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Effects of Puerperal Metritis on Uterine Blood Flow, Acute Phase Proteins and Selected Biochemical Parameters in Aleppo Goats

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ABSTRACT The aim of this study was to determine the effects of puerperal metritis on uterine blood flow, acute-phase proteins, and certain biochemical parameters in Aleppo goats. In the study, researchers divided 30 multiparous Aleppo goats, which were in the first 10 days postpartum and had not been exposed to any medical treatment, into two equal groups: Group 1 (n=15) included goats with a healthy uterus throughout the postpartum period, whereas Group 2 (n=15) consisted of goats diagnosed with puerperal metritis. Before any intervention, Pulsatility Index (PI) and Resistance Index (RI) were measured using Doppler ultrasonography. Blood samples were then collected to analyze White Blood Cell (WBC) count, Interleukin-6 (IL-6), Tumor Necrosis Factor-Alpha (TNF-α), Serum Amyloid A (SAA), Procalcitonin (PCT), Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase (GGT), urea, and creatinine levels. The obtained data were evaluated using an independent samples t-test. Body temperature, respiratory rate, heart rate, and WBC values were significantly higher in the puerperal metritis group compared to the control group ($p<0.001$). In goats with metritis, comparison with the control group revealed that PI and RI values were low ($p<0.001$), and IL-6, TNF-α, SAA, PCT, AST, GGT, urea and creatinine levels were high ($p<0.001$). In conclusion, puerperal metritis induces substantial alterations in uterine blood flow and results in marked variations in acute-phase proteins and biochemical markers in goats.

Keywords: *Doppler, Goat, Metritis.*

ÖZ

Halep Keçilerinde Puerperal Metritisin Uterus Kan Akımı, Akut Faz Proteinleri ve Seçilmiş Biyokimyasal Parametreler Üzerine Etkileri

Bu çalışmanın amacı, Halep keçilerinde puerpreal metritisin uterus kan akımı, akut faz proteinleri ve bazı biyokimyasal parametreler üzerine etkilerini belirlemektir. Çalışmada, postpartum ilk 10 gün içerisinde bulunan ve herhangi medikal tedaviye maruz kalmamış multipar 30 Halep keçisi eşit iki gruba ayrıldı: Grup 1 (n=15), doğum sonrası dönemde sağlıklı uterusa sahip keçileri içerirken, Grup 2 (n=15), doğum sonrası puerperal metritis tanısı koyulan keçilerden oluşturuldu. Herhangi bir uygulama yapılmadan önce, Doppler ultrasonografi muayenesi yapılarak Pulsatilite İndeksi (PI) ve Rezistans İndeksi (RI) ölçüldü. Daha sonra Beyaz Kan Hücresi (WBC) sayımı, İnterlökin-6 (IL-6), Tümör Nekroz Faktör Alfa (TNF-α), Serum Amiloid A (SAA), Prokalsitonin (PCT), Aspartat Aminotransferaz (AST), Gama-Glutamil Transferaz (GGT), üre ve kreatinin düzeylerini analiz etmek için kan örnekleri toplandı. Elde edilen veriler bağımsız örnekler t-testi kullanılarak değerlendirildi. Vücut sıcaklığı, solunum hızı, kalp hızı ve WBC değerleri puerperal metritis grubunda kontrol grubuna kıyasla anlamlı derecede yüksek belirlendi ($p<0.001$). Metritisli keçilerde kontrol grubuna kıyasla PI ve RI değerlerinin düşük ($p<0.001$), IL-6, TNF-α, SAA, PCT, AST, GGT, üre ve kreatinin düzeylerinin yüksek olduğu belirlendi ($p<0.001$). Sonuç olarak puerperal metritisin keçilerde uterus kan akımında önemli değişikliklere neden olduğu ve akut faz proteinleri ile biyokimyasal belirteçlerde belirgin varyasyonlara yol açtığı görüldü.

Anahtar Kelimeler: *Doppler, Keçi, Metritis.*

INTRODUCTION

Puerperal metritis is characterized by inflammation of the uterus (Kurt et al. 2019) and is among the most prevalent reproductive illnesses in goats (Kulsum et al. 2020). The principal etiology of the condition is uterine infection resulting from dystocia and retained placenta (Majeed 1994). Puerperal metritis is an important cause of infertility and has been associated with decreased milk production, increased culling rates, and economic losses

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(Majeed 1994; Sayeed et al. 2020). The incidence of the disease in goats brought to the hospital was recorded as 23.4% (Kulsum et al. 2020), although the prevalence is 4.5% (Sayeed et al. 2020). Puerperal metritis is clinically defined by elevated body temperature and the occurrence of a malodorous, red-brown, watery vaginal discharge (Kurt et al. 2019).

The use of Doppler ultrasound to monitor uterine involution in postpartum goats allows the assessment of both uterine size (morphometry) and blood flow parameters such as resistance and pulsatility indices, blood flow volume, and blood flow velocity. These measurements provide detailed information about the progression of uterine involution and the restoration of normal uterine blood flow (Madboulyn et al. 2024). Elmetwally and Bollwein (2017) revealed that Doppler ultrasonography effectively assesses postpartum uterine hemodynamic alterations in sheep and goats. Additionally, they stated that changes in uterine blood flow during the postpartum period could be an important parameter for evaluating the completion of uterine involution and the resumption of ovarian follicular activity. Blood biochemical analyses are commonly used in general health assessments, as they provide crucial information about an animal's nutritional status, health condition, and well-being (Faraz et al. 2019). The severity of tissue damage can be assessed by deviations of specific blood parameters from their normal ranges (Abebe et al. 2002).

Cytokines belong to a class of soluble proteins with the capacity to regulate cellular and tissue functions. They are locally produced, have a short half-life, and exert their effects through autocrine, paracrine, or endocrine pathways (Khalid et al. 2015). IL-6, a pro-inflammatory cytokine, is released by macrophages and T lymphocytes, attaching to receptors on hepatocytes and functioning as a powerful pyrogen that induces the synthesis of acute-phase proteins (APP) (Petersen et al. 2004). Serum cytokine concentrations can be analyzed to evaluate the innate immune response during infection or inflammation. Acute-phase proteins are a category of blood proteins, predominantly synthesized by the liver, that contribute to safeguarding infected organisms from pathological harm, preserving homeostasis, and inhibiting antibody-independent microbial proliferation. The concentrations of acute-phase proteins may be affected by several pathogenic (including viral and non-infectious disorders) and physiological (such as food, age, sex, pregnancy, lactation, and environmental factors) variables (Nazifi et al. 2012). Since they are associated with disease severity, they can be considered prognostic biomarkers and serve as an important criterion for monitoring herd health (Miglio et al. 2018).

This study aims to investigate the effects of puerperal metritis on uterine blood flow, acute-phase proteins, and certain biochemical parameters in Aleppo goats.

MATERIAL AND METHODS

This study was approved by the Local Ethics Committee for Animal Experiments of Harran University under permit number 2025/003/10.

Selection of Animals and Experimental Protocol

This randomized controlled clinical study included 30 goats that met our study criteria, selected from a total of 458 Aleppo goats brought to the Obstetrics and Gynecology Clinic of the Animal Hospital at Harran University, Faculty of Veterinary Medicine. Our study was conducted over a three-year period (2022-2025). During this period, a total of 458 goats were examined during their postpartum period. The majority of the examined goats were brought for routine postpartum examinations within the first 10 days. These examinations were conducted in a planned manner to ensure early detection of potential postpartum complications. Clinical metritis was detected in 458 goats (n=30) when forming the metritis group. Of these animals, those meeting the study criteria were included in the puerperal metritis group. Examination of the control and study groups was conducted in January–February and March–April. To minimize the influence of seasonal factors, both groups were examined during the same periods. The goats used in the study were between 2 and 4 years old and had similar body condition scores. The goats used in the study were selected from goats that had previously given birth normally and had given birth to singletons. Goats that were at most 10 days (7±3) postpartum and had not received any postpartum treatment were included in the study. The goats were divided into two groups according to whether they developed puerperal metritis. The first group (Group 1, n=15) consisted of goats with healthy uteruses in the postpartum period, and the second group (Group 2, n=15) consisted of goats that developed puerperal metritis. The medical history revealed that all goats in the puerperal metritis group underwent dystocia intervention. The distinction between goats with puerperal metritis and healthy goats was made based on clinical findings, ultrasonographic examination, and vaginal discharge characteristics. The healthy goat group used in the study was selected from the healthy goats brought to the clinic for routine postpartum checkups. All goats selected for the control and puerperal metritis groups were referred to the Internal Medicine Clinic for evaluation of comorbidities after genital examination. Goats with any other health problems, a history of illness lasting longer than 48 hours, or those in hypothermia or coma were excluded from the study. Goats in the puerperal metritis group received 3 mL of intramuscular (IM) antibiotics (Synulox/Zoetis® containing 140 mg amoxicillin trihydrate and 35 mg clavulanic acid per mL) for 5 days, 5 mL of vitamins (Nervit Composite/Vetas® containing 100 mg vitamin B_1 and 10 mg vitamin B_6 per mL) for 5 days, a single dose of 2 mL PGF2α (10 mg Dinoprost tromethamine, Dinolytic, Pfizer®), and fluid therapy for 3 days. All goats regained their health at the end of the treatments.

Doppler Ultrasound Examination

Uterine artery Doppler examination was performed transrectally. The goats were restrained in a standing position before the transrectal scanning evaluations, and no sedative medication was administered. Ultrasonographic examination was conducted in a quiet environment by the same experienced operator using a linear transducer (5–10 MHz) (Sonosite Edge II, USA). For the anatomical assessment of the uterine artery, the urinary bladder was first identified using B-mode ultrasound, followed by differentiation of the uterine tissue, and the uterine artery to be measured was identified using color Doppler. Blood flow velocity waveforms were obtained from the proximal portion of the uterine artery (before bifurcation) after mapping the orientation of the iliac vessels (Elmetwally et al. 2016). Pulsed Doppler ultrasonography of the uterine artery was performed to determine the Pulsatility Index (PI) and Resistance Index (RI). These parameters were automatically calculated by the Doppler device software

using mathematical formulas or the Pourcelot index. Measurements were recorded after obtaining a minimum of three consecutive healthy arterial waveforms. To minimize potential thermal and cavitation effects during Doppler ultrasonography, an interval of approximately 60 seconds was allowed between each 30-second measurement. The entire examination was completed within 5 minutes.

Collection of Blood Samples and Laboratory Analysis

After the Doppler ultrasound examination of all the goats used in the study, blood samples were collected from the jugular vein into tubes without anticoagulants. The collected blood samples were centrifuged at 3000 rpm for 10 minutes for separating the serum. The obtained serum samples were stored at -20 °C until the day of analysis. The levels of AST, GGT, urea, and creatinine in the blood serum collected from the goats in the study groups were determined using the Seamaty SMT-120V (Notavet, Izmir, Türkiye) biochemical analyzer. Serum Amyloid A level (Goat Serum Amyloid A (SAA) ELISA Kit, MBS262875, Mybiosource, USA), Interleukin 6 level (Goat Interleukin 6 (IL6) ELISA Kit, MBS2021391, Mybiosource, USA), Tumor Necrosis Factor-alpha level (Goat Tumor Necrosis Factor Alpha (TNF-alpha) ELISA Kit, MBS263127, Mybiosource, USA), and Procalcitonin level (Goat Procalcitonin (PCT) ELISA Kit, MBS260198, Mybiosource, USA) were measured using commercial kits.

Statistical Analysis

Statistical analyses were conducted utilizing SPSS version 26 software. The distribution normality of data was evaluated by visual methods (histograms and probability plots) and analytical techniques (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive analyses were conducted utilizing mean and standard error for variables exhibiting a normal distribution. Given that all parameters had a normal distribution, group comparisons were conducted using the independent samples t-test. The overall error margin for statistical significance was established at 5%.

RESULTS

The vital signs and WBC values of healthy and puerperal metritis goats are presented in Table 1. Significant differences were observed between the groups in body temperature, respiratory rate, heart rate, and WBC values ($p<0.001$). Body temperature, respiratory rate, heart rate, and WBC values were higher in the puerperal metritis group compared to the control group ($p<0.001$).

Table 1: Mean vital signs and WBC values of healthy and puerperal metritis goats (Mean±Standard error of mean).

	Control (n=15) $\bar{X} \pm SEM$	Metritis (n=15) $\bar{X} \pm SEM$	p value
Temperature (°C)	38.7±0.4[a]	40.5±0.11[b]	<0.001
HR (min)	74.06±0.62[a]	88.40±1.03[b]	<0.001
RR (min)	16.8±0.47[a]	26.86±0.61[b]	<0.001
WBC ($10^3/\mu L$)	8.15±0.14[a]	21.53±0.78[b]	<0.001

[a,b] Different letters in the same line indicate a statistically significant difference. Heart rate (HR), Respiratory rate (RR), White blood cells (WBC).

The Doppler ultrasound measurement values of healthy and puerperal metritis goats are presented in Table 2, Figure 1 and Figure 2 Significant differences were observed between the groups in PI and RI values ($p<0.001$). The Pulsatility Index and RI values were lower in the puerperal metritis group compared to the control group ($p<0.001$).

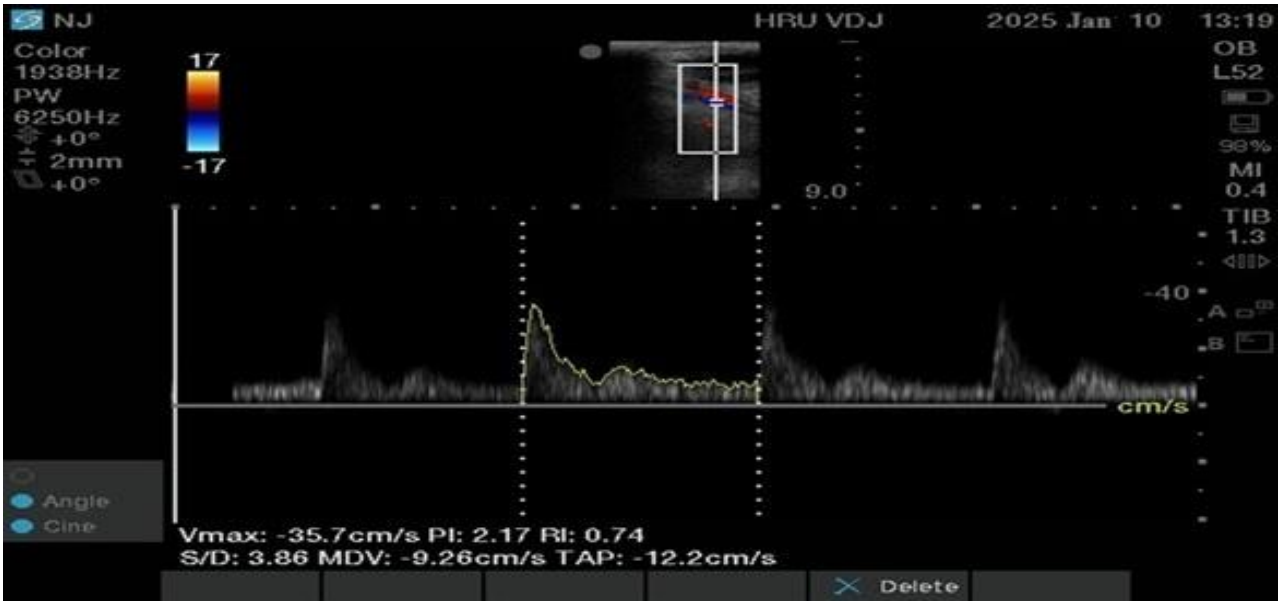

Figure 1: Uterine artery Doppler image of a goat with a healthy uterus on day 4 postpartum. PI value: 2.17, RI value: 0.74.

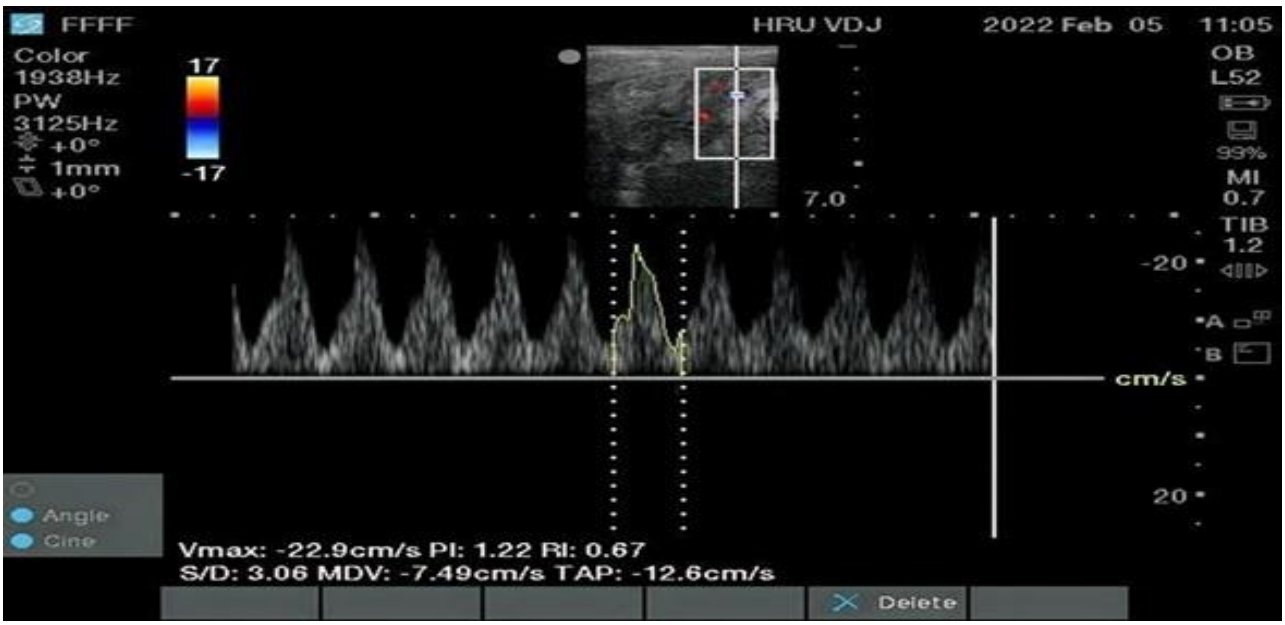

Figure 2: Uterine artery Doppler image of a goat with puerperal metritis on day 3 postpartum. PI value: 1.22, RI value: 0.67.

Table 2: Mean Doppler ultrasound measurement values of healthy and puerperal metritis goats (Mean±Standard error of mean).

	Control (n=15) $\overline{X} \pm$ SEM	Metritis (n=15) $\overline{X} \pm$ SEM	p value
PI	2.03±0.023[a]	1.58±0.025[b]	<0.001
RI	0.81±0.019 [a]	0.59±0.015[b]	<0.001

[a,b] Different letters in the same line indicate a statistically significant difference. Pulsatility Index (PI), Resistance Index (RI).

Table 3: Mean proinflammatory cytokine, acute phase proteins and biochemical parameter values of healthy and puerperal metritis (Mean±Standard error).

	Control (n=15) $\overline{X} \pm$ SEM	Metritis (n=15) $\overline{X} \pm$ SEM	p value
IL-6 (pg/mL)	18.25±0.33[a]	69.96±1.58[b]	<0.001
TNF-α (pg/mL)	7.51±0.13[a]	21.12±0.59[b]	<0.001
SAA (µg/mL)	7.64±0.13[a]	80.92±2.27 [b]	<0.001
PCT (pg/mL)	588.26±9.54[a]	1204.53±23.11[b]	<0.001
AST (U/L)	54.87±0.45[a]	88.67±1.88[b]	<0.001
GGT (U/L)	19.37±0.33[a]	40.60±0.91[b]	<0.001
Urea (mg/dL)	15.47±0.24[a]	28.67±0.61[b]	<0.001
Creatinin (mg/dL)	0.62±0.16[a]	1.59±0.25[b]	<0.001

[a,b] Different letters in the same line indicate a statistically significant difference. Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α), Serum amyloid A (SAA), Procalcitonin (PCT), Aspartate aminotransferase (AST), Gamma glutamine transferase (GGT).

The proinflammatory cytokine, acute phase proteins, and biochemical parameter values of healthy and puerperal metritis goats are presented in Table 3. Significant differences were observed between the groups in IL-6, TNF-α, SAA, PCT, AST, GGT, urea, and creatinine values (p<0.001). IL-6, TNF-α, SAA, PCT, AST, GGT, urea, and creatinine values were higher in the puerperal metritis group compared to the control group (p<0.001).

DISCUSSION AND CONCLUSION

Puerperal metritis, like other gynecological disorders, leads to economic losses in small ruminant farming (Kulsum et al. 2020). Metritis can develop as a result of various bacterial infections (Doumtsop et al. 2021) and causes inflammation in all layers of the uterine wall, leukocyte infiltration, edema, and degeneration of the myometrium (Mikulková et al. 2020), ultimately leading to infertility. While postpartum acute metritis has been relatively well-studied in cows, there is limited research on the topic in goats. The presented study investigates the effects of puerperal metritis on uterine blood flow, acute phase proteins, and certain biochemical parameters in Aleppo goats, providing clinicians with information to develop diagnostic and treatment protocols.

The significant increase in body temperature, heart rate, and respiration rate in the puerperal metritis group can be attributed to the interaction between the host's immune response and bacterial endotoxins. This link triggers a series of events that elevate body temperature. Our clinical observations correspond with previous results in cows diagnosed with clinical metritis (Perumal et al. 2020). Our investigation revealed elevated WBC levels in the puerperal metritis group. The elevation of white blood cells signifies the body's reaction to infection and reflects the activation of the immune system (Moriyama and Nishida 2021). Likewise, prior research has indicated a notable elevation in WBC levels among septic patients (Di et al. 2020; Karon et al. 2017).

Doppler ultrasonography has effectively evaluated hemodynamic alterations in the uterus during the early postpartum phase in sheep and goats (Elmetwally and Bollwein 2017). A low Resistance Index RI value signifies increased perfusion to a particular organ, whereas a high Pulsatility Index (PI) value denotes reduced perfusion to distant tissues (Varughese et al. 2013). Sharma et al. (2019) found reduced (PI) and (RI) during uterine infection in comparison to estrus. Tinkanen and Kunjansuu (1992) demonstrated that bacterial infections in pelvic organs in women induce hyperemia, resulting in increased uterine blood flow and decreased (PI) and (RI) values in the uterine artery. Sayed et al. (2024) showed reduced PI

and RI levels in buffaloes with endometritis relative to the control group in their research. Debertolis et al. (2016) observed reduced (PI) and (RI) values during generated acute inflammation in the endometrium of cows. Furthermore, subsequent to the eradication of uterine infection, an elevation in PI and RI values and a reduction in uterine blood flow was seen (Honnens et al. 2008). Consistent with the literature, the present study also found lower PI and RI values in goats with puerperal metritis. Puerperal metritis induces inflammation that may result in dilatation of the uterine arteries and reduced vascular resistance, leading to decreased PI and RI levels. Moreover, the infection process might enhance blood circulation to the afflicted region to promote healing and immunological response. This augmented blood flow may also facilitate the decrease in PI and RI.

TNF-α and IL-6 are recognized cytokines linked to the inflammatory system (Kushibiki 2011). Moreover, TNF-α possesses several immune system activities, including antibacterial activity and participation in inflammation (Kushibiki 2011). Sayed et al. (2024) observed increased concentrations of TNF-α and IL-6 in buffaloes suffering from endometritis compared to the control group. The present study, in alignment with other research, detected increased concentrations of TNF-α and IL-6 in goats suffering from puerperal metritis (Adnane et al. 2017; Bhadaniya et al. 2019), thereby confirming the crucial role of IL-6 as an inflammatory mediator in response to infection. In women diagnosed with clinical endometritis, relatively higher concentrations of proinflammatory cytokines have been shown to result from excessive production of TNF-α and IL-6 in the endometrium, which plays a role in maintaining the chronic inflammatory process (Tortorella et al. 2014). The higher concentrations of inflammatory responses or cytokines in IL-6 compared to the control group are thought to be associated with unique inflammatory processes caused by atypical pathogenic responses (Salama et al. 2009).

Acute phase proteins, as with other infections, increase during metritis, and this inflammatory response may occur before the appearance of clinical symptoms, with protein levels increasing gradually. Serum amyloid A (SAA) is associated with chronic subclinical endometritis, inflammatory indicators, and reproductive issues. SAA has garnered attention due to its rapid plasma rise, short half-life, and variable amounts in reproductive disorders like as metritis and mastitis (Trela et al. 2022). In our study, increased serum amyloid A (SAA) levels were observed in goats with puerperal metritis, in accordance with existing literatüre. These findings highlight the importance of APPs in the early identification of uterine infections, allowing timely intervention and prevention of metritis.

This work represents the first use of PCT, a novel diagnostic biomarker in animals, to elucidate the inflammatory responses induced by puerperal metritis and to employ it as a diagnostic indicator in afflicted goats. The release of PCT is initiated by endotoxins or inflammatory mediators generated in reaction to bacterial infections. Consequently, it might be essential in differentiating between viral and bacterial infections (Joo et al. 2011; Schuetz et al. 2011). Likewise, PCT is employed in human medicine for the diagnosis of sepsis (Schuetz et al. 2011). PCT has been identified as a predictor of inflammatory immune response indicators, including body temperature and leukocyte composition. Akkuş et al. (2024) examined PCT levels in sheep with mastitis in their study, suggesting that elevated PCT levels could be a new biomarker for the diagnosis of mastitis. Devi et al. (2024) conducted studies to measure PCT levels as a potential clinical biomarker in goats with pneumonia and healthy controls and determined that PCT was higher in pneumonia groups and decreased with treatment. In accordance with the literature, in the presented study, PCT was found to be higher in goats with puerperal metritis compared to those with healthy uteri. PCT production is triggered by endotoxins or mediators (TNF-α, IL-6) produced in response to bacterial infections. Serum levels are strongly correlated with the prevalence and severity of bacterial infections (Schuetz et al. 2011).

In the presented study, based on liver function results, an increase in AST and GGT enzyme activities, indicating liver damage, was observed in the puerperal metritis group. Elevated serum liver enzyme activities have been described in metritis cows (Paiano et al. 2019), which suggests that the animals' livers are under excessive functional load. Elevated GGT levels signify acute hepatic injury (Bobe et al. 2004). Moreover, higher serum activity of the AST enzyme was also noted with the high GGT enzyme activity. These data indicate that goats with acute metritis undergo alterations in liver function. Rising serum AST activity is considered a marker of liver tissue damage (Heidarpour et al. 2012). Intense septicemia caused by uterine infection can lead to impairments in liver function. This may contribute to an increase in the absorption of systemic toxins, which can cause liver tissue damage, and an elevation in liver enzyme activities (González et al. 2011). Moreover, the increase in liver enzyme activities may also arise from a decrease in food intake or prolonged fasting, which can contribute to liver tissue damage in relation to the increased energy demand during the early postpartum period (Paiano 2019).

Nasreldin et al. (2020) observed elevated urea and creatinine levels in sheep exhibiting preclinical and clinical endometritis relative to the control group. Kaufmann et al. (2010) and Kaya et al. (2016) indicated that serum urea levels were elevated in cows with endometritis, with these levels rising in accordance with the severity of the condition. Consistent with the literature, in the present study, both urea and creatinine levels were found to be higher in goats with puerperal metritis compared to the control group. Metritis triggers a systemic inflammation that can affect multiple organ systems, including the kidneys. Inflammation can induce alterations in metabolic pathways and elevate the synthesis of waste products, including urea and creatinine (Cui et al. 2019; Giuliodori et al. 2013). Moreover, the inflammation and infection related to puerperal metritis can compromise renal function and result in decreased clearance of urea and creatinine from the bloodstream. This may lead to the buildup of these compounds in the serum owing to the kidneys' ineffectiveness in filtering waste materials.

There are several limiting factors in our study. First, the selection of goats from clinical cases, particularly because the goats in the clinical group presented with advanced disease when they visited our clinic, made it difficult to gain a clear understanding of the role of the measured parameters in the early diagnosis of puerperal metritis in goats. Additionally, since the goats in the control group did not receive treatment, the effect of treatment on the measured parameters could not be evaluated. These factors have limited the generalizability of the findings and the evaluation of their effects on a broader population.

In conclusion, puerperal metritis in goats leads to significant changes in uterine blood flow, acute phase proteins, and certain biochemical parameters. These

findings contribute to a better understanding of the effects of puerperal metritis. By using more animals, it has been concluded that the changes in the parameters measured during the postpartum period in healthy and puerperal metritis goats can be considered as early markers and recovery criteria. This approach may provide valuable contributions to the early diagnosis and improvement of treatment processes for puerperal metritis.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: ÖY
Supervision/Consultancy: ÖY, TA
Data Collection and/or Processing: ÖY, TA
Analysis and/or Interpretation: ÖY, TA
Writing the Article: ÖY, TA
Critical Review: ÖY, TA

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