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Original article

Longitudinal metabolic and progesterone dynamics in estrus-synchronized ewes: influence of pregnancy outcome and litter size

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Abstract

Metabolic status plays a critical role in reproductive efficiency in small ruminants; however, longitudinal information describing metabolic adaptations from estrus synchronization through gestation and the immediate postpartum period remains limited. This study investigated temporal changes in selected serum metabolic and hormonal parameters in estrus-synchronized ewes and compared pregnant (PR) and non-pregnant (NPR) animals, with particular emphasis on the influence of litter size. A total of 235 ewes were synchronized during the breeding season. Of these, 150 animals (75 PR and 75 NPR) were included in the main comparative analysis, while a subset of 45 ewes (30 PR and 15 NPR) was monitored longitudinally across gestation and the early postpartum period. Blood samples were collected during synchronization (D-14 and D0), pregnancy (D35, 60, 120, and 145), and on Day 1 postpartum (1 dpp). Serum glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), aspartate aminotransferase (AST), and progesterone concentrations were determined. Glucose concentrations at D-14 were significantly higher in ewes that subsequently established pregnancy ($p<0.05$). During gestation, NEFA and BHB concentrations increased progressively, with the highest values observed during late pregnancy, particularly in ewes carrying multiple fetuses ($p<0.05$). Serum AST activity increased moderately toward late gestation and the postpartum period but remained within physiological reference ranges. Progesterone concentrations were markedly higher in PR ewes from D35 onward ($p<0.05$) and were positively associated with litter size. These findings demonstrate stage-dependent metabolic adaptations and suggest that assessing pre-mating metabolic status and monitoring metabolism during late gestation may provide useful information for reproductive management, particularly in ewes carrying multiple fetuses.

Keywords: energy metabolism, ewe, litter size, progesterone, synchronization

Introduction

Blood biochemical parameters are widely used as diagnostic and prognostic tools in veterinary medicine and livestock production, providing valuable information on metabolic status, health, and reproductive performance (Van Saun 2023). In small ruminants, reproductive efficiency is closely linked to metabolic homeostasis, particularly during periods of increased physiological demand such as estrus synchronization, pregnancy, and the periparturient period (Matsuyama and Kimura 2015, Chaves et al. 2024) Pregnancy, although a physiological process, imposes substantial metabolic demands. These demands increase markedly due to fetal growth, placental development, and mammary gland maturation, especially during late gestation and the transition to lactation (Bauman and Currie 1980).

As pregnancy progresses, maternal nutrient partitioning increasingly favors the developing fetus and mammary tissue, often resulting in elevated metabolic demands and, in some cases, negative energy balance (Scaramuzzi et al. 2006). In ewes, this imbalance is particularly pronounced in animals carrying multiple fetuses and represents a major predisposing factor for pregnancy toxemia, a condition characterized by hypoglycemia, ketonemia, and excessive lipid mobilization (Sargison et al. 1994, Mongini and Van Saun 2023). Serum concentrations of glucose, non-esterified fatty acids, and ketone bodies such as beta-hydroxybutyrate are considered sensitive indicators of energy balance and are commonly used to assess metabolic stress in ruminants (Cascone et al. 2022).

The liver plays a central role in maintaining metabolic homeostasis during pregnancy by regulating gluconeogenesis, lipid metabolism, and hormone clearance (Sun et al. 2016). Aspartate aminotransferase is frequently used as an indirect indicator of hepatic metabolic load and tissue integrity (Campos-Gaona et al. 2024) and may increase in association with enhanced lipolysis, hepatic steatosis, or energy deficiency (Lubojacka 2005). In addition to metabolic parameters, reproductive hormones – particularly progesterone (P4) – are essential for the establishment and maintenance of pregnancy. Progesterone concentrations increase throughout gestation and are influenced by placental development, fetal number, and metabolic clearance rates (Ranilla et al. 1994, Al-Gubory et al. 1999, Rosales-Nieto et al. 2021). Low circulating P4 concentrations have been associated with impaired embryo development and reduced fertility (Al Jaryan et al. 2023), whereas increased feed intake may enhance hepatic blood flow and accelerate steroid metabolism, thereby reducing circulating P4 concentrations (Sangsritavong et al. 2002).

Given the close interaction between metabolism and reproduction, serum metabolic and hormonal parameters are expected to vary across different reproductive stages (González-García et al. 2014). However, most studies have focused on isolated physiological periods, and comprehensive longitudinal data spanning estrus synchronization, pregnancy, and the immediate postpartum period in clinically healthy ewes remain limited. Furthermore, comparative evaluations between pregnant and non-pregnant ewes and assessments of the influence of litter size under field conditions remain limited and somewhat controversial. Recent metabolomics-based studies have shown that blood metabolites may be useful for predicting sheep pregnancy and litter size or explaining treatment-related changes during early pregnancy. Goldansaz et al. (2022) identified serum metabolite panels for predicting pregnancy and litter size, particularly around Day 50 of gestation, whereas Zhang et al. (2024) reported that GnRH treatment after progesterone-based estrus synchronization altered peri-implantation metabolic pathways and was associated with a reduced pregnancy rate in ewes. However, these approaches are mainly based on high-throughput metabolomic platforms and are focused on early gestational prediction or peri-implantation mechanisms. Consequently, less is known about how routinely measurable metabolic and hormonal parameters change longitudinally from estrus synchronization to late gestation and the immediate postpartum period, particularly when pregnancy outcome and litter size are evaluated together under field conditions.

Therefore, the objectives of the present study were to evaluate longitudinal changes in selected serum metabolic and hormonal parameters from estrus synchronization through pregnancy and the immediate postpartum period, to compare pregnant and non-pregnant ewes, and to assess the influence of litter size on metabolic adaptation. We hypothesized that these parameters would differ between pregnant and non-pregnant animals, with the most pronounced alterations occurring during late gestation, particularly in multiple pregnancies.

Materials and Methods

Animals, management, and housing

The study was conducted between 2019 and 2021 on a semi-intensive sheep farm located in northern Türkiye. A total of 235 ewes (Tahirova, Merino, and Bafra breeds) and 12 fertile rams were maintained under uniform management conditions. Animals were housed indoors on straw bedding and had daily access to a fenced grazing area. The diet consisted of corn,

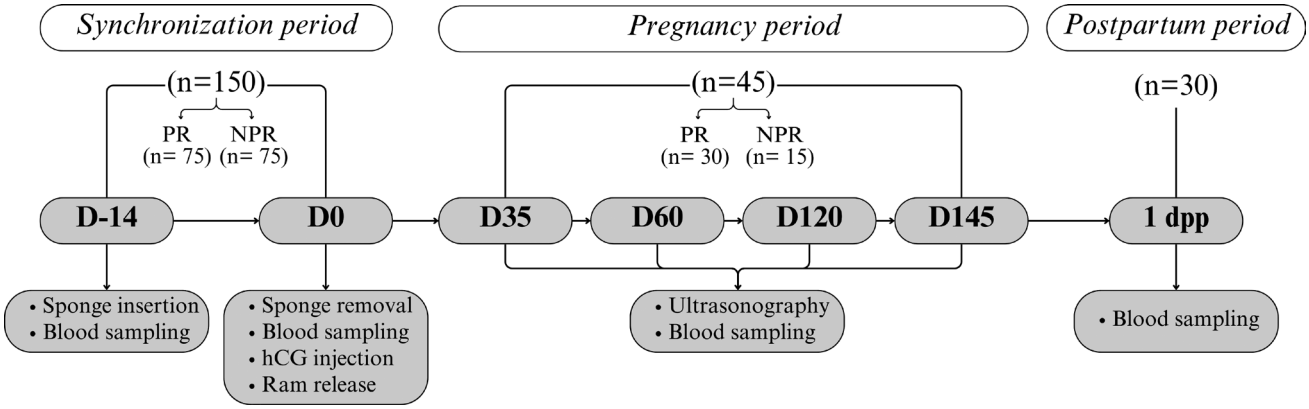

Fig 1. Schematic overview of the experimental design and animal selection throughout the study. From 235 synchronized ewes, pregnancy was confirmed in 160 animals, while 75 were classified as non-pregnant. Subsets of these populations were randomly selected for longitudinal biochemical monitoring during the synchronization and pregnancy periods. dpp: Day postpartum, PR: Pregnant, NPR: Non-pregnant.

crushed barley, corn silage, barley pulp, straw, grass silage, hay, and a commercial vitamin – mineral premix. Water and mineral licks were provided ad libitum.

Ewes included in the study were clinically healthy, multiparous animals between two and four years of age. All animals were maintained under identical housing, feeding, and management conditions throughout the study. Therefore, we minimized variation related to management, nutrition, age, and parity as much as possible before randomly selecting the study subgroups. Before enrollment, all animals underwent physical, gynecological, and ultrasonographic examinations, while rams were subjected to andrological evaluation. Animals showing clinical or reproductive abnormalities were excluded. This study involved non-experimental clinical veterinary practices; therefore, we did not seek ethical approval because it was not required under national regulations (HADMEK 15.02.2014/28914). We obtained informed consent from the farm owner.

Estrus synchronization and mating protocol

Flushing was implemented six weeks prior to sponge insertion through improved forage quality and supplementation with zinc, selenium, and vitamin E for both ewes and rams. A total of 235 ewes were synchronized during the breeding season (April–July) using intravaginal P4-impregnated sponges (60 mg medroxyprogesterone acetate; Esponjavet®, Hipra, Spain) inserted for 14 days (D-14). At sponge removal (D0), ewes received an intramuscular injection of 500 IU human chorionic gonadotropin (Oviser®, Hipra, Spain). Following sponge removal, rams were introduced for controlled natural mating. Each ewe was allowed to mate up to three times at 12-h intervals.

We confirmed pregnancy ultrasonographically in 160 ewes and classified 75 animals as non-pregnant. Due to logistical and economic constraints associated with large-scale longitudinal sampling, we used a random number table to select subsets of animals from the eligible study population, thereby minimizing selection bias and obtaining representative study subgroups. To obtain balanced experimental groups, we randomly selected 75 animals from the pregnant population. Accordingly, during the synchronization period, we collected blood samples from pregnant (PR; n=75) and non-pregnant (NPR; n=75) animals. During the pregnancy period, we collected blood samples from a subset consisting of PR (n=30) and NPR (n=15) animals (Fig. 1).

Among the pregnant ewes, litter size (singleton or multiple pregnancy) was determined based on fetal counts obtained during late gestation ultrasonographic examinations.

Pregnancy diagnosis and litter size determination

Pregnancy diagnosis was performed using transrectal ultrasonography (Hasvet 838®, Veterinary Ultrasound, equipped with a 7.5 MHz linear probe) at D35 and D60 post-mating. Ewes were classified as PR or NPR ewes based on the presence of an embryonic vesicle and detectable fetal heartbeat. Additional ultrasonographic examinations at D120 and D145 were performed to confirm pregnancy and determine litter size.

Blood sampling and laboratory analyses

Blood samples were collected at D-14 and D0 during the synchronization period, and at D35, D60, D120, and D145 during the pregnancy period. In addition, blood samples were obtained on Day 1 postpartum (1 dpp) from 30 ewes in the PR group. Samples were collected from the jugular vein into blood collection tubes with and without EDTA (Fig. 1). Blood samples were centrifuged at 3000 × g for 15 min at room tem-

perature. Serum was separated, divided into two or three aliquots, and stored at -20°C until analysis.

All analyses were performed according to the manufacturers' instructions. Serum glucose (RX series GL3815, Randox Laboratories Ltd., UK), serum NEFA (RX Monza, Randox Laboratories Ltd., UK), and serum BHB (RX Monza, Randox Laboratories Ltd., UK) concentrations and serum activities of AST (RX series AS3804, Randox Laboratories Ltd., UK) were measured using an automated biochemical analyzer (Randox RX Imola, Randox Laboratories Ltd., UK). The intra-assay and inter-assay coefficients of variation were <2% and <3% for glucose, <5% and <7% for NEFA, and <5% and <6% for BHB, <3% and <5% for AST, respectively. Serum P4 concentrations were determined using a chemiluminescent immunoassay (Architect i2000SR, Abbott Diagnostics, Ireland; kit code TK7K77). The intra-assay and inter-assay coefficients of variation for the assay were <5% and <8%, respectively.

Statistical analysis

We performed all statistical analyses using SPSS Statistics Version 27.0.1.0 (IBM Corp., USA). We recorded progesterone values below the detection limit (<0.1 ng/mL) as 0.1 ng/mL for statistical evaluation. We assessed the normality of the data distribution using the Shapiro–Wilk test. Because not all variables were normally distributed, we primarily used non-parametric methods and present numerical results as medians with interquartile ranges unless otherwise stated. For graphical presentation, we present the data as mean ± standard deviation (SD). We compared the PR and NPR groups using the Mann-Whitney U test and evaluated within-group differences across time points using the Wilcoxon signed-rank test. We analyzed differences in glucose concentrations according to litter size using two-way repeated-measures analysis of variance (ANOVA) within a general linear model after logarithmic transformation of the data, followed by Tukey's post hoc test. We analyzed differences in AST, NEFA, BHB, and P4 concentrations according to litter size during the synchronization and pregnancy periods using the Kruskal-Wallis test. We considered $p<0.05$ statistically significant. Although linear mixed-effects models are generally recommended for repeated-measures data, we did not apply them because the study included relatively small longitudinal subgroup sizes, non-normally distributed variables, unequal group sizes, and postpartum measurements that were available only for pregnant ewes. Therefore, we considered separate non-parametric analyses the most appropriate analytical approach for the structure and distribution of the available data.

Results

A total of 122 lambs were born from 75 ewes. Of these, 49.3% (37/75) were single births, 38.7% (29/75) were twin births, and 12.0% (9/75) were triplet births.

Serum glucose concentrations

Serum glucose concentrations exhibited significant temporal variation across the study period ($p<0.05$). During the synchronization period, glucose concentrations at D-14 were significantly higher in PR ewes compared with NPR ewes ($p<0.05$), whereas no significant difference was observed between groups at D0 (Fig. 2A).

In PR ewes, glucose concentrations gradually declined from early to late pregnancy, reaching the lowest values at D145. A slight increase was observed on 1 dpp; however, glucose concentrations remained lower than those recorded during the synchronization period. In contrast, NPR ewes showed relatively stable glucose concentrations throughout the study period (Fig. 2A).

When PR ewes were evaluated according to litter size, those carrying multiple fetuses exhibited lower glucose concentrations during late gestation (D120 and D145) compared with singleton pregnancies; however, these differences were not statistically significant (Fig. 2B).

Serum non-esterified fatty acids concentrations

Serum NEFA concentrations remained within physiological reference ranges during the synchronization period and early pregnancy in both NR and NPR ewes. No significant differences were observed between groups at D-14, D0, or D35 (Fig. 2C).

From mid-pregnancy onwards, NEFA concentrations increased progressively in PR ewes, with the highest values observed during late gestation (D120 and D145). These increases were significantly higher compared with early pregnancy values ($p<0.05$) (Fig. 2C).

Ewes carrying multiple fetuses showed significantly elevated NEFA concentrations during late gestation compared with singleton pregnancies ($p<0.05$). In contrast, NEFA concentrations in NPR ewes remained relatively stable throughout the study period (Fig. 2D).

Serum beta-hydroxybutyrate concentrations

Serum BHB concentrations showed a pattern similar to that observed for NEFA. During the synchronization period and early pregnancy, BHB concentrations were low and comparable between PR and NPR ewes (Fig. 2E).

In PR ewes, BHB concentrations increased signifi-

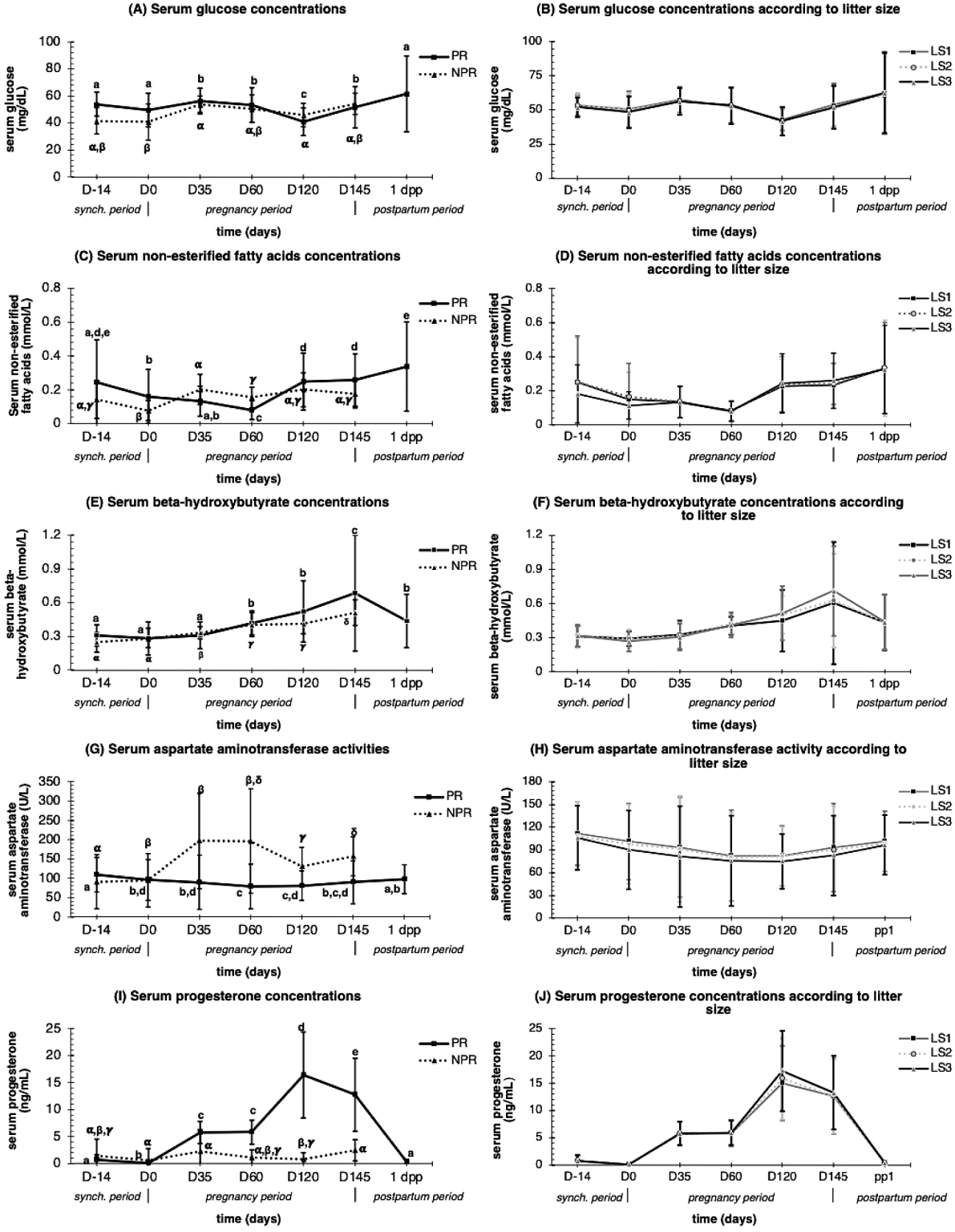

Fig 2. Temporal changes in metabolic parameters and progesterone concentrations during the synchronization, pregnancy, and postpartum periods. Panels A, C, E, G, and I show comparisons between pregnant (PR) and non-pregnant (NPR) ewes, whereas panels B, D, F, H, and J present data according to litter size (LS1–LS3). Values are expressed as mean ± SD. Different lowercase letters $^{(a–e)}$ and Greek letters $^{(\alpha–\varepsilon)}$ indicate significant temporal differences within the PR and NPR groups, respectively ($p<0.05$).

cantly during late pregnancy (D120 and D145; $p<0.05$), with the highest values observed at D145 (Fig. 2E).

Ewes carrying multiple fetuses exhibited significantly higher BHB concentrations during late gestation compared with singleton pregnancies ($p<0.05$). Postpartum BHB concentrations decreased compared with late gestation but remained higher than early pregnancy values (Fig. 2F).

Serum aspartate aminotransferase activities

Serum AST activities showed moderate fluctuations throughout the study period. No significant differences between PR and NPR ewes were observed during the synchronization period or early pregnancy (Fig. 2G).

In PR ewes, AST activities increased slightly during late gestation and reached peak values on 1 dpp. This postpartum increase was statistically significant compared with mid-pregnancy values ($p<0.05$). However, AST activities remained within established reference ranges throughout the study period (Fig. 2H).

Serum progesterone concentrations

Serum P4 concentrations differed markedly between PR and NPR animals throughout the study. During the synchronization period, no significant differences were observed between groups (Fig. 2I).

From D35 onwards, P4 concentrations were significantly higher in PR ewes compared with NPR ewes at all sampling points ($p<0.001$). In pregnant ewes, P4 concentrations increased progressively, reaching peak levels at D120, followed by a decline at D145 and a sharp decrease 1 dpp (Fig. 2I).

Ewes carrying multiple fetuses exhibited significantly higher P4 concentrations at D60 and D120 compared with singleton pregnancies ($p<0.05$). In GNP ewes, P4 concentrations showed variable patterns with intermittent elevations but remained consistently lower than those observed in PR ewes (Fig. 2J).

Discussion

The present study provides a longitudinal evaluation of metabolic and hormonal changes from estrus synchronization through pregnancy and immediate postpartum period in ewes under field conditions. Although several studies have described metabolic alterations during specific stages of gestation, longitudinal data covering the period from synchronization to late pregnancy remain limited. The findings support the hypothesis that metabolic status differs between PR and NPR animals and that the metabolic demands of gestation – particularly during late pregnancy and in multiple pregnancies – are associated with measurable changes in serum metabolic and hormonal parameters.

These findings complement recent metabolomics-based studies on sheep pregnancy and litter size. Goldansaz et al. (2022) showed that serum metabolite panels, including D-glucose, L-carnitine, methanol, L-arginine, and urea for pregnancy detection and methionine and L-carnitine for litter size prediction, could classify pregnancy status and fetal number most effectively around Day 50 of gestation. In the present study, we did not aim to develop predictive metabolomic models; however, the higher pre-mating glucose concentrations in ewes that subsequently became pregnant and the greater late-gestation NEFA and BHB responses in multiple pregnancies support the concept that pregnancy outcome and fetal number are reflected in maternal circulating metabolic profiles. Similarly, Zhang et al. (2024) reported that metabolic alterations during the peri-implantation period after P4-based estrus synchronization were associated with pregnancy outcome in ewes. Unlike these metabolomics-based studies, the present work extends the interpretation to routinely measurable metabolic and hormonal parameters monitored longitudinally from synchronization through late gestation and 1 dpp under field conditions.

Pre-mating metabolic status and pregnancy outcome

One of the most notable findings of the present study was the significantly higher glucose concentrations at D-14 in ewes that subsequently became pregnant. This observation suggests that pre-mating energy status, particularly carbohydrate availability, may be associated with pregnancy establishment. Adequate glucose availability is essential for follicular development, oocyte competence, and early embryonic survival, and improved energy balance before breeding has been associated with higher conception rates in small ruminants (Scaramuzzi et al. 2006, Dupont et al. 2014). In line with this interpretation, Goldansaz et al. (2022) included D-glucose in a validated pregnancy-associated serum biomarker panel, further supporting the relevance of carbohydrate metabolism to pregnancy outcome. However, whereas their predictive model was based on Day 50 samples, the present study detected this difference before mating, suggesting that pre-breeding glucose status was associated with subsequent pregnancy establishment. Although NEFA and BHB concentrations did not differ between groups during the synchronization period, their low and stable values indicate that the animals were not experiencing negative energy balance at the time of mating. These findings suggest that relatively subtle differences in carbohydrate metabolism, rather than pronounced lipid mobilization, may be more closely associated with subsequent pregnancy establishment during the peri-conception period in ewes. This may indicate that moderate variations in energy availability before breeding are initially reflected in carbohydrate metabolism. In contrast, markers of lipid mobilization such as NEFA and BHB typically increase only when a more pronounced negative energy balance develops (Bouvier-Muller et al. 2016).

Metabolic adaptations during pregnancy

As pregnancy progressed, significant increases in NEFA and BHB concentrations were observed in PR ewes, supporting the hypothesis that gestation is associated with increased metabolic demand (Ji et al. 2023). These changes reflect enhanced lipolysis and ketogenesis in response to the growing energy requirements of the fetus and the reduction in ruminal capacity caused by uterine expansion (Sargison et al. 1994, Cascone et al. 2022, Mongini and Van Saun 2023).

The more pronounced increases observed in ewes carrying multiple fetuses highlight the additional metabolic burden imposed by higher fetal numbers. This observation is consistent with previous studies indicating that litter size is a major determinant of metabolic stress and a key risk factor for pregnancy toxemia in small ruminants (Sargison et al. 1994, González-García et al. 2014, Cascone et al. 2022). Multiple pregnancies substantially increase fetal energy requirements, which can intensify maternal lipid mobilization during late gestation. Despite the increase in ketone body concentrations, no clinical signs of pregnancy toxemia were observed in the present study, suggesting that the animals were able to maintain metabolic adaptation under the management conditions applied. These findings are consistent with greater maternal metabolic adaptation during late gestation, particularly in multiple pregnancies, as gestational metabolic demands increase.

Glucose dynamics during gestation

In contrast to NEFA and BHB, serum glucose concentrations showed only moderate variation during pregnancy and did not differ significantly between PR and NPR animals after mating. The gradual decline in glucose concentrations toward late gestation likely reflects increased fetal glucose utilization rather than impaired maternal glucose regulation, which is consistent with previous reports in gestating ewes (Dupont et al. 2014, Cascone et al. 2022). These findings indicate that glucose alone may have limited diagnostic value for detecting negative energy balance during pregnancy and emphasize the importance of evaluating multiple metabolic indicators simultaneously (Mongini and Van Saun 2023).

Although glucose concentrations tended to be lower in ewes carrying multiple fetuses during late gestation, the differences were not statistically significant. This may reflect the strong homeostatic regulation of circulating glucose in ruminants, where increased fetal glucose demand is often compensated by enhanced maternal gluconeogenesis.

Hepatic metabolic response

Serum AST activity showed mild increases during late pregnancy and the immediate postpartum period but remained within physiological reference ranges. This suggests that hepatic metabolic capacity was largely maintained despite the increased metabolic demands associated with gestation. The postpartum rise in AST activity may be related to physiological stress associated with parturition and the onset of lactation rather than to pathological liver damage, as reported in previous studies in small ruminants (Lubojacka et al. 2005, Onasanya et al. 2015, Singh et al. 2022).

The absence of a significant effect of litter size on AST activity further suggests that, in clinically healthy ewes receiving adequate nutritional support, hepatic adaptation to pregnancy-associated metabolic demands is generally well regulated.

Progesterone dynamics and reproductive status

Progesterone dynamics clearly differentiated PR from NPR ewes from D35 onward, supporting its role in pregnancy maintenance rather than early diagnosis. Consistent with previous reports, P4 concentrations remain lower in non-pregnant ewes than in pregnant animals (Al Mousawe et al. 2024, Ozturan et al. 2025), while the progressive increase during gestation reflects sustained luteal activity and placental steroidogenesis (Ranilla et al. 1994, Al-Gubory et al. 1999, Rosales-Nieto et al. 2021).

Higher P4 concentrations observed in multiple pregnancies are consistent with the increased placental mass and endocrine activity associated with larger fetal numbers. Similar patterns have been reported in small ruminants Ranilla et al. (1994) and Rosales-Nieto et al. (2021), with differences becoming more pronounced at Days 40, 60, and 80 of gestation (Gur et al. 2011, Ozturan et al. 2025). However, variability in NPR ewes likely reflects physiological luteal activity rather than pregnancy-specific endocrine patterns. This finding underscores the limited diagnostic value of isolated P4 measurements for early pregnancy diagnosis and highlights the importance of ultrasonographic confirmation (Kaçar et al. 2021).

Progesterone metabolism is also influenced by nutritional status and hepatic blood flow. Increased feed intake may enhance hepatic blood flow and accelerate steroid metabolism, thereby affecting circulating P4 concentrations (Sangsritavong et al. 2002).

Metabolic reproductive interactions

Overall, the present findings indicate that metabolic and hormonal regulation from estrus synchronization

through gestation and the immediate postpartum period are closely interconnected in ewes. While clinically healthy animals appear capable of adapting to the metabolic demands of pregnancy, late gestation – particularly in multiple pregnancies – represents a period of increased metabolic load characterized by enhanced lipid mobilization, elevated ketone body concentrations, and sustained endocrine activity.

The observed associations between litter size and several metabolic parameters are consistent with fetal number being an important factor associated with maternal metabolic adaptation during gestation. Increased fetal demand associated with multiple pregnancies may amplify maternal energy requirements and promote metabolic adjustments aimed at maintaining fetal growth and pregnancy maintenance.

An additional strength of the present study is the longitudinal design spanning estrus synchronization, pregnancy, and the immediate postpartum period, allowing metabolic and hormonal adaptations to be evaluated across multiple reproductive stages under field conditions.

Despite these strengths, we acknowledge several limitations. First, we analyzed repeated measurements using separate non-parametric tests rather than linear mixed-effects models that explicitly account for within-animal correlations. Consequently, our analyses may not have fully accommodated correlations among repeated observations, and readers should consider this limitation when interpreting the results. Second, although we randomly selected the longitudinal subgroup from the eligible study population, it represented only a subset of the original cohort, which may have reduced the statistical power of some longitudinal comparisons. Third, we included animals from different breeds in the study population but could not evaluate potential breed-related variation in the statistical analysis. Although we maintained all animals under identical management and feeding conditions and selected animals of similar age and parity, we did not record body condition score or individual feed intake. We therefore could not include these variables in the analyses. Accordingly, we cannot completely exclude residual confounding related to breed or individual nutritional status. Finally, although we analyzed several metabolic parameters, we did not apply adjustments for multiple comparisons. Therefore, readers should interpret our findings within the context of these methodological considerations and the observational design of the study, which precludes conclusions regarding causality or predictive performance.

Conclusion

This study demonstrates pronounced longitudinal changes in metabolic and hormonal parameters from estrus synchronization through pregnancy and the immediate postpartum period in ewes. In particular, the progressive elevation of NEFA and BHB concentrations during late gestation – especially in ewes carrying multiple fetuses – reflects the substantial metabolic demands associated with advanced pregnancy.

The observation that ewes with higher pre-mating glucose concentrations had higher pregnancy rates indicates an association between pre-breeding metabolic status and subsequent pregnancy establishment. However, given the observational design of the present study, these findings should not be interpreted as evidence of causality or predictive performance. Progesterone dynamics clearly differentiated PR from NPR ewes during gestation and were influenced by litter size, consistent with greater endocrine activity associated with multiple pregnancies.

Collectively, these findings support the potential value of optimized nutritional management before breeding and metabolic monitoring during late gestation and may provide useful information for reproductive management in sheep production systems.

Author Declarations

Ethics approval

As this study involved non-experimental clinical veterinary practices, ethical approval was not required under national regulations (HADMEK 15.02.2014/28914). Informed consent was obtained from the farm owner.

Use of generative artificial intelligence

Generative artificial intelligence was used only for language editing. All scientific content and interpretations are the responsibility of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Al-Gubory KH, Solari A, Mirman B **(1999)** Effects of luteectomy on the maintenance of pregnancy, circulating progesterone concentrations and lambing performance in sheep. Reprod Fertil Dev 11: 317-322.

Al-Jaryan IL, Al-Thuwaini TM, Merzah LH, Alkhammas AH

(2023) Reproductive physiology and advanced technologies in sheep reproduction. Rev Agric Sci 11: 171-180.

Al-Mousawe A, Ibrahim N **(2024)** Diagnosis of pregnancy in Iraqi Awassi ewes through progesterone hormone measurement and ultrasonography following induction of fertile estrus with sulpiride. Egypt J Vet Sci 55: 945-953.

Bauman DE, Currie WB **(1980)** Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci 63: 1514-1529.

Bouvier-Muller J, Allain C, Enjalbert F, Tabouret G, Portes D, Caubet C, Tasca C, Foucras G, Rupp R **(2016)** Response to dietary-induced energy restriction in dairy sheep divergently selected for resistance or susceptibility to mastitis. J Dairy Sci 99: 480-492.

Campos-Gaona R, Correa-Orozco A, Salamanca-Carreño A, Vélez-Terranova M **(2024)** Index development to comprehensive assess liver function during the dairy cows' transition period in low-tropic conditions. Animals 14(14): 2056.

Cascone G, Licitra F, Stamilla A, Amore S, Dipasquale M, Salonia R, Antoci F, Zecconi A **(2022)** Subclinical ketosis in dairy herds: impact of early diagnosis and treatment. Front Vet Sci 9: 895468.

Chaves AS, Silva F, Valentim R, Quintas H **(2024)** Body condition in small ruminants – effects of nutrition on the hypothalamic–pituitary–gonad axis and ovarian activity that controls reproduction. Physiologia 4: 213-225.

Dupont J, Scaramuzzi RJ, Reverchon M **(2014)** The effect of nutrition and metabolic status on the development of follicles, oocytes and embryos in ruminants. Animal 8: 1031-1044.

Goldansaz SA, Markus S, Plastow G, Wishart DS **(2022)** Predictive blood biomarkers of sheep pregnancy and litter size. Sci Rep 12: 10307.

González-García E, De Figuereido VG, Foulquie D, Jousserand E, Autran P, Camous S, Tesniere A, Bocquier F, Jouven M **(2014)** Circannual body reserve dynamics and metabolic profile changes in Romane ewes grazing on rangelands. Domest Anim Endocrinol 46: 37-48.

Gur S, Turk G, Demirci E, Yuce A, Sonmez M, Ozer S, Aksu E **(2011)** Effect of pregnancy and foetal number on diameter of corpus luteum, maternal progesterone concentration and oxidant/antioxidant balance in ewes. Reprod Domest Anim 46: 289-295.

Ji X, Liu N, Wang Y, Ding K, Huang S, Zhang C **(2023)** Pregnancy toxemia in ewes: a review of molecular metabolic mechanisms and management strategies. Metabolites 13: 149.

Kaçar C, Kaya S, Kuru M, Erkılıç EE, Öğün M, Oral H, Demir MC **(2021)** Determination of natural antibodies, beta-hydroxybutyric acid, and non-esterified fatty acid levels in the serum of peripartum Tuj and Hemşin sheep. Vet World 14: 1002-1006.

Lubojacka V, Pechova A, Dvořák R, Drastich P, Kummer V, Poul J **(2005)** Liver steatosis following supplementation with fat in dairy cow diets. Acta Vet Brno 74: 217-224.

Matsuyama S, Kimura K **(2015)** Regulation of gonadotropin secretion by monitoring energy availability. Reprod Med Biol 14: 39-47.

Mongini A, Van Saun RJ **(2023)** Pregnancy toxemia in sheep and goats. Vet Clin North Am Food Anim Pract 39: 275-291.

Onasanya GO, Oke FO, Sanni TM, Muhammad AI **(2015)** Parameters influencing haematological, serum and bio-chemical references in livestock animals under different management systems. Open J Vet Med 5: 181-189.

Ozturan HG, Aslan S, Zabitler Tepik F, Darbaz I, Sayiner S, Wehrend A **(2025)** Concentrations of progesterone (p4), anti-müllerian hormone (amh), and haptoglobin (hp) in pregnant and non-pregnant ewes and their association with fetal mortality, maternal weight, and twinning rate. Vet Sci 12: 463.

Ranilla MJ, Sulon J, Carro MD, Mantecón AR, Beckers JF **(1994)** Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in churra and merino sheep. Theriogenology 42: 537-545.

Rosales-Nieto CA, Ehrhardt R, Mantey A, Makela B, Byrem, Veiga-Lopez A **(2021)** Preconceptional diet manipulation and fetus number can influence placenta endocrine function in sheep. Domest Anim Endocrinol 74: 106577.

Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC **(2002)** High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J Dairy Sci 85: 2831-2842.

Sargison ND, Scott PR, Penny CD, Pirie RS, Kelly JM **(1994)** Plasma enzymes and metabolites as potential prognostic indices of ovine pregnancy toxaemia – a preliminary study. Br Vet J 150: 271-277.

Scaramuzzi RJ, Campbell BK, Downing JA, Kendall NR, Khalid M, Muñoz-Gutiérrez M, Somchit A **(2006)** A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reprod Nutr Dev 46: 339-354.

Singh R, Singh A, Beigh SA, Sharma N, Singh V **(2022)** Effect of physiological status and parity on metabolic and trace element profile of crossbred Rambouillet sheep of Himalayan region. Trop Anim Health Prod 54: 63.

Sun F, Cao Y, Cai C, Li S, Yu C, Yao J **(2016)** Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PLoS One 11: e0160659.

Van Saun RJ **(2023)** Metabolic profiling in ruminant diagnostics. Vet Clin North Am Food Anim Pract 39: 49-71.

Zhang J, Sun S, Bai X, Yang N, Liu Y, Wu X, Li X **(2024)** Metabolomics analysis of the effect of GnRH on the pregnancy rate of ewes with estrus synchronization scheme based on progesterone. Front Vet Sci 11: 1442931.