2019;50(1):63–67.
49. Rahman, ANMA. Hormonal changes in the uterus during pregnancy lessons from the ewe: a review. Journal of Agricultural and Rural Development. 2008;4(1):1–7. doi: 10.3329/jard.v4i1.761.