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Serum calcium and glucose as predictors of postpartum reproductive complications

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ABSTRACT

Transition period in dairy cows is a critical phase that increases susceptibility to reproductive disorders. The study evaluated whether serum calcium and glucose levels around calving can predict common postpartum complications like retained placenta, metritis, dystocia, and ovarian cysts. Blood samples were collected from 82 multiparous Holstein cows prepartum and at calving. Serum calcium and glucose were compared between healthy and diseased cows using t-tests. ROC analysis was used to establish predictive thresholds. No significant difference was found in serum glucose concentrations between healthy and diseased cows at either time point. Serum calcium was significantly lower at parturition in cows with metritis, retained placenta, and in the overall unhealthy group. ROC analysis identified prepartum glucose and calcium as predictors for dystocia and retained placenta, respectively. Calcium at parturition were predictive of metritis and general health status. Logistic regression revealed that cows with prepartum glucose below 49.5 mg/dL had an 8-fold increased odds of dystocia. Similarly, prepartum calcium below 8.4 mg/dL was associated with an 11-fold increase in the odds of retained placenta. At parturition, calcium levels below 7.85 mg/dL and 7.9 mg/dL increased the odds of metritis and overall morbidity by 10 and 7 times, respectively. Calcium status around calving was a key determinant of postpartum reproductive health in dairy cows. Reduced calcium levels were strongly associated with metritis, retained placenta, and overall morbidity (presence of at least one disorder), while glucose showed limited predictive value except for dystocia. Monitoring calcium dynamics could support early detection, targeted intervention, and improved herd health.

Keywords: Calcium, Dairy Cow, Glucose, Postpartum Diseases, ROC analysis

Transition period around parturition is a critical phase in dairy cows, marked by metabolic and homeostatic changes that increase susceptibility to health and reproductive disorders (Bruinjé and LeBlanc, 2025). Negative energy balance in early lactation predisposes cows to uterine diseases, including metritis, endometritis, vaginal discharge, and retained placenta (Serbetci *et al.* 2024). Subclinical hypocalcemia, despite lacking clinical signs, also impairs reproductive performance (Berean *et al.* 2025). A strong association exists between subclinical hypocalcemia and endometritis (Yue *et al.* 2022). This condition is common in multiparous Holstein cows, with a prevalence of approximately 50%, and has been linked to impaired reproductive outcomes and an increased risk of postpartum health events (Seely and McArt, 2023, Serrenho *et al.* 2021).

However, recent studies indicate that calcium measurements during the first days in milk may lack predictive power for future health outcomes (McArt and Neves, 2020; Menta *et al.* 2021). Consistently, Karimi Dehkordi and Yahyaei (2020) demonstrated that calcium levels in the transition period can predict reproductive performance in Holstein cows (Karimi Dehkordi and Yahyaei 2020).

Alterations in blood calcium and phosphorus have been identified as important indicators of reproductive disorders, including retained fetal membranes, in dairy cattle (Alrawy, 2024). Glucose, beyond its metabolic role, reflects overall energy balance and efficiency (Bicalho *et al.* 2017).

Elevated glucose on day 3 postpartum has been associated with metritis (Bicalho *et al.* 2017), while inadequate glucose impairs fertility, prolonging calving intervals and increasing repeat breeding (Ahmed *et al.* 2017). In support, Pishvaei *et al.* (2021) reported that ketone bodies affect ovarian function by altering reproductive hormones and follicular size (Pishvaei *et al.* 2021). Similarly, Karimi Dehkordi *et al.* (2016) identified thresholds of NEFA and β-hydroxybutyrate (BHBA) strongly linked to poor reproductive outcomes (Karimi Dehkordi *et al.* 2016).

Reproductive disorders during the peripartum period reduce herd productivity by prolonging days open, delaying ovarian resumption, and impairing fertility. Despite established links between metabolic imbalance and reproduction, the predictive value of calcium and glucose remains uncertain. Therefore, this study aimed

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to investigate the relationship between prepartum and parturition serum calcium and glucose concentrations and the incidence of dystocia, retained placenta, metritis, and ovarian cysts in multiparous Holstein cows.

MATERIALS AND METHODS

Animals and management: The present research was conducted on a commercial dairy farm housing 300 cows, located in Shahrekord, Iran. A total of 82 multiparous Holstein cows, with an average daily milk yield of 38.51 ± 1.23 kg, were selected for this investigation. Enrollment occurred at 14 days prior to the expected calving date, and monitoring continued through parturition. The study cohort had an average parity of 3.5 ± 1.2 and a body condition score (BCS) of 3.12 ± 0.25 (on a 5-point scale where 1 = emaciated and 5 = obese) at the time of enrollment. Throughout the postpartum period, cows were housed in individual stalls equipped with sand bedding and ensured adequate ventilation. Nutritional needs were met through a total mixed ration (TMR), and water was available *ad libitum*. Milking was performed three times per day at consistent intervals (07:30, 14:30, and 22:30).

Blood sampling and analysis: Blood was drawn from the tail vein of the enrolled cows at two defined time points: once at 14 days before the expected calving date and again on the calving day at 09:00 h. After centrifugation, the serum was separated and preserved at −20 °C until further analysis. Serum calcium concentration was assessed using atomic absorption spectrophotometry (Analyst 200; Perkin-Elmer, Waltham, MA, USA). Glucose levels were determined with an automated biochemical analyzer (Technicon RA 1000; Bayer, NY, USA) using commercial diagnostic kits (Pars Azmoon Co., Tehran, Iran), following the manufacturer's instructions.

Health monitoring and disease definitions: All cows enrolled in the study were clinically examined once daily from the day of calving until 40 days postpartum to monitor reproductive health status. Diagnostic definitions were based on established criteria as follows:

Retained Placenta (RP): Defined as failure to expel fetal membranes within 24 h after parturition (Sheldon *et al.* 2006).

Metritis (MET): Diagnosed when cows showed fetid purulent or reddish-brown vaginal discharge accompanied by systemic signs, including fever (rectal temperature >39.5 °C), depression, and reduced milk yield within the first 21 days in milk (DIM) (Sheldon *et al.* 2006).

Ovarian Cysts (OC): Identified by transrectal ultrasonography between 21 and 35 DIM and defined as non-ovulatory follicles larger than 1.7 cm in diameter that persisted for more than 10 consecutive days without corpus luteum formation.

Dystocia (DYS): Defined as prolonged or difficult parturition that required manual or mechanical assistance.

For analytical purposes, cows were classified in two ways:

Disorder-specific classification: Animals were categorized as healthy or diseased with respect to each disorder (RP, MET, OC, and DYS).

Overall health classification: Animals were grouped as healthy (absence of all reproductive disorders) or diseased (presence of at least one of the above disorders).

Serum glucose and calcium concentrations were determined at 14 days before calving and on the day of parturition, and values were compared between groups.

Statistical analysis: All statistical analyses were performed using SPSS software (version 22; SPSS Inc., Chicago, IL, USA). Data distribution was tested for normality using the Kolmogorov–Smirnov test. Serum calcium and glucose concentrations were compared between the defined health groups using independent samples t-tests. A p-value < 0.05 was considered statistically significant.

To evaluate the predictive capacity of serum calcium and glucose at different sampling times, receiver operating characteristic (ROC) curves were generated. For each metabolite, the optimal cutoff threshold was determined from the ROC point that maximized sensitivity and specificity. Predictive performance was further quantified by calculating the area under the curve (AUC). Sensitivity was defined as the proportion of affected animals correctly classified above the threshold, while specificity represented the proportion of healthy animals correctly identified below the threshold. Likelihood ratios (LRs) were also calculated to provide additional diagnostic insight.

Associations between serum metabolite concentrations and the risk of postpartum disorders were further examined using binary logistic regression models. Results were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (CIs) to quantify the strength of associations.

RESULTS AND DISCUSSION

This comprehensive study investigated the relationship between peripartum serum levels of calcium and glucose and the incidence of postpartum disorders in Holstein dairy cows. Our analysis revealed distinct, disorder-specific metabolic patterns that provided crucial insights for clinical management.

Regarding calcium metabolism, the most significant findings were observed at parturition. Cows that developed metritis exhibited substantially lower serum calcium concentrations at calving (7.4 ± 0.15 mg/dL) compared to healthy controls (8.09 ± 0.07 mg/dL; p = 0.005). Similarly, cases of retained placenta showed markedly reduced calcium levels (7.3 ± 0.1 mg/dL vs. 8.0 ± 0.07 mg/dL in controls; p=0.003). When examining cows affected by at least one postpartum disorder (dystocia, metritis, retained placenta, or ovarian cysts), a consistent pattern of hypocalcemia emerged. At parturition, serum calcium levels were significantly lower in diseased cows (7.6 ± 0.09 mg/dL) compared to healthy controls (8.1 ± 0.08 mg/dL; p = 0.01).

ROC curve analysis further confirmed the predictive

value of calcium measurements. A calcium concentration <7.85 mg/dL at parturition strongly predicted metritis (AUC = 0.833), with 80% sensitivity and 72.2% specificity, increasing the odds of metritis ten-fold (OR = 10.0; 95% CI: 2.8–35.7; p ≤ 0.05). Similarly, calcium <7.9 mg/dL at calving predicted the occurrence of at least one peripartum disorder (AUC = 0.879) with 91.7% sensitivity and 79.3% specificity (OR = 7.0; 95% CI: 2.5–19.6). In addition, prepartum calcium <8.4 mg/dL predicted retained placenta with high accuracy (AUC = 0.946), demonstrating 75% sensitivity and 89.2% specificity, and increased the risk eleven-fold (OR = 11.0; 95% CI: 3.2–37.8; p ≤ 0.05).

Logistic regression further supported these findings: each 1 mg/dL increase in calcium at parturition was associated with a 20% reduction in the odds of metritis (OR = 0.8; 95% CI: 0.68–0.94; p = 0.01) and a 27% reduction in the odds of any peripartum disorder (OR = 0.73; 95% CI: 0.59–0.90; p = 0.004). Prepartum calcium increases of 1 mg/dL were associated with a 29.8% decrease in the odds of retained placenta (OR = 0.702; 95% CI: 0.53–0.93; p = 0.01).

These results reinforced the fundamental role of hypocalcemia in the pathogenesis of periparturient diseases. Calcium is crucial for smooth muscle tone and immune function, and its insufficiency compromises uterine contractility and neutrophil activity, thereby impairing placental expulsion and early immune defense, increasing susceptibility to metritis and retained placenta (Sammad *et al.* 2022; Bruinjé and LeBlanc, 2025).

Although the calcium-to-phosphorus ratio has been proposed as an indicator of mineral imbalance, we deliberately focused on serum calcium alone. This decision was based on the direct pathophysiological role of hypocalcemia in uterine contractility and immune function (Wilkens *et al.* 2020; Sammad *et al.* 2022), the confounding factors affecting the ratio (e.g., dietary phosphorus, renal excretion, PTH-mediated reabsorption), and the strong predictive performance of calcium alone in our ROC analysis (AUC = 0.946 for retained placenta and 0.833 for metritis). Additionally, measuring a single metabolite is more practical and cost-effective for routine herd monitoring. Future studies may explore whether the calcium-to-phosphorus ratio offers additional value beyond calcium alone.

The present findings of a strong association between low calcium levels and increased risk of postpartum complications were consistent with the results of Caixeta *et al.* (2017), who demonstrated that subclinical hypocalcemia in the first 3 days of lactation was associated with impaired reproductive performance in dairy cows.

A strong association between subclinical hypocalcemia and reproductive disorders such as endometritis has also been reported previously (Yue *et al.* 2022; Seely & McArt, 2023; Serrenho *et al.* 2021). Moreover, alterations in calcium and phosphorus have been linked to retained fetal membranes in dairy cattle (Alrawy, 2024), further supporting our findings.

This finding was consistent with recent research by Wilkens *et al.* (2020), who demonstrated that subclinical hypocalcemia in the prepartum period significantly impairs uterine smooth muscle contractility and immune cell function, thereby predisposing cows to retained fetal membranes. Furthermore, Esposito *et al.* (2014) had highlighted that the interactions between metabolic diseases and immune response are particularly critical in transition dairy cows, where hypocalcemia contributes to immunosuppression and increases susceptibility to uterine infections. These mechanisms explain how hypocalcemia predisposes cows to retained placenta and metritis.

Our findings are in agreement with the results of Karimi Dehkordi and Yahyaei (2020), who evaluated the diagnostic value of calcium concentrations during the transition period in Holstein cows. They demonstrated that lower calcium levels were significantly associated with reduced fertility, including prolonged days open and lower conception rates at first service, as well as increased risk of postpartum disorders. These results highlighted that calcium status

Table 1. Serum calcium levels in healthy and diseased cows

Disease	Group	Number	Prepartum (mg/dL)	At parturition (mg/dL)	P-value
MET	Healthy	72	8.50 ± 0.06	8.09 ± 0.07	0.005
	Diseased	10	8.30 ± 0.12	7.40 ± 0.15	
RP	Healthy	74	8.50 ± 0.06	8.00 ± 0.07	0.003
	Diseased	8	8.20 ± 0.10	7.30 ± 0.10	
DYS	Healthy	78	8.50 ± 0.06	8.00 ± 0.07	0.3
	Diseased	4	8.40 ± 0.10	7.60 ± 0.40	
OC	Healthy	60	8.40 ± 0.06	8.00 ± 0.06	0.9
	Diseased	22	8.60 ± 0.10	8.00 ± 0.20	
Unhealthy	Healthy	58	8.50 ± 0.08	8.10 ± 0.08	0.01
	Diseased	24	8.30 ± 0.07	7.60 ± 0.09	

MET = metritis; RP = retained placenta; DYS = dystocia; OC = ovarian cysts; Diseased = cows affected by at least one of the defined disorders.

Table 2. ROC analysis of serum calcium levels

Disease	Critical Threshold[1]	Se (%)	Sp (%)	SE	AUC	P-value
			Prepartum			
MET	8.55	80	47.2	0.115	0.667	0.2
DYS	8.6	100	41	0.092	0.557	0.7
RP	8.4	75	89.2	0.038	0.946	0.004
OC	8.65	63.6	40	0.104	0.438	0.5
Unhealthy	8.6	83.3	48.3	0.085	0.651	0.1
			At Parturition			
MET	7.85	80	72.2	0.066	0.833	0.006
DYS	8	100	33.3	0.188	0.712	0.3
RP	7.5	75	59.5	0.104	0.706	0.1
OC	7.9	45.5	60	0.124	0.511	0.9
Unhealthy	7.9	91.7	79.3	0.053	0.879	<0.001

[1]Highest combined specificity (SP) and sensitivity (Se), mg/dL, Se: epidemiologic sensitivity, Sp: epidemiologic specificity, SE: standard error, AUC: area under the curve

serves as a reliable predictor of reproductive performance, further supporting the predictive role of calcium observed in the present study.

In terms of glucose, our analysis revealed a different pattern for glucose metabolism. Although mean serum glucose concentrations at parturition were lower in the diseased group (57.3 ± 3.2 mg/dL) compared to controls (63.9 ± 2.1 mg/dL), this difference showed only a strong trend without reaching statistical significance ($p = 0.07$). ROC analysis, however, identified prepartum glucose <49.5 mg/dL as a significant predictor of dystocia (AUC = 0.917), with 100% sensitivity and 89.7% specificity, increasing the odds by 8-fold (OR = 8.0; 95% CI: 2.5–25.6; $p \leq 0.05$).

Although in the present study, the mean serum glucose concentrations did not differ significantly between healthy cows and those affected by postpartum disorders, ROC analysis revealed that a specific glucose cut-off could serve as a strong predictor of dystocia. This apparent discrepancy is logical, as mean comparisons only capture overall differences between groups and may fail to reach significance when substantial overlap exists within the physiological range. By contrast, ROC analysis evaluates the full distribution of data and identifies a threshold at which sensitivity and specificity are maximized (Zhou *et al.* 2014).

Low glucose levels may be related to the negative energy balance common in high-producing dairy cows around calving, which has been shown to predispose cows to uterine diseases (Serbetci *et al.* 2024) and impair fertility (Ahmed *et al.* 2017). Elevated glucose concentrations postpartum have been linked to metritis (Bicalho *et al.* 2017), supporting the role of glucose as a marker of energy balance and reproductive performance. Our finding that low prepartum glucose predicted dystocia aligned with these earlier observations and highlighted the metabolic vulnerability of cows during this period. The importance of prepartum glucose levels, identified in

Table 3. Serum glucose levels in healthy and diseased cows

Disease	Group	n	Prepartum (mg/dL)	At Parturition (mg/dL)	P-value
MET	Healthy	72	63.10 ± 2.30	62.60 ± 1.90	0.3
	Diseased	10	64.80 ± 5.30	57.80 ± 4.60	
RP	Healthy	74	63.00 ± 2.10	62.20 ± 1.80	0.5
	Diseased	8	66.20 ± 8.40	60.20 ± 7.90	
DYS	Healthy	78	68.10 ± 2.10	62.60 ± 1.80	0.06
	Diseased	4	64.00 ± 1.00	49.00 ± 0.10	
OC	Healthy	60	63.50 ± 2.50	62.10 ± 2.10	0.8
	Diseased	22	62.60 ± 4.00	61.70 ± 3.70	
Unhealthy	Healthy	58	64.20 ± 2.50	63.90 ± 2.10	0.07
	Diseased	24	61.00 ± 4.00	57.30 ± 3.20	

MET = metritis; RP = retained placenta; DYS = dystocia; OC = ovarian cysts; Diseased = cows affected by at least one of the defined disorders.

Table 4. ROC analysis of serum glucose levels

Disease	Critical threshold[1]	Se (%)	Sp (%)	SE	AUC	P-value
Prepartum						
MET	72	80	30.6	0.115	0.436	0.6
DYS	49.5	100	89.7	0.045	0.917	0.04
RP	57	50	56.8	0.160	0.443	0.7
OC	62.5	63.6	53.3	0.102	0.515	0.8
Unhealthy	63.5	66.7	48.3	0.102	0.586	0.3
At Parturition						
MET	63	80	47.2	0.118	0.619	0.3
DYS	53	100	76.9	0.051	0.897	0.06
RP	63	75	45.9	0.051	0.897	0.06
OC	64.5	72.7	43.3	0.106	0.524	0.8
Unhealthy	56	66.7	69	0.097	0.097	0.07

[1]Highest combined specificity (SP) and sensitivity (Se), mg/dL, Se: epidemiologic sensitivity, Sp: epidemiologic specificity, SE: standard error, AUC: area under the curve

this study for predicting dystocia was consistent with the research by Akhtar *et al.* (2022), who demonstrated that supplementation of glucose precursors in the peripartal period can improve metabolic health and performance in dairy cows.

Furthermore, metabolic indicators such as ketone bodies have been shown to influence ovarian function and reproductive hormones (Pishvaei *et al.* 2021), while thresholds of NEFA and BHBA were reported as predictors of poor reproductive outcomes (Karimi Dehkordi *et al.* 2016). These findings further emphasized the importance of considering glucose alongside other energy metabolites in reproductive health monitoring.

In our study, no significant associations were found between serum glucose or calcium levels and the development of ovarian cysts. This suggested that the etiology of this condition may be more influenced by hormonal imbalances in the weeks following calving rather than immediate periparturient metabolic status. However, considering that energy balance and metabolic stress strongly influence ovarian function (Bruinjé and LeBlanc, 2025), it is possible that indirect effects of metabolites contribute over time.

Recent research indicates that insulin resistance and adipokine levels play a crucial role in the pathogenesis of ovarian cysts. Specifically, alterations in insulin signaling pathways have been associated with impaired follicular development and steroidogenesis, leading to cyst formation (Gareis *et al.* 2023). Additionally, the expression of adiponectin and its receptors in ovarian follicle has been shown to influence reproductive function, with lower levels potentially contributing to ovarian dysfunction (Forootan *et al.* 2020). These findings highlight the importance of considering both metabolic and hormonal interactions over simple metabolic markers when assessing the risk of ovarian cysts in dairy cows.

This study demonstrated that calcium status around calving plays a pivotal role in determining postpartum reproductive health in multiparous Holstein cows and can serve as a reliable biomarker for predicting several common disorders. Specifically, calcium emerged as the primary biomarker for predicting metritis and retained placenta with the following accuracy: prepartum calcium < 8.4 mg/dL predicted retained placenta with excellent accuracy (AUC = 0.946, sensitivity = 75%, specificity = 89.2%, odds ratio = 11.0); calcium at parturition < 7.85 mg/dL predicted metritis with good accuracy (AUC = 0.833, sensitivity = 80%, specificity = 72.2%, odds ratio = 10.0); and calcium at parturition < 7.9 mg/dL predicted overall morbidity (presence of at least one disorder) with high accuracy (AUC = 0.879, sensitivity = 91.7%, specificity = 79.3%, odds ratio = 7.0). In contrast, glucose showed limited predictive value for most reproductive disorders; however, prepartum glucose < 49.5 mg/dL could serve as a useful biomarker for predicting dystocia with excellent accuracy (AUC = 0.917, sensitivity = 100%, specificity = 89.7%, odds ratio = 8.0). For ovarian cysts, neither calcium nor glucose demonstrated significant predictive ability, indicating that the metabolic biomarkers measured in this study are not suitable predictors for this disorder. These findings underscore the value of ROC-derived cutoffs for early risk stratification, which might be overlooked by simple group mean comparisons. From a practical perspective, routine monitoring of calcium dynamics, alongside prepartum glucose assessment, can support timely intervention strategies aimed at reducing postpartum complications, improving reproductive performance, and enhancing overall herd productivity.

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