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Nutrition Affects Embryo Survival, Progesterone, Leptin, Leptin Receptor, and Progesterone Receptor Membrane Component 1 in Goats

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ABSTRACT

Embryo mortality remains a significant limitation on reproductive efficiency in goats, especially in the first month of pregnancy. Maternal nutrition plays an important role in regulating the endocrine and molecular mechanisms essential for pregnancy maintenance. Thus, this study aimed to determine the effects of short-term concentrate supplementation on circulating leptin, neuropeptide Y (NPY), and progesterone concentrations, *LEPR* and *PGRMC1* expression, and early embryo survival in female Boer goats. Twenty female Boer goats were randomly allocated in a completely randomized design to either a Control group receiving a maintenance diet supplying 4.45 MJ ME/day or a supplemented group receiving additional concentrate to supply 8.90 MJ ME/day, equivalent to twice the maintenance energy requirement. The supplemented diet provided twice the maintenance energy requirement for 25 days, beginning five days before ovulation and extending into early pregnancy. Blood plasma sampled every 2 days, from Days -5 to 27, was assayed for leptin, neuropeptide-Y, and progesterone. On Day 27 after mating, the expression of *PGRMC1* and *LEPR* was assessed in pituitary tissue, follicles, and corpora lutea (CL). Pregnancy and embryo loss were monitored using progesterone concentration and transrectal ultrasonography. Data were analyzed using independent t-tests, repeated-measures analysis, Spearman correlation, and Chi-square tests. Nutritional supplementation significantly increased the circulating concentrations of leptin, neuropeptide-Y, and progesterone ($p<0.05$). It also increased the expression of *PGRMC1* 3.7-fold in luteal tissue and 2.3-fold in follicular tissue, and increased the expression of *LEPR* 5.0-fold in pituitary tissue and 3.3-fold in luteal tissue. Plasma progesterone concentration was positively correlated with plasma leptin concentration ($r = 0.63$; $p<0.05$) and with *PGRMC1* expression ($r = 0.65$; $p<0.05$). Compared with the Control diet, the supplemented diet increased the pregnancy rate from 55.6% to 87.5% and reduced embryo loss from 44.4% to 10.0% despite having no effect on ovulation rate (1.00 ± 0.21 vs. 1.25 ± 0.24, $p>0.05$). It was concluded that nutritional supplementation enhances luteal function, progesterone synthesis, and embryo survival, and that the effects were mediated by circulating leptin and improved expression of *PGRMC1* and *LEPR*.

Keywords: embryo mortality; goat; LEPR; nutrition; PGRMC1

INTRODUCTION

In goats, conception rates are typically high, often exceeding 70%–90% depending on management and breed (Samir *et al.*, 2016; Robertson *et al.*, 2020). However, up to 30% of embryos are lost, mostly before implantation and within the first month of pregnancy, so only 60%–70% of conceptions remain viable by day 30 of gestation (Samir *et al.*, 2016; Köse *et al.*, 2021). A possible explanation is luteal insufficiency, due to premature luteal regression or luteal dysfunction, so too little progesterone is secreted to support pregnancy (Dar *et al.*, 2017). Over-nutrition has long been linked to an increase in the metabolic clearance of progesterone secreted by the corpus luteum, perhaps decreasing embryo survival (O'Connell *et al.*, 2013) and therefore neutralizing the benefits of improved ovulation rate (Meza-Herrera *et al.*, 2019).

The situation with nutrition is complex because well-timed supplements, often termed 'flushing', can improve reproductive outcomes by providing specific metabolic signals during critical stages of the

reproductive cycle in goats and sheep (reviews: Martin, 2022). Indeed, a supplement can increase the number of embryos in goats (Meza-Herrera *et al.*, 2019; Astuti *et al.*, 2020). In contrast, poor nutritional management of goats during the peri-conceptional period can impair early embryo survival by creating a sub-optimal environment for oocyte maturation and embryo development (Widiyono *et al.*, 2022). Caloric and protein restriction suppress hypothalamic–pituitary–gonadal (HPG) axis activity by reducing gonadotropin-releasing hormone (GnRH) pulsatility, leading to decreased luteinizing hormone (LH) secretion, impaired follicular development, and suboptimal corpus luteum function. In addition, nutrient deficiency alters metabolic hormone profiles, including reduced leptin and increased neuropeptide-Y (NPY), which further modulate reproductive hormone secretion and ovarian function. Collectively, these endocrine disruptions can impair oocyte competence, fertilization, and early embryo survival.

Luteal structures and progesterone secretion are supported by the hypothalamic-pituitary axis, driven by gonadotropin-releasing hormone (GnRH), so treatment with exogenous GnRH has shown promise in promoting embryonic survival (Köse *et al.*, 2021; Wittayarat *et al.*, 2024). Evidence is also emerging of a role for progesterone receptor membrane component 1 (*PGRMC1*), a multifunctional protein localized in granulosa and luteal cells in various species, including goats (Mohammed *et al.*, 2025). The intracellular roles of *PGRMC1* include vesicle trafficking, progesterone signaling, mitotic spindle regulation, cell cycle control, and, perhaps most importantly in the context of the present study, steroidogenesis (Solairaja *et al.*, 2022). The synthesis of progesterone involves cholesterol conversion via P450 enzymes, and it is likely that *PGRMC1* influences a P450-mediated pathway. However, there is limited information on how nutrition affects *PGRMC1* expression and its role in mediating nutritional impacts on progesterone secretion in goats.

Metabolic hormones also play a central role in linking nutritional status with reproductive function. Neuropeptide-Y (NPY), a key regulator of energy balance, can influence reproductive processes both centrally and directly at the ovarian level via its receptors, affecting follicular development and survival (Urata *et al.*, 2023). Leptin, an adiposity signal, is similarly implicated in reproductive regulation, with its receptor (*LEPR*) expressed in ovarian follicles and the corpus luteum. Leptin has been shown to modulate steroidogenesis and progesterone secretion in other species (Nakano *et al.*, 2022; Zhang *et al.*, 2025), yet its role in regulating luteal function and early pregnancy in goats remains inadequately characterized.

Despite extensive research on nutritional management and reproductive performance, a critical gap persists in understanding how flushing modulates endocrine–molecular pathways, particularly the interaction between metabolic hormones (leptin, NPY), luteal regulators (*PGRMC1*), and progesterone secretion during early pregnancy in goats. Therefore, the objectives of this study were to (i) evaluate the effect of nutritional flushing on circulating concentrations of progesterone, leptin, and NPY, (ii) determine the expression of *PGRMC1* and *LEPR* in pituitary and ovarian tissues (follicle and corpus luteum), and (iii) assess the relationships between these endocrine and molecular parameters and reproductive performance in female goats during early pregnancy. We hypothesized that nutritional flushing increases circulating leptin, reduces NPY, upregulates *PGRMC1* and *LEPR*, and thereby improves progesterone secretion and embryo survival.

MATERIALS AND METHODS

Animals and Experimental Design

This study was conducted in accordance with animal ethics guidelines and was approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (Ethics Approval/ Registration Number: UPM/IACUC/AUP-R064/2016).

A total of twenty healthy female Boer goats, aged between 2 and 3 years (multiparous), with an average body weight of 27.0 ± 0.9 kg and body condition score (BCS) of 2.3 ± 0.1, were used in this study. The animals were housed individually in pens measuring 1.5 m × 0.5 m and were randomly allocated into two dietary treatment groups: (1) a Control group (n=10) receiving a basal maintenance diet, and (2) a Supplemented group (n=10) receiving the same basal diet with additional commercial goat pellet concentrate to provide twice the maintenance energy requirement.

The synchronization and feeding protocols were performed as previously described by Mohammed *et al.* (2025). All animals underwent oestrus synchronization using a controlled internal drug release device (CIDR; Eazi-Breed® CIDR®, Pfizer Australia) containing 0.3 g progesterone, which was inserted intravaginally for 18 consecutive days (Days -18 to 0). The feeding intervention for the Supplemented group began on Day -5 and continued for a total of 25 days (Figure 1). Natural mating was conducted from Days 2 to 4 by

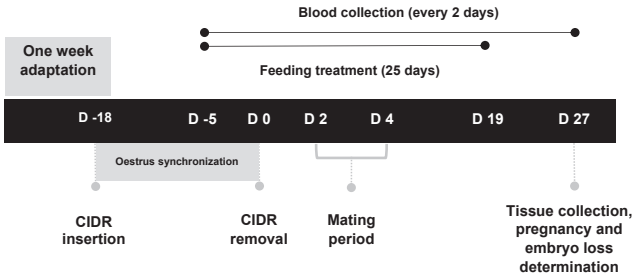

Figure 1. Experimental design and sampling schedule for female Boer goats receiving either a maintenance diet or short-term concentrate supplementation supplying twice the maintenance energy requirement. Oestrus was synchronized using a CIDR device from Day −18 to Day 0. Dietary supplementation was provided from Day −5 to Day 19, mating was conducted from Days 2 to 4, blood samples were collected from Day −5 to Day 27, and pituitary and ovarian tissues were collected on Day 27.

introducing fertile bucks to all does. Ovulation rates were assessed on Day 19 using trans-rectal ultrasound, pregnancy and embryo loss were monitored using P4 concentration and trans-rectal ultrasonography on Day 27.

Blood samples for plasma progesterone analysis were collected at two-day intervals from Day -5 to Day 27. Samples were centrifuged post-collection immediately, and the plasma was stored at −20 °C until analysis. On Day 27, all does were humanely sacrificed. Ovaries and brains were harvested immediately; CL tissues were collected from both ovaries, while the anterior pituitary was isolated from the brain. All tissue samples were preserved in RNALater® (Qiagen, USA) and stored at −20 °C for subsequent RNA extraction and quantitative RT-PCR analysis.

Feeding Treatment

All goats had *ad libitum* access to clean drinking water throughout the experimental period. The animals were assigned to one of two dietary treatments: the Control group (n = 10), which received a maintenance-level diet supplying 4.45 MJ/day of metabolizable energy (ME), and the Supplemented group (n = 10), which was fed the same basal maintenance diet with additional concentrate supplementation to provide a total of 8.90 MJ/day of ME, equivalent to twice the maintenance energy requirement. The basal diet, offered to both groups, comprised 70% Napier grass (*Pennisetum purpureum*) and 30% commercial goat pellet concentrate, formulated on a dry matter basis. Feed was provided twice daily at fixed times (morning and afternoon) to ensure consistency in feeding management and intake patterns. Feed refusals were collected and weighed daily to monitor actual feed intake and ensure accurate estimation of nutrient consumption. All feed offered was generally consumed, with minimal refusals observed throughout the experimental period. Out of the 20 animals initially included in the experiment, data from three does (1 Control and 2 Supplemented) were excluded from statistical analysis due to abnormal feeding behavior, resulting in a final dataset used for analysis. The nutritional composition of the feed, including metabolizable energy, crude protein, and dry matter content of Napier grass and the commercial concentrate, is presented in Table 1. The nutrient requirement was calculated according to the nutrient requirements for small ruminants described by the National Research Council (NRC, 2007).

Determination of Reproductive Performance

Ovulation rate was evaluated on Day 19 post-breeding by counting the number of corpora lutea (CL) on both ovaries using a 7.5 MHz linear transducer (SSD-900; Aloka, Tokyo, Japan). Pregnancy rate was calculated as the proportion of does confirmed pregnant via transrectal ultrasound on Day 27 and supported by serial plasma progesterone measurements, with levels >2 ng/mL on Day 21 post-breeding indicative of pregnancy. Embryo count and viability were assessed using ultrasound on Day 27, and embryo loss was determined by comparing the number of detected embryos to the expected number based on ovulation data. Additionally, any sudden decline in progesterone concentrations to <2 ng/mL during the experimental period was interpreted as indicative of embryo loss.

Table 1. Nutrient composition of Napier grass and commercial goat pellet concentrate used in the dietary treatments, based on proximate analyses

Type of feed	Nutrient composition		
	DM (%)	ME (MJ/kg)	CP (%)
Napier grass	25.10	6.47	3.40
Concentrate	89.57	12.35	15.22

Note: DM = Dry matter; ME = Metabolizable energy; CP = Crude protein.

Hormone Determination

Plasma concentrations of leptin and NPY were quantified using commercial kits from QayeeBio (Shanghai, China), while progesterone was measured using an ELISA kit from ENZO Life Sciences Inc. (USA), according to the manufacturer's protocol. The sensitivity of the assays was 15.6 pg/mL for leptin, 6.25 pg/mL for NPY, and 8.57 pg/mL for progesterone. The intra- and inter-assay variations were 5.7% and 6.0% (Leptin), 8.1% and 11.8% (NPY), and 7.9% and 6.2% (progesterone).

Determination of *PGRMC1* and *LEPR* Expression

RNA extraction and cDNA synthesis. Total RNA was extracted from each sample using the RNeasy Mini Kit (Qiagen, USA) following the manufacturer's instructions. Genomic DNA was removed by on-column DNase digestion using RQ1 RNase-Free DNase (Promega, USA). RNA integrity was confirmed by the presence of clear 28S and 18S rRNA bands on a 1% agarose gel. RNA concentration and purity were measured using a NanoVue™ Plus Spectrophotometer, with all samples showing OD 260/280 ratios between 1.8 and 2.0, indicating good quality. For each sample, 1 μg of total RNA was reverse-transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, USA), and the cDNA was stored at -20 °C until qPCR analysis.

Primers. The design of primers for amplifying the goat *LEPR* gene was based on gene sequences from multiple species retrieved from the National Center for Biotechnology Information (NCBI) GenBank database. Multiple sequence alignment was performed using ClustalW2 with selected sequences and the full-length *LEPR* gene to identify conserved exon regions. Primers were designed within these regions using Primer3 software. Primers for *PGRMC1* were adopted from Mohammed *et al.* (2025), while those for the reference genes *GAPDH* and *β-actin* were obtained from Celestino *et al.* (2010); the *ubiquitin* primer was sourced from Frota *et al.* (2011). All primer sequences and their corresponding accession numbers are presented in Table 2.

Quantitative RT-PCR. The RT-qPCR master mix was prepared using the KAPA SYBR FAST qPCR Kit Master Mix (2X) Universal (KAPA Biosystems, USA), and the reactions were run on a LightCycler 480 system (Roche, USA). Each 20 µL reaction contained 10 µL SYBR master mix, 0.4 µL each of forward and reverse primers (200 nM), 2 µL cDNA (100 ng), and 7.2 µL of nuclease-free water. The cycling program included enzyme activation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s. Negative controls, using water instead of cDNA, were included to check for any contamination. Melting curve analysis confirmed specific amplification, and the PCR product size was verified on a 2% agarose gel.

Relative gene expression was calculated using the $^{2^{-\Delta\Delta}}$Ct method (Lixiang *et al.,* 2025), with the Control group serving as the calibrator. All RT-qPCR reactions were performed in technical triplicate, and mean Ct values were used for downstream analysis to minimize intra-assay variation. Amplification efficiencies of the reference genes were 98.3% for β-actin, 96.9% for GAPDH, and 97.9% for ubiquitin, all within the acceptable MIQE range (90%–110%), supporting their suitability for relative quantification. Normalization was performed using the geometric mean of the three reference genes (GAPDH, β-actin, and ubiquitin) to reduce potential bias associated with single-gene normalization. The expression stability of these genes was assessed across all samples based on Ct consistency, and no significant variation was observed among experimental groups, confirming their stability and appropriateness for normalization.

Statistical Analysis

Statistical analyses were performed using SPSS version 26. The effects of the nutritional supplement on *PGRMC1* and *LEPR* mRNA levels were evaluated using an independent t-test, while plasma progesterone concentrations were analyzed with a repeated-measures multivariate test. Spearman's rank correlation was used to assess relationships between variables. Ovulation rate, pregnancy rate, and embryo loss rate were compared using the Chi-square test. Quantitative RT-PCR results are presented as fold change ± SD relative to the Control group (set to 1), and plasma progesterone concentrations are shown as mean ± SEM. Statistical significance was set at $p \leq 0.05$.

RESULTS

Ovulation Rate, Pregnancy Rate, and Embryo Loss

Nutritional supplementation for 25 days did not significantly affect ovulation rate (1.00 ± 0.21 versus 1.25 ± 0.24 in the Control), indicating that short-term supplementation did not influence follicular recruitment or ovulatory response. However, despite similar ovulation rates, the pregnancy rate was markedly greater in the supplemented group (87.5 ± 0.88%) compared with the Control group (55.5 ± 0.82; $p<0.05$%). This divergence between ovulation and pregnancy outcomes indicates that the beneficial effects of supplementation were mediated primarily through post-ovulatory mechanisms rather than increased ovulatory output. The improved pregnancy rate was reflected in embryo recovery data. Supplemented does produced significantly more embryos (9.6 ± 0.71) than Control does (5.7 ± 1.42), with embryo loss substantially reduced by supplementation, declining from 44% in Controls to only 10% in supplemented does (Table 3). These findings strongly suggest that nutritional supplementation enhanced embryo survival during early gestation rather than increasing fertilization per se.

Table 2. Primer sequences used for RT-qPCR analysis of *LEPR*, *PGRMC1*, and reference genes in pituitary, follicular, and luteal tissues of female Boer goats

Gene	Primer sequence (5′ → 3′)	Amplicon size (bp)	Accession no./ Reference
LEPR	F: TGGCTGGAAGATGTGGGAAA R: TAAAGGGTAAGCACTGAGGGAC	177	XM018045226.1
PGRMC1	F: CCTGAGAGACGGGTAAAACAT R: GTCCCCAAACAGAGAAAGTAGG	297	XM013976238.1
B-actin	F: ACCACTGGCATTGTCATGGACTCT R: TCCTTGATGTCACGGACGATTTCC	200	AF481159/ Celestino *et al*. (2010)
GADPH	F: TGTTTGTGATGGGCGTGAACCA R: ATGGCGTGGACAGTGGTCATAA	154	AJ431207/ Celestino *et al*. (2010)
Ubiquitin	F: GAAGATGGCCGCACTCTTCTGAT R: ATCCTGGATCTTGGCCTTCACGTT	150	GI:57163956/ Frota *et al*. (2011)

Note: F= forward primer; R= reverse primer.

Table 3. Ovulation, embryo number, and embryo loss in female Boer goats receiving either a maintenance diet or short-term concentrate supplementation supplying twice the maintenance energy requirement

Group	Number of corpus luteum	Number of embryos			Embryo loss (%)
		0	1	2	
Control (n = 9)	9	4	5	0	44[a]
Supplemented (n=8)	10	1	5	2	10[b]

Note: Means in the same column with different superscripts differ significantly ($p<0.05$).

Nutritional Supplementation and Concentrations of Leptin, NPY, and Progesterone

Plasma concentrations of leptin, NPY, and progesterone for the Supplemented (flushing) and Control groups are presented in Figure 2. Overall, does receiving supplementation exhibited higher circulating leptin and progesterone concentrations but lower NPY concentrations compared with Controls, reflecting an improved metabolic and endocrine milieu.

From Days -5 to 11, leptin concentrations were similar between groups (Figure 2A). However, from Day 13 to Day 19, leptin levels were significantly greater in supplemented does than in Controls ($p<0.05$). Although leptin concentrations declined in both groups following cessation of supplementation on Day 19, values remained significantly elevated in the supplemented group through Day 27 ($p<0.05$). This sustained elevation indicates that the metabolic effects of supplementation persisted beyond the feeding period. The pattern of NPY concentration (Figure 2B) was inverse to that of leptin. Supplemented does consistently exhibited lower NPY concentrations than Controls, although differences were not significant from Days -5 to 9. From Day 11 to Day 19, NPY concentrations were significantly lower in supplemented does, with the greatest divergence observed on Day 19, after which concentrations declined in both groups. Reduced NPY suggests attenuation of the negative energy balance signal and reduced inhibitory input to the reproductive axis. Plasma progesterone concentrations were low and similar between groups until approximately Day 9, after which concentrations gradually increased (Figure 2C). From Day 13 until the end of the sampling period on Day 27, progesterone concentrations were significantly greater in supplemented does than in Controls ($p<0.05$). Moreover, the result shows that the concentrations of NPY and leptin were significantly negatively correlated (Figure 3A; $r = –0.51$, $p<0.05$) and the concentrations of progesterone and leptin were significantly positively correlated (Figure 3B; $r = 0.63$, $p<0.05$). The apparent negative relationship between the concentrations of NPY and progesterone was not significant (Figure 3C). The association between elevated leptin, suppressed NPY, and increased progesterone strongly supports the role of improved metabolic status in enhancing luteal function.

Expression of *LEPR* and *PGRMC1*

Gene expression analysis revealed that the effects of nutritional supplementation extended beyond systemic endocrine changes to include tissue-specific molecular adaptations. Compared with Control does, *LEPR* expression was significantly upregulated in pituitary (5.03-fold) and luteal (3.32-fold) tissues in supplemented does (Figure 4; $p<0.05$), with no significant change observed in ovarian follicles. Increased pituitary *LEPR* expression suggests enhanced central sensitivity to leptin, potentially amplifying its stimulatory effects on gonadotropin secretion. Concurrent upregulation in luteal tissue indicates a direct ovarian action of leptin during the luteal phase. In contrast, *PGRMC1* expression

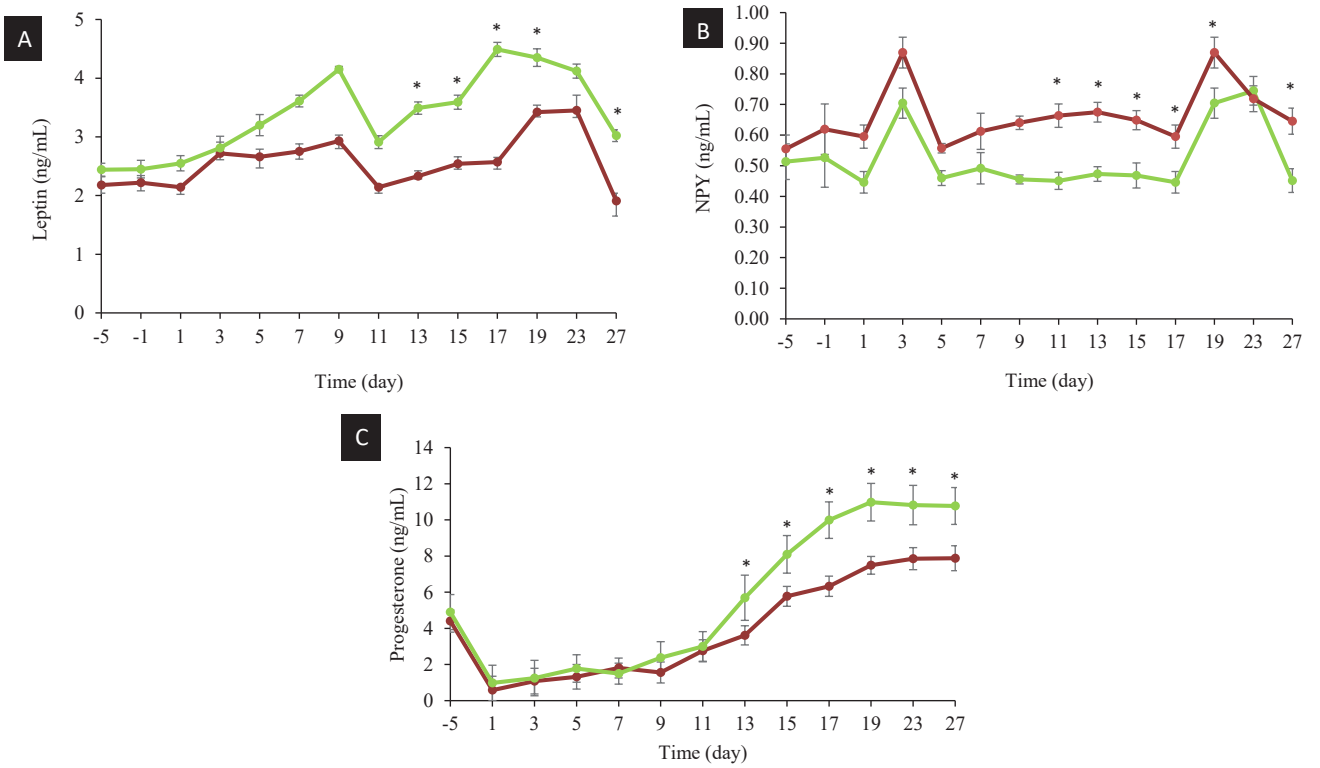

Figure 2. Plasma concentrations of (A) leptin, (B) neuropeptide Y, and (C) progesterone in female Boer goats receiving either a maintenance diet (Control; red line; n = 9) or short-term concentrate supplementation supplying twice the maintenance energy requirement (Supplemented; green line; n = 8). Supplementation was provided from Day −5 to Day 19. Values are presented as mean ± SEM. An asterisk indicates a significant difference between dietary treatments at the corresponding time point ($p<0.05$).

in pituitary tissue was unaffected by supplementation (Figure 5), but was significantly increased in follicular (2.31-fold) and luteal (3.75-fold) tissues ($p<0.05$).

Correlations between Hormone Concentration and Gene Expression

Correlation analyses further support the functional relevance of these molecular adaptations. A moderate positive correlation was observed between *PGRMC1* expression and plasma progesterone concentration ($r = 0.65$, $p<0.05$), indicating that increased *PGRMC1* expression is associated with enhanced luteal progesterone output. *LEPR* expression also showed a moderate positive correlation with progesterone ($r = 0.61$, $p<0.05$), suggesting that leptin signaling contributes to luteal steroidogenesis.

Linear regression analysis (Figure 6) confirmed that expression of both genes significantly predicted progesterone concentration. *PGRMC1* expression accounted for approximately 75% of the variation in progesterone levels ($R^2 = 0.75$), while *LEPR* expression explained about 57% ($R^2 = 0.57$). These findings underscore the importance of both leptin signaling and progesterone receptor-associated pathways as key mediators linking nutritional status to luteal function.

DISCUSSION

The absence of a significant effect on ovulation rate suggests that the improved pregnancy outcomes were not due to increased ovulatory activity, but rather to post-ovulatory mechanisms. The combination of unchanged ovulation rate, higher pregnancy rate, and reduced embryonic loss indicates improved luteal function and endocrine support during early gestation. These findings suggest that nutritional supplementation may enhance embryo survival during

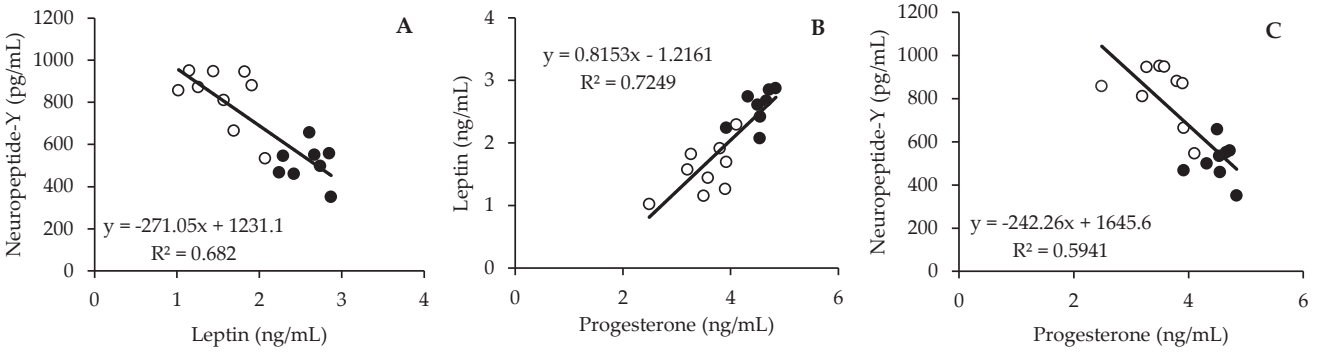

Figure 3. Relationships among plasma concentrations of neuropeptide Y, leptin, and progesterone in female Boer goats receiving either a maintenance diet (Control) or short-term concentrate supplementation supplying twice the maintenance energy requirement (Supplemented). Each point represents an individual observation. Control and Supplemented goats are represented by white and black symbols, respectively.

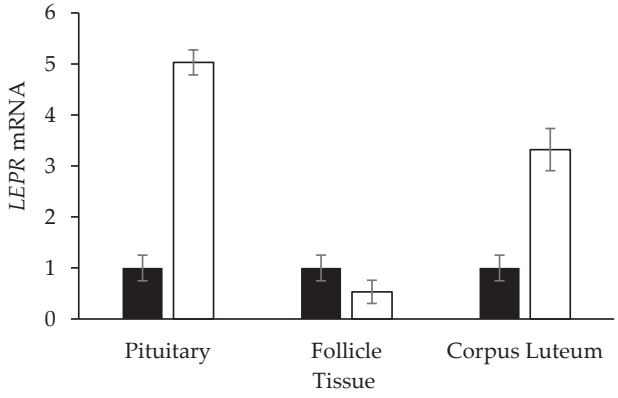

Figure 4. Relative expression of the leptin receptor gene (*LEPR*) in pituitary, follicular, and luteal tissues collected on Day 27 from female Boer goats receiving either a maintenance diet (Control) or short-term concentrate supplementation supplying twice the maintenance energy requirement (Supplemented). Expression values were normalized to the geometric mean of GAPDH, β-actin, and ubiquitin and are presented as fold change ± SD relative to the Control group. An asterisk indicates a significant difference between dietary treatments ($p<0.05$). ■, Control; □, Supplemented.

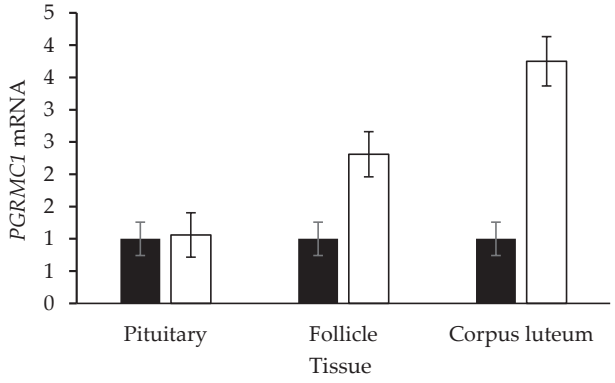

Figure 5. Relative expression of progesterone receptor membrane component 1 (*PGRMC1*) in pituitary, follicular, and luteal tissues collected on Day 27 from female Boer goats receiving either a maintenance diet (Control) or short-term concentrate supplementation supplying twice the maintenance energy requirement (Supplemented). Expression values were normalized to the geometric mean of GAPDH, β-actin, and ubiquitin and are presented as fold change ± SD relative to the Control group. An asterisk indicates a significant difference between dietary treatments ($p<0.05$). ■, Control; □, Supplemented

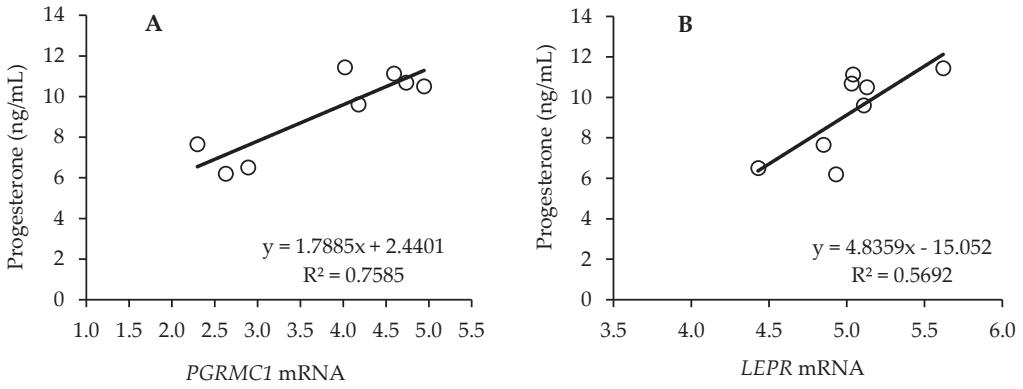

Figure 6. Relationships between plasma progesterone concentration and the relative expression of (A) *PGRMC1* and (B) *LEPR* in luteal tissue collected from female Boer goats receiving either a maintenance diet or short-term concentrate supplementation supplying twice the maintenance energy requirement. Each point represents an individual animal. The solid line represents the fitted linear regression.

early pregnancy rather than fertilisation success. This interpretation is consistent with previous reports linking early embryonic mortality to luteal insufficiency and inadequate progesterone secretion, which impair uterine receptivity and increase the risk of implantation failure or embryonic loss (Bustamante-Andrade *et al.,* 2021). However, it should be noted that the relatively small sample size may limit the detection of subtle effects on ovulation rate, and thus minor treatment effects cannot be entirely excluded.

In contrast to earlier assumptions that increased dietary energy could elevate hepatic progesterone clearance and increase embryo losses in sheep (Viñoles *et al.,* 2015), the present findings are consistent with studies in Australian Cashmere goats, where high-protein diets did not reduce progesterone concentrations despite elevated leptin and insulin (ShikhMaidin *et al.,* 2014), and in pigs, where increased energy intake did not negatively affect embryonic survival in most experiments (Leal *et al.,* 2019).

The observed endocrine changes are consistent with established leptin–NPY interactions linking nutrition and reproduction. Elevated leptin reflects improved energy balance and suppresses hypothalamic NPY expression, thereby relieving inhibition of GnRH neurons and facilitating LH secretion, a key driver of luteal progesterone synthesis (Takle & Legesse, 2017). Recent studies further demonstrate that improved nutritional status enhances leptin sensitivity, strengthening its inhibitory effect on NPY and reinforcing reproductive prioritization (Zhang *et al.,* 2020; Asgari *et al.,* 2025). Although NPY is primarily recognized for its central role, increasing evidence indicates that it also acts peripherally within the ovary, where it can suppress steroidogenesis (Urata *et al.,* 2020). Thus, the reduction in circulating NPY in supplemented does may contribute to a more favorable luteal environment by alleviating inhibitory effects on progesterone synthesis. Nevertheless, the present study did not directly assess hypothalamic or intra-ovarian NPY expression, and therefore, mechanistic inferences regarding central versus peripheral actions should be interpreted cautiously.

In addition to its central actions, leptin exerts direct ovarian effects. Leptin receptors are abundantly expressed in ovarian tissues, including the CL (Gallelli *et al.,* 2019; Macedo *et al.,* 2019; Martins *et al.,* 2021), where leptin enhances the activity of key steroidogenic enzymes such as StAR, P450scc, and 3β-HSD (Chaudhari *et al.,* 2020). During the luteal phase, elevated leptin concentrations may activate these local pathways, complementing leptin-induced stimulation of the hypothalamic–pituitary–gonadal axis. Recent evidence suggests that these central and peripheral mechanisms act synergistically under conditions of improved metabolic status, potentially leading to enhanced progesterone synthesis and luteal competence (Batista *et al.,* 2013).

The upregulation of *LEPR* in luteal tissue suggests a dual mechanism whereby leptin acts both centrally and locally to enhance reproductive function. Locally, leptin signaling within the corpus luteum may promote angiogenesis, enhance steroidogenic enzyme activity, and support luteal cell survival. This interpretation is consistent with previous findings in goats demonstrating *LEPR* expression in luteal tissue and showing that leptin enhances expression of angiogenic factors such as VEGF, FGF2, and angiopoietin 1 in early luteal cultures, thereby supporting luteal vascularisation and functional maintenance (Garcia, 2017). Similar regulation of VEGF/FGF2/Ang1 by leptin is reported in caprine and porcine CL and summarized in a recent review (Flores *et al.,* 2022; Młyczyńska *et al.,* 2022).

Additionally, *PGRMC1* is suggested to influence progesterone synthesis through non-genomic pathways that enhance steroidogenesis by increasing cholesterol synthesis. This occurs through two mechanisms: first, *PGRMC1* directly binds to insulin-induced gene-1 (INSIG1) and sterol regulatory element-binding protein cleavage-activating protein (SCAP), increasing SCAP transcription, thereby enhancing steroid synthesis via *StAR* (Manna *et al.,* 2016; Stocco & Selvaraj, 2017). Second, *PGRMC1* stabilizes the P450 protein CYP51/ lanosterol demethylase (McGuire *et al.,* 2021), thereby regulating intracellular cholesterol production, which serves as the precursor for progesterone biosynthesis

(Asperger *et al.,* 2020). Through these pathways, *PGRMC1* facilitates the sustained production of progesterone, which is essential for embryo survivability. It should be noted, however, that gene expression data were not complemented by protein-level or functional assays, which would be required to confirm the activation of these pathways.

Furthermore, *PGRMC1* is recognized as a mediator of progesterone action in follicles (Lodde *et al.,* 2022). Progesterone inhibits follicular growth and granulosa cell mitosis, with substantial evidence supporting its anti-mitotic effects (Sueldo *et al.,* 2015). Increased *PGRMC1* expression in supplemented does may be associated with slower follicular development by suppressing intracellular signaling pathways such as p-ERK1/2, p-p38, and p-NF-κB, which are involved in cell differentiation and steroidogenesis (Yuan *et al.,* 2019). This suppression of steroidogenic enzymes (StAR and cholesterol side-chain cleavage enzyme) may reduce oestradiol production, diminishing negative feedback on gonadotropin-releasing hormone (GnRH) secretion and thereby enhancing LH secretion. This increase in LH may further support CL function and progesterone production.

Overall, the combined endocrine, molecular, and reproductive data suggest that nutritional supplementation enhances reproductive performance primarily by improving luteal function rather than increasing ovulation rate. This effect is mediated through coordinated systemic changes in metabolic hormones and local up-regulation of key genes involved in leptin signaling and progesterone synthesis. Nevertheless, the relatively small sample size, particularly for molecular and correlation analyses, may limit statistical power, and the observed associations should therefore be interpreted as indicative rather than definitive. Future research should focus on elucidating downstream signaling pathways associated with *LEPR* and *PGRMC1* in the corpus luteum, particularly their interactions with steroidogenic acute regulatory protein (StAR) and mitochondrial cholesterol transport. Additionally, investigation of whether the benefits of nutritional supplementation extend into later gestation and influence offspring development would further clarify its long-term reproductive value.

CONCLUSION

Short-term nutritional supplementation improved progesterone levels and luteal function in goats during early pregnancy. This suggests that nutritional flushing helps create a better hormonal environment for supporting early embryo development. These findings highlight the importance of targeted nutrition during early gestation as a practical strategy to support reproductive success in goats.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used artificial intelligence tools to assist in drafting, editing, and improving the clarity of the manuscript. After using this tool, the authors critically reviewed and revised the content as needed and take full responsibility for the final content of the publication.

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