MJAVL
Manas Journal of Agriculture Veterinary and Life Sciences
ISSN/e-ISSN 1694-7932
Volume 16 (Issue 1) (2026) Pages 22-30

Research Article

Effect of Prepartum Iron Injection on Somatic Cell Count and Microbiological Profile of Ewe Milk: A Pilot Study

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ABSTRACT

Mastitis, defined as inflammation of the mammary tissue caused by various pathogenic microorganisms, leads to significant economic losses. Iron, besides its numerous metabolic roles, can promote pathogen growth under in vitro and suitable in vivo conditions. However, iron bioavailability in the body is tightly regulated by the immune system and iron-binding proteins. This study aimed to determine the effects of parenteral iron administration on day 130 of gestation in ewes on milk somatic cell count (SCC) and bacterial and fungal loads on postpartum days 5 and 30. Twenty-two clinically healthy singleton pregnant Kıvırcık ewes were randomly assigned to an experimental (n=11, Fe^{3+}) and a control group (n=11, 0.9% NaCl). Blood samples were collected at injection (day 130 of gestation), within 3 days postpartum, and on postpartum day 30; milk samples were obtained on postpartum days 5 and 30. Serum ferritin levels, milk SCC, and bacteriological analyses were performed using conventional methods. Pre-injection ferritin levels differed significantly between groups (P=0.022), representing a methodological limitation. A moderate positive correlation between serum ferritin and SCC was observed in the experimental group; however, no significant differences were found between groups. Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and fungal agents were isolated from milk samples. In conclusion, prepartum parenteral iron administration in ewes had no significant effect on SCC; it partially reduced E. coli and fungal isolates but had no effect on S. aureus. As a pilot study, these findings indicate the need for further research with larger sample sizes.

ARTICLE INFO
Received: 30.03.2026
Accepted: 03.06.2026

Keywords: *Iron, Mastitis, Microbiological profile, Sheep, Somatic cell count*

Koyunlarda Doğum Öncesi Demir Enjeksiyonunun Süt Somatik Hücre Sayısı ve Mikrobiyolojik Profil Üzerine Etkisi: Pilot Çalışma

ÖZET

Çeşitli patojen mikroorganizmalar tarafından oluşturulan ve meme dokusunun yangısı olarak tanımlanan mastitis, önemli ekonomik kayıplara neden olmaktadır. Demir, birçok metabolik süreçte rol almanın yanında, *in vitro* ortamlarda ve uygun *in vivo* koşullarda patojen mikroorganizmaların üremesini destekleyen bir mineraldir. Ancak vücut içinde demir biyoyararlanımı bağışıklık sistemi ve demir bağlayıcı proteinler tarafından düzenlenmektedir. Bu çalışmada, koyunlarda gebeliğin 130. gününde uygulanan parenteral demirin, doğum sonrası 5. ve 30. günlerdeki süt somatik hücre sayısı (SHS) ile bakteri ve mantar sayıları üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada klinik olarak sağlıklı görünen toplam 22 adet tekiz gebe Kıvırcık koyun kullanılmış ve hayvanlar rasgele deney (n: 11, Fe^{3+}) ve kontrol (n: 11, %0.9 NaCl) gruplarına ayrılmıştır. Kan örnekleri enjeksiyon anında (gebeliğin 130. günü), doğum sonrası ilk üç gün içinde ve postpartum 30. günde; süt örnekleri ise doğum sonrası 5. ve postpartum 30. günlerde alınmıştır. Serum ferritin düzeyi, süt SHS sayısı ve bakteriyolojik analizler konvansiyonel yöntemlere göre yapılmıştır. Gruplar arasında enjeksiyon öncesi ferritin düzeylerinin anlamlı farklılık gösterdiği (*P*=0,022) ve bunun metodolojik bir kısıtlılık oluşturduğu değerlendirilmiştir. Deney grubunda serum ferritini ile SHS arasında orta düzeyde pozitif bir korelasyon saptanmış, ancak gruplar arası anlamlı bir etkinin olmadığı görülmüştür. Süt örneklerinden *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* ile mantar etkenleri izole edilmiştir. Sonuç olarak, koyunlara doğum öncesi uygulanan parenteral demirin SHS üzerinde anlamlı bir etkisi bulunmamış; *E. coli* ve mantar izolatlarında kısmi azalma sağlarken, *S. aureus* üzerine etkisi gözlenmemiştir. Bu pilot çalışma sonucunda daha fazla hayvanın kullanılacağı benzer çalışmalara ihtiyaç duyulduğu değerlendirilmiştir.

MAKALE BİLGİSİ
Geliş: 30.03.2026
Kabul: 03.06.2026

Anahtar kelimeler: *Demir, Mastitis, Mikrobiyolojik profil, Koyun, Somatik hücre sayısı*

Cite this article as: Saat, N., Öztürk, B., Ayalp-Erkan, A., Çetinkaya, YB. & İlhan, Z. (2026). Effect of prepartum iron injection on somatic cell count and microbiological profile of ewe milk: A pilot study. *Manas Journal of Agriculture Veterinary and Life Sciences, 16(1),* 22-30. https://doi.org/10.53518/mjavl.1919139

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INTRODUCTION

Mastitis, defined as inflammation of the mammary gland, represents one of the most prevalent and economically consequential conditions affecting sheep production worldwide. The disease most commonly arises from infection by pathogenic microorganisms, with *Staphylococcus aureus* being the predominant causative agent, followed by members of the *Enterobacteriaceae* family, *Streptococcus* spp., *Corynebacterium* spp., *Mannheimia haemolytica*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, fungi, and yeasts. Less frequently, mastitis may also result from physical trauma, allergic reactions, or neoplastic processes. Based on clinical presentation, the condition is classified as peracute, acute, chronic, or subclinical (Jesse et al., 2023; İlhan, 2018; Menzies & Ramanoon, 2001). In sheep, mastitis causes significant economic losses through reduced milk yield, deterioration of milk composition, and the necessity of withholding milk following antibiotic treatment. Furthermore, intramammary infections affect both dairy and meat-producing flocks, as impaired lactation leads to suboptimal neonatal growth (Chambers et al., 2024; Cuccuru et al., 2011). Beyond production losses, clinical mastitis induces behavioral changes including restlessness, anorexia, and signs of pain, while even subclinical forms may compromise animal welfare (Gelasakis et al., 2015).

Early and accurate diagnosis is fundamental to effective mastitis management. Available diagnostic approaches include clinical examination, mammary ultrasonography, endoscopic evaluation, bacteriological and immunological analysis of milk, cytological examination, and measurement of milk electroconductivity (Fragkou et al., 2014). Among these, somatic cell count (SCC) is widely recognized as the most reliable indicator of the inflammatory response in the ovine mammary gland. SCC reflects the concentration of leukocytes predominantly neutrophils (approximately 95%) in milk, and is influenced by lactation stage, physiological status, parity, age, breed, days in milk, daily milking frequency, and the number of nursing lambs (Gonzalo et al., 1994; Králíčková et al., 2013; Olechnowicz et al., 2010). Unlike bovine milk, for which SCC thresholds are well established (2×10^5- 4×10^5 cells/mL), no universally accepted reference ranges exist for sheep milk. Proposed thresholds for distinguishing healthy from infected udders range from 250 - 300×10^3 cells/mL (Olechnowicz et al., 2010), while values between 600,000 and 800,000 cells/mL are generally regarded as indicative of active infection (Conington et al., 2008; Saat et al., 2021). Despite these limitations, SCC remains the most widely employed research parameter for mastitis surveillance in sheep.

Iron is an essential trace element that participates in a broad array of critical physiological functions, including oxygen transport via hemoglobin, oxidative phosphorylation through the electron transport chain, and phagocytic activity of immune cells. In addition to its roles in host metabolism, iron is indispensable for pathogen proliferation; bacterial ribonucleotide reductase, a key enzyme in DNA synthesis, requires iron for its catalytic activity, and numerous viruses similarly depend on host iron stores for replication (Deniz et al., 2025; Drakesmith & Prentice, 2008; Marx, 2002; Saat & Ayalp Erkan, 2023). During infection, the host and pathogen compete for available iron, and the host-mediated sequestration of iron, clinically manifested as hypoferremia and anemia of chronic disease, constitutes a well-characterized innate defense strategy (Kent, 1994; Weinberg, 1996). Iron-binding proteins such as haptoglobin, transferrin, and lactoferrin modulate both systemic and local immune responses, and alterations in their concentrations during infection can influence susceptibility and disease severity (Gennero et al., 2010; Newton et al., 2007; Weinberg, 1996).

In cattle, serum ferritin has been established as a marker for body iron stores as well as for inflammatory and malignant conditions (Schnell et al., 2015). While iron and ferritin have long been utilized in human medicine as indicators of inflammation, their application in veterinary diagnostics, particularly outside of respiratory diseases, remains limited (Değirmençay et al., 2022). Given the dual role of iron in both host immunity and microbial pathogenesis, understanding how iron supplementation influences mammary gland health in sheep is of considerable scientific and practical relevance. Against this background, the present study aimed to evaluate the effect of prenatal iron administration on milk somatic cell count during the first postpartum month in pregnant ewes. The aim of this study was to evaluate the effects of parenteral iron administration on day 130 of gestation in sheep on postpartum milk somatic cell count (SCC) and microbial isolation.

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MATERIALS and METHODS

Ethical Approval

Balikesir University Animal Experiments Local Ethics Committee approved the experimental procedures used (reference number: 2024/7-3 Approval Date: 01.08.2024).

Ewes

Ewes were selected from the herd at Balıkesir University Animal Husbandry Application and Research Center (Coordinates: N 39.4828793086999, E 28.03785333404705). The herd had ad libitum access to water. They grazed on pasture and additionally consumed barley and concentrated feed. The welfare criteria were met with 2.5 m^2 per sheep. A total of 50 ewes were initially screened using transrectal ultrasonography (Fujifilm, Sonosite Edge II Ultrasound, Japan) to confirm pregnancies (Romano & Christians, 2008). The gestational age was determined based on a controlled mating protocol. Ewes were synchronized using intravaginal progesterone sponges, and the ram was introduced to the flock on a defined date and removed after a fixed period. Parturition date was subsequently estimated from the recorded date of ram introduction, allowing confirmation that selected ewes were at approximately 130 days of gestation at the time of enrolment. Transrectal ultrasonography was additionally performed at the time of grouping to confirm pregnancy, and verify gestational age estimates. Of the 50 ewes screened, 28 were excluded due to twin pregnancies (n: 19), or clinical health problems (n: 9). A total of 22 clinically healthy, singleton-pregnant Kıvırcık ewes, aged 2 to 5 years, were selected for the study. The selected ewes were randomly divided into two groups: those receiving iron injections (n: 11, experimental, (E)) and those receiving placebo (n: 11, control, (C)). The sheep in the experimental group were given 2 mL of intramuscular iron (Fe^{+3}) (Ferrum, Abdiibrahim, Türkiye), while the control group received a placebo of 0.9% NaCl (Neoflex, Turktipsan, Türkiye).

Collecting samples

Blood samples were collected at three time points: the 130^{th} day of gestation (pre-lambing/at time of injection), within the first 3 days after lambing, and on the 30^{th} day after lambing. All blood collections were performed between 08:00 and 10:00 h to minimize diurnal variation. Milk samples were collected at two time points. The first sampling was performed within the first 5 days after lambing, once colostrum had transitioned to normal milk characterized by a translucent, white, and watery appearance and stable California Mastitis Test (CMT) reactivity, and the second sampling was conducted on day 30 after lambing. Blood samples were collected from the jugular vein of the sheep into non-anticoagulated, vacuum tubes (Vacuette, Greiner Bio-One, Austria). For milk samples, the udders were cleaned with an alcohol wipe beforehand, the first few streams of milk were discarded, and then milk was collected for somatic cell count and microbiological analysis (Koc et al., 2026). The collected blood samples were brought to the laboratory within 1 hour, centrifuged at 3,500 rpm for 10 minutes, and the serum was stored at -20°C. The collected milk samples were transferred to the laboratory within 2 hours for analysis.

Bacteriological and mycological analysis

Milk samples were immediately inoculated onto sheep blood agar supplemented with 5% sheep blood (Merck, 110896) and MacConkey agar (Merck 1.05465, Germany), and the plates were incubated aerobically at 37 °C for 24-72 hours. In addition, samples were inoculated onto Sabouraud dextrose agar supplemented with chloramphenicol (bioMérieux 46979, France) and incubated aerobically for 5-7 days. Bacterial identification was carried out based on macroscopic and microscopic morphology, as well as conventional biochemical tests. The isolates were characterized using catalase, coagulase, DNase, oxidase, and aesculin tests, in addition to carbohydrate fermentation assays, including mannitol, glucose, and lactose. Gram-negative bacterial isolates were further identified using the Microgen GN-ID A and B system (Quinn et al., 2011). Fungal agents in the milk samples were assessed by macroscopic and microscopic examination after staining with lactophenol cotton blue (Merck, 113741.0100) (İlhan, 2015; Larone, 2011).*Somatic cell count analysis*

The samples were tested using a flow cytometry-based laser scanning automated SCC device (Combi FTS, Bentley®, USA). In this study, taking EU criteria as a reference, milk samples with an SCC value $>4\times10^5$ cells/mL were considered mastitis (Saat et al., 2021).

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Ferritin analysis

Serum ferritin concentrations were determined using the RX Monaco device (Randox Laboratories Ltd., United Kingdom) by spectrophotometric methods (Baydar et al., 2026).

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). The normality of data distribution was verified using the Kolmogorov–Smirnov test ($p>0.05$). Differences across time points within and between groups were assessed using two-way repeated measures ANOVA, followed by Bonferroni post-hoc correction. The relationship between serum ferritin levels and milk SCC was evaluated using Spearman's rank correlation coefficient (r_s). Statistical significance was defined at $p<0.05$; values between 0.05 and 0.10 were considered to reflect a statistical tendency.

RESULTS

Bacteriological and mycological findings

Milk samples collected during the first five days after lambing from the experimental group (n: 11) revealed the presence of *S. aureus* in 100% of the animals. *P. aeruginosa* and *K. pneumoniae* were detected in 54.5% and 36.3% of the samples, respectively, while *E. coli* and fungal isolates (including yeasts) were detected at higher rates of 54.5% and 18.1%, respectively. In the control group (n: 11), *S. aureus* was also present in 100% of the ewes, with *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and fungal isolates (including yeasts) detected in 54.5%, 36.3%, 18.1%, and 18.1% of the samples, respectively.

Microbiological culture analysis of milk samples collected at one month after lambing revealed that *S. aureus* remained present in all animals (100%) in both groups. In the experimental group, *P. aeruginosa* and *K. pneumoniae* were detected in 63.6% and 54.5% of the samples, respectively, while fungal isolates (including yeasts) were present in 36.3%. Notably, *E. coli* was not detected in any samples from this group. In the control group, *P. aeruginosa*, *K. pneumoniae*, fungal isolates (including yeasts), and *E. coli* were detected in 90.9%, 81.8%, 54.5%, and 18.1% of the samples, respectively. Fungi (excluding yeasts) were additionally identified in 9.1% of the samples (Table 1).

Table 1. *Microbiological analysis results obtained from milk samples collected at the time of lambing and one month after lambing in experimental and control groups of ewes*

Bacterial / Fungal agents	Experimental (n: 11) % First five days after lambing	Control (n: 11) % First five days after lambing	Experimental (n: 11) % One month after lambing	Control (n: 11) % One month after lambing
S. aureus	100	100	100	100
P. aeruginosa	54.5	54.5	63.6	90.9
K. pneumoniae	36.3	36.3	54.5	81.8
E. coli	54.5	18.1	0	18.1
Fungal isolates (including yeasts)	18.1	36.3	36.3	63.6

Ferritin levels in groups

In the control group, serum ferritin concentrations remained stable across all three collection time points ($p>0.05$). In the experimental group, ferritin levels increased progressively following iron administration, reaching a significantly higher concentration at one month postpartum compared to the pre-injection baseline ($P=0.034$). Notably, pre-injection ferritin concentrations differed significantly between groups (C: 2.66±0.48 ng/mL vs. E: 0.97±0.48 ng/mL; $P=0.022$), indicating that baseline iron status was not equivalent prior to intervention — a finding that should be considered when interpreting intergroup comparisons. At the time of lambing, a trend toward higher ferritin was observed in the experimental group relative to controls (2.88±0.68 vs. 1.12±0.68 ng/mL; $P=0.084$). By one month postpartum, the difference was statistically significant (E: 2.64±0.40 ng/mL, C: 1.35±0.40 ng/mL; $P=0.037$) (*Figure 1.*).

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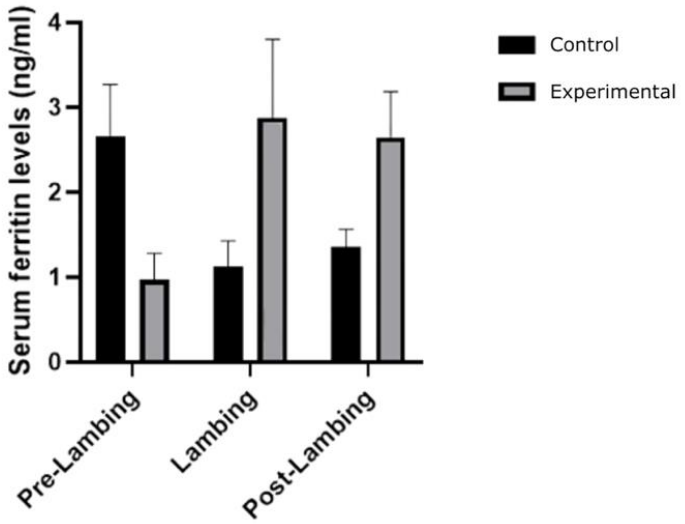


Figure 1. *Serum ferritin levels of the control and experimental groups at pre-lambing (130th day of gestation), lambing (first 3 days after lambing), and post lambing (30th days after lambing) periods.*

Somatic cell count levels in groups

When examining milk SCC at first five days after lambing, no significant difference was found between control and experimental groups (C: 855,727±233,117 cells/mL; E: 614,636±233,117 cells/mL; p>0.05). Similarly, no statistical difference was found in SCC at one month after lambing between the control (C: 343,727±248,755 cells/mL) and experimental (E: 491,909±248,755 cells/mL) groups (p>0.05), (*Figure 2.*).

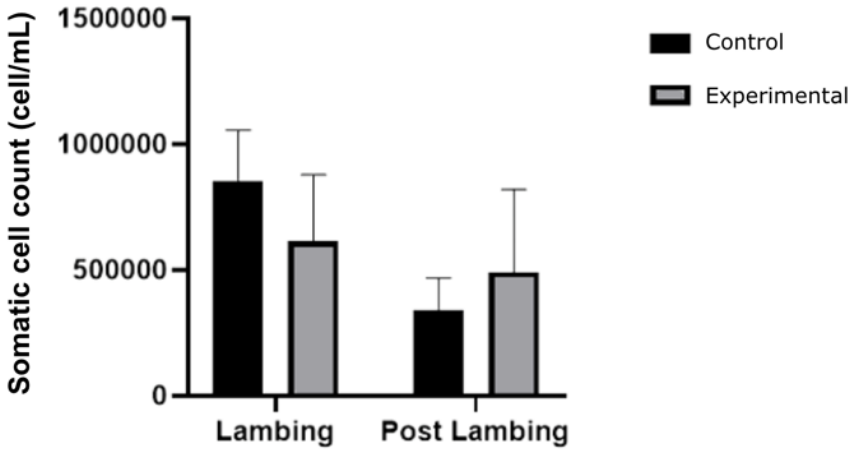


Figure 2. *Somatic cell counts (cell/mL) of the control and experimental groups at lambing (five days after lambing) and post-lambing (30th days after lambing) period*

Relationship between ferritin and SCC in groups

Despite statistically significant differences in serum ferritin between the experimental and control groups, no corresponding significant difference in SCC was observed. Spearman's correlation analysis revealed a moderate positive association between serum ferritin and SCC within the experimental group (r_s=0.626; however, this did not reach statistical significance (p>0.05). Intergroup analysis, encompassing both the experimental and control groups, confirmed that blood ferritin levels did not significantly predict milk SCC regardless of iron treatment (Table 2.).

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Table 2. *Ferritin (ng/mL) and SCC (cell/mL) levels in ewes during the pre-lambing (130th day of gestation), lambing (first 3 days after lambing), and post-lambing (30th days after lambing) periods.*

Groups	Pre Lambing		Lambing		Post Lambing		p
	Ferritin	SCC	Ferritin	SCC	Ferritin	SCC	
Experimental (n: 11)	0.97±0.48^{*b}	-	2.88±0.68	614,636±233,117	2.64±0.40^{*a}	491,909±248,755	p<0.05
Control (n: 11)	2.66±0.48^{a}	-	1.12±0.68	855,727±233,117	1.35±0.40^{b}	343,727±248,755	p>0.05

*:p=0.034, The significance between different letters (a,b) in the same column is statistically important. SCC: Somatic cell count

DISCUSSION

Mastitis is one of the most significant diseases affecting dairy animals worldwide, causing decreased milk production, altered milk composition, and substantial economic losses. Beyond its effects on animal health and welfare, mastitis also poses a risk to milk quality and safety due to the potential presence of pathogenic microorganisms. Effective prevention and management of mastitis are therefore essential for maintaining both productivity and public health in the dairy industry (Chambers et al., 2024; İlhan, 2018; Jesse et al., 2023; Králíčková et al., 2013).

According to Food and Agriculture Organization (FAO) data, total milk production in 2022 was reported to be 930 million tons, of which 46.5 million tons, or approximately 5%, was sheep milk. While sheep milk does not reach the high production volumes of cow milk, it still holds a significant place in global dairy production and remains an important resource for both nutrition and the economy.

Iron is a trace mineral with important functions such as oxygen transport and storage. Iron containing enzymes play roles in regulating phagocytosis and immunoglobulin production. Additionally, through acute-phase proteins like transferrin and haptoglobin, as well as lactoferrin, a glycoprotein also known as lactotransferrin, iron contributes to the regulation of non-specific immunity, thus playing a role in both general and local defense systems (Khaleghnia et al., 2021).

In current study, ferritin levels in the control group remained relatively low at the time of parturition and during the postpartum period. Similarly, (Kilinc et al., 2022) observed a natural decline in serum iron levels from gestation to the postpartum period in cows , which is consistent with the findings in our control group. The probable reason for this physiological decline in both studies may be the depletion of maternal iron stores driven by the high metabolic demands of fetal development and the onset of lactation. Minor variations between the studies can be attributed to the different biomarkers assessed (ferritin - serum iron) and species differences.

In the current study, the experimental group showed progressively increasing ferritin values following iron administration, despite having lower baseline levels prior to injection. This pre-existing difference between groups before the intervention represents a methodological limitation, and future studies should consider stratified randomization based on pre-intervention iron status. In a study by Khaleghnia et al. (2021), it was reported that in calves with low iron reserves, the injected iron was likely transferred to iron-utilizing organs as quickly as possible, resulting in low blood serum iron levels immediately after injection (Khaleghnia et al., 2021). The pattern observed in our experimental group is consistent with this progressive iron mobilization rather than immediate serum accumulation, and the similarity in findings is thought to be due to the shared metabolic handling of iron across ruminant species.

Regarding somatic cell count, no significant difference was found between the iron-injected and control groups at either time point. Mohri et al. (2010) similarly found that parenteral iron administration had no significant effect on blood parameters in calves (Mohri et al., 2010), which is consistent with our findings. The probable reason is that iron injection at the administered dose does not cause a significant change in neutrophil recruitment to the mammary gland. Baydar & Dabak (2014), on the other hand, reported a significant difference in serum iron levels between cows with mastitis and healthy cows (Baydar & Dabak, 2014), which does not match with this study. The reason may be that the cited study evaluated naturally occurring infection-driven iron reduction, whereas the present study examined the effect of externally administered iron in a different species.

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Orino et al. (2006) reported no correlation between mastitis and serum iron levels in their study on cows, which is similar to the findings of this study. The reason is thought to be that the natural ferritin-binding ability of the mammary gland is not substantially altered by parenteral iron administration at the dose used.

Upon analyzing the microbial agents responsible for mastitis in sheep, *S. aureus* was found to be the predominant microorganism across all groups and time points in the current study. In contrast, Ahmed et al., 2020 reported *E. coli* as the most prevalent pathogen (50%), followed by *S. aureus* (25%). Another study by Abdalhamed et al., 2018 documented *S. aureus* prevalence at only 31.1%, with considerably lower rates of other pathogens compared to the present findings. These discrepancies among studies may be attributed to differences in animal husbandry conditions, sampling time points, and the number of animals included in each study.

Several limitations of this study should be acknowledged. First, the unequal baseline ferritin levels between groups before injection may have reduced the reliability of intergroup comparisons. Stratified randomization based on pre-intervention iron status would have been methodologically preferable. Second, formal clinical or laboratory diagnosis of mastitis was not performed throughout the study period; therefore, discussions linking SCC to mastitis status are necessarily indirect. Third, the study evaluated only one postpartum time point (30 days), limiting conclusions about the longitudinal effects of iron administration on SCC. Fourth, the sample size (n=11 per group) may have limited statistical power to detect modest differences in SCC. Future studies with larger sample sizes, matched baseline iron levels, confirmed mastitis diagnoses, and extended follow-up periods are needed to clarify the relationship between iron supplementation and udder health in sheep.

CONCLUSION

Intramuscular iron injection on day 130 of gestation did not significantly affect milk somatic cell counts in ewes during the first postpartum month. Neither iron treatment nor serum ferritin levels were significant predictors of milk SCC, although a moderate positive correlation between ferritin and SCC was observed within the experimental group. Parenteral iron supplementation had limited impact on milk microbial composition; while reductions in *E. coli* and fungal isolates were noted, high prevalence of *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* persisted. These findings suggest that prenatal iron administration alone is not sufficient to prevent mastitis. Future well powered studies with stratified allocation, confirmed mastitis diagnosis, and extended postpartum monitoring are needed to clarify the potential role of iron supplementation in optimizing udder health and milk quality in sheep.

Compliance with Ethical Standards

This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in MJAVL belongs to the author(s).

Ethical Approval

Balikesir University Animal Experiments Local Ethics Committee approved the experimental procedures used (reference number: 2024/7-3 Approval Date: 01.08.2024).

Conflict of Interest

There is no financial conflict of interest with any institution, organization, or individual regarding our article titled "Effect of prepartum iron injection on somatic cell count and microbiological profile of ewe milk: A pilot study" and there is no conflict of interest among the authors.

Author Contributions

Aslihan AYALP ERKAN and Buse OZTURK conceived the presented idea. Nevzat SAAT developed the theory and performed the calculations. Aslihan AYALP ERKAN and Buse OZTURK conducted the experiment. All authors contributed to the collection and preservation of the samples. Ziya İLHAN performed to laboratory analysis. Yusuf Bilal CETINKAYA and Aslihan AYALP ERKAN wrote the manuscript. All authors discussed the results and contributed to the final manuscript. Nevzat SAAT supervised the project.

Funding

Balıkesir University Scientific Research Projects Unit supports project number 2022/019. Some consumables obtained from this project were also used in this study.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Statement of Use for Artificial Intelligence and Its Types

Artificial intelligence (ChatGPT, OpenAI, ABD) was used to provide support for the academic translation of the Turkish text into English.

Approval for Publication

All authors declare that they have seen and approved the final version of the submitted manuscript

Acknowledgements

We would like to express our sincere gratitude to the management and staff of the Balikesir University Livestock Research and Application Centre, the Board of Directors and staff of the Balikesir Sheep and Goat Breeders' Association, the staff of the Diagnostic and Analysis Laboratory of the Faculty of Veterinary Medicine at Balikesir University (Republic of Türkiye Ministry of Agriculture and Forestry Working Permit Number: TAL-55), the students of the Faculty of Veterinary Medicine at Balikesir University, and the staff of the Balikesir Cattle Breeders' Association. Furthermore, artificial intelligence assistance (ChatGPT, OpenAI, USA) was used for the academic translation of the Turkish manuscript into English.

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