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Hematological profile and CBC-derived inflammatory indices in clinically healthy Holstein cows during the transition period

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Summary

The transition period is characterized by physiological, metabolic, and immunological adaptations that influence hematological parameters in dairy cows. This study evaluated changes in complete blood count (CBC) parameters and CBC-derived inflammatory indices in clinically healthy Holstein cows during the transition period. Twenty clinically healthy multiparous Holstein cows were evaluated longitudinally. Blood samples were collected during late gestation, on the day of calving, and at 20 days postpartum. Hematological parameters and CBC-derived inflammatory indices were analyzed using an automated veterinary hematology analyzer. Data were analyzed using the Friedman test followed by Wilcoxon signed-rank post hoc comparisons with Holm-Bonferroni correction. Red blood cell count, hemoglobin, hematocrit, and mean corpuscular volume decreased significantly during early lactation, whereas mean corpuscular hemoglobin concentration increased postpartum. Red cell distribution width was highest during late pregnancy. The monocyte-to-lymphocyte ratio and large immature cell percentage peaked at calving, whereas the platelet-to-lymphocyte ratio increased postpartum. No significant changes were detected in the remaining inflammatory indices. Clinically healthy Holstein cows undergo physiological hematological adaptations during the transition period. Changes in erythrocyte parameters, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and large immature cell percentage may provide supportive information for monitoring physiological responses around calving.

Keywords: complete blood count, CBC-derived inflammatory indices, hematology Holstein cows, transition period, dairy cattle

The transition period, encompassing late pregnancy, parturition, and the onset of lactation, represents one of the most critical physiological stages in dairy cows. The transition period is generally defined as the three weeks before and three weeks after parturition (4). During this period, cows undergo profound metabolic, endocrine, and immunological adaptations associated with the transition from the dry period to lactation (24). Rapid fetal growth during late gestation induces marked physiological adjustments to meet the increasing nutritional and metabolic demands of both the dam and the developing fetus (2, 23, 29). Although parturition is a natural physiological event, it imposes considerable physiological stress and requires extensive metabolic adaptation, some of which begins before calving (4). Following calving, abrupt physiological changes accompany the initiation of lactation, increasing the susceptibility of dairy cows to metabolic and infectious diseases during this critical stage (10, 14).

Assessment of hematological profiles is widely used to evaluate systemic health status and physiological alterations associated with different reproductive stages in dairy cows (5). In recent years, hematological analysis has become an indispensable diagnostic tool in bovine medicine (9). In clinical practice, hematological parameters are interpreted together with complementary diagnostic findings to assess production- and reproduction-related disorders. Accurate interpretation of these parameters may provide valuable information for the early detection of disease, monitoring of physi-

ological adaptation, and assessment of prognosis (12). In addition to conventional hematological parameters, complete blood count (CBC)-derived inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the mean platelet volume-to-platelet ratio (MPV/PLT), have recently gained increasing attention as practical indicators of systemic inflammatory responses. Parturition is accompanied by marked endocrine and immune adaptations, including changes in circulating neutrophil numbers and function (15, 25). These hematological alterations may also be reflected in CBC-derived indices, such as NLR and PLR, which have been investigated in transition dairy cows in relation to inflammatory and health status (8). In recent years, studies have been conducted on the use of inflammatory indices obtained from complete blood counts as novel predictive and prognostic markers in various diseases and pathological conditions in humans, dogs, and cows (8, 16, 27).

In cattle, CBC-derived inflammatory indices have also been investigated in different clinical and reproductive contexts (3, 13). However, studies evaluating CBC-derived inflammatory indices longitudinally throughout the transition period remain scarce. A better understanding of these physiological variations may improve the interpretation of hematological findings during the transition period and facilitate the differentiation of normal adaptive responses from pathological alterations. Therefore, it was hypothesized that hematological parameters and CBC-derived inflammatory indices vary according to reproductive stage, reflecting physiological adaptations that occur throughout the transition period. Accordingly, the aim of this study was to investigate changes in hematological parameters and CBC-derived inflammatory indices in clinically healthy Holstein cows during late pregnancy, parturition, and early lactation.

Material and methods

This prospective observational study was conducted on 20 clinically healthy Holstein cows from a private dairy farm located in Tekirdağ Province, Türkiye. The cows had a mean age of 3.33 ± 1.45 years, a mean parity of 2.07 ± 1.10, a mean daily milk yield of 30.73 ± 3.59 kg/day, and a mean body condition score (BCS) of 3.50 ± 0.19. Pregnancy status and gestational stage were confirmed by ultrasonographic examination (Hasvet WED-3100V; Hasvet, Istanbul, Türkiye), and cows in the last three weeks of gestation were enrolled in the study. Blood sampling was performed between January and February 2026. All animals were housed in free-stall barns under identical management conditions, received the same nutritional and reproductive management, and had *ad libitum* access to transition-period total mixed rations (TMR) and fresh drinking water. Total mixed rations were offered twice daily, and cows were milked twice daily using a DeLaval milking system.

Cows showing clinical signs of systemic or reproductive disease, including dystocia, retained fetal membranes, mastitis, lameness, or other clinically detectable disorders, were excluded from the study.

Ethical approval was obtained from the Tekirdağ Namık Kemal University Local Animal Experiments Ethics Committee (Approval No. T2026-2913, dated 15 January 2026). Written informed consent for blood collection was obtained from the farm owner before the initiation of the study.

The first blood samples were collected into EDTA tubes immediately after ultrasonographic examination at a mean of 20 ± 5 days before parturition. The second blood samples were collected on the day of calving, and the third samples were obtained at 20 days postpartum (early lactation). At all sampling time points, blood samples were collected into lavender-top blood collection tubes containing K3-EDTA. All samples were transported to the laboratory within 2 hours of collection and analyzed using an automated hematology analyzer (Hasvet VH5R; Hasvet, Istanbul, Türkiye).

The following hematological parameters were measured: red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV), white blood cell count (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BASO), platelet count (PLT), and mean platelet volume (MPV).

Complete blood count-derived inflammatory indices were calculated using absolute cell counts. The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The platelet-to-lymphocyte ratio (PLR) was calculated by dividing the absolute platelet count by the absolute lymphocyte count, whereas the platelet-to-neutrophil ratio (PNR) was obtained by dividing the absolute platelet count by the absolute neutrophil count. The monocyte-to-lymphocyte ratio (MLR) was calculated by dividing the absolute monocyte count by the absolute lymphocyte count. In addition, atypical lymphocytes (ALY; ALY_count and ALY_pct), automatically identified by the analyzer, were also recorded and evaluated.

An a priori sample size calculation was performed using the GPower software. Because GPower does not provide a direct power analysis option for the Friedman test, the sample size calculation was performed using the closest parametric approximation, namely the repeated-measures analysis of variance (repeated-measures ANOVA) model. The analysis assumed an effect size of $f = 0.50$, a significance level of $\alpha = 0.05$, a statistical power of 80%, one experimental group, three repeated measurements, a nonsphericity correction (ε) of 1, and a conservative correlation between repeated measurements. Based on these assumptions, the minimum required sample size was calculated as 20 animals.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro-Wilk test. As the data were not normally distributed, repeated measurements were analyzed using the Friedman

test. When a significant overall difference was detected, pairwise comparisons were performed using the Wilcoxon signed-rank test with Holm-Bonferroni correction. Results are presented as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$.

Results and discussion

Significant differences were observed in several hematological parameters across the evaluated reproductive periods (Tab. 1 and 2).

Among erythrocyte parameters, RBC, HGB, and HCT values differed significantly between the study periods ($p < 0.01$; Tab. 1). Pairwise comparisons demonstrated that RBC values were significantly lower during early lactation than during both pregnancy ($p = 0.002$) and parturition ($p = 0.040$). Similarly, HGB and HCT values were significantly lower during early lactation than during pregnancy (Tab. 2).

MCV also differed significantly between the study periods, with lower values observed during early lactation than during pregnancy and parturition, whereas MCHC showed the opposite pattern and was significantly higher during early lactation ($p < 0.05$; Tab. 1). Pairwise comparisons confirmed these differences (Tab. 2). No significant differences were detected in MCH ($p > 0.05$). RDW-CV differed significantly between the reproductive periods, with the highest values recorded during pregnancy. This difference was primarily attributable to the pregnancy–parturition comparison ($p = 0.008$; Tab. 2).

Among leukocyte-related parameters, ALY values were significantly lower during early lactation than during pregnancy and parturition ($p < 0.05$; Tab. 1). BASO counts and the monocyte-to-lymphocyte ratio (MLR) were significantly lower during pregnancy than during parturition (Tab. 2). No significant differences

Tab. 1. Hematological parameters in cows during pregnancy (pre), parturition (mid), and lactation (post) periods

Parameter	Unit	Pregnancy (mean ± SD)	Parturition (mean ± SD)	Lactation (mean ± SD)	p-value	Kendall's W
WBC	10^9/L	6.86 ± 1.70	6.89 ± 2.46	7.28 ± 1.38	0.420	0.058
LYM_pct	%	49.95 ± 7.75	45.41 ± 10.99	47.14 ± 7.44	0.282	0.084
MON_pct	%	9.77 ± 1.45	13.58 ± 5.06	10.52 ± 1.86	0.091	0.160
NEU_pct	%	37.91 ± 8.27	39.80 ± 12.76	40.47 ± 7.12	0.549	0.040
EOS_pct	%	2.31 ± 2.03	0.92 ± 0.71	1.76 ± 1.89	0.127	0.138
BASO_pct	%	0.065 ± 0.053	0.151 ± 0.116	0.115 ± 0.089	0.101	0.153
LYM_count	10^9/L	3.381 ± 0.893	3.038 ± 0.880	3.385 ± 0.630	0.165	0.120
MON_count	10^9/L	0.665 ± 0.185	0.886 ± 0.413	0.766 ± 0.208	0.420	0.058
NEU_count	10^9/L	2.659 ± 0.978	2.975 ± 1.664	3.002 ± 1.041	0.189	0.111
EOS_count	10^9/L	0.148 ± 0.132	0.062 ± 0.053	0.116 ± 0.120	0.127	0.138
BASO_count	10^9/L	0.0038 ± 0.0033	0.0090 ± 0.0068	0.0079 ± 0.0068	0.044*	0.208
MLR	ratio	0.198 ± 0.033	0.291 ± 0.096	0.232 ± 0.067	0.027*	0.241
NLR	ratio	0.799 ± 0.287	0.980 ± 0.510	0.910 ± 0.361	0.138	0.132
ALY_pct	%	0.711 ± 0.537	0.557 ± 0.298	0.430 ± 0.160	0.057	0.191
ALY_count	10^9/L	0.0418 ± 0.0154	0.0358 ± 0.0178	0.0305 ± 0.0117	0.031*	0.231
RBC	10^{12}/L	5.94 ± 0.48	5.93 ± 1.36	5.06 ± 0.51	<0.001***	0.573
HGB	g/dL	9.89 ± 0.68	9.31 ± 1.41	8.24 ± 0.94	<0.001***	0.516
HCT	%	29.33 ± 2.12	27.27 ± 5.58	23.41 ± 2.76	0.001**	0.458
MCV	fL	49.57 ± 3.72	49.05 ± 5.21	46.41 ± 4.06	0.002**	0.404
MCH	pg	16.64 ± 1.26	16.43 ± 2.02	16.27 ± 1.53	0.178	0.115
MCHC	g/dL	33.68 ± 0.61	33.57 ± 1.56	35.19 ± 0.84	<0.001***	0.713
RDW_CV	%	18.92 ± 0.87	18.38 ± 1.01	18.29 ± 1.36	0.016*	0.275
PLT	10^9/L	242.67 ± 109.27	261.20 ± 119.53	331.47 ± 152.66	0.041*	0.213
PNR	ratio	92.90 ± 46.53	113.79 ± 65.59	118.54 ± 59.45	0.127	0.138
PLR	ratio	69.36 ± 27.51	86.87 ± 30.24	98.06 ± 43.80	0.031*	0.231
MPV	fL	9.28 ± 0.81	8.18 ± 0.98	7.88 ± 0.86	<0.001***	0.636
LIC_pct	%	0.329 ± 0.161	0.932 ± 1.057	0.255 ± 0.273	0.127	0.138
LIC_count	10^9/L	0.0235 ± 0.0142	0.0718 ± 0.0981	0.0182 ± 0.0191	0.155	0.124

Explanations: Data are presented as Mean ± SD. Comparisons between periods were performed using the Friedman test. Effect size was expressed as Kendall's W. * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$.

Tab. 2. Pairwise comparisons for parameters found significant in the Friedman test (Wilcoxon signed-rank test; Holm-Bonferroni adjusted p-values) and effect size (r)

Parameter	Unit	Comparison	Z	p-value	r
BASO	%	Pregnancy – Parturition	–2.4815	0.039*	0.6407
MLR	ratio	Pregnancy – Parturition	–2.7264	0.019*	0.7039
RBC	10^{12}/L	Pregnancy – Lactation	–3.3794	0.002**	0.8726
RBC	10^{12}/L	Parturition – Lactation	–2.4706	0.040*	0.6379
HGB	g/dL	Pregnancy – Lactation	–3.0985	0.006**	0.8000
HCT	%	Pregnancy – Lactation	–3.3226	0.003**	0.8579
MCV	fL	Pregnancy – Lactation	–2.9818	0.009**	0.7699
MCV	fL	Parturition – Lactation	–2.4143	0.047*	0.6234
MCHC	g/dL	Pregnancy – Lactation	–3.2652	0.003**	0.8431
MCHC	g/dL	Parturition – Lactation	–3.3801	0.002**	0.8727
RDW_CV	%	Pregnancy – Parturition	–3.0126	0.008**	0.7779
PLT	10^9/L	Pregnancy – Lactation	–2.5274	0.034*	0.6526
MPV	fL	Pregnancy – Parturition	–3.2063	0.004**	0.8569
MPV	fL	Pregnancy – Lactation	–3.1458	0.005**	0.8408

Explanations: The Wilcoxon signed-rank test was applied. p-values were adjusted for multiple comparisons using the Holm correction. Effect size was calculated using the formula $r = Z/\sqrt{n}$ (n = 20). * – $p < 0.05$, ** – $p < 0.0$

were observed for the remaining leukocyte parameters ($p > 0.05$).

Platelet-related parameters also exhibited significant changes across the reproductive periods. PLT values were significantly higher during early lactation than during pregnancy ($p < 0.05$; Tab. 1), while pairwise analysis confirmed a significant increase in PLT ($p = 0.034$; Tab. 2). In contrast, MPV values were significantly higher during pregnancy than during both parturition and early lactation (Tab. 2). No significant differences were found for PNR, LIC%, or LIC between the three periods ($p > 0.05$). PLR differed significantly in the overall Friedman test, but no pairwise comparison remained statistically significant after Holm-Bonferroni correction.

The transition period is considered one of the most critical stages in the life cycle of dairy cows. During this phase, which marks the onset of lactation, cows undergo substantial physiological adaptations to support the demands of milk production (7). Beyond influencing clinical status, the transition period has a pronounced impact on hematological parameters (6). The findings of the present study contribute to understanding these physiological alterations.

In the current study, erythrocyte count (RBC), hemoglobin (HGB), and hematocrit (HCT) levels were significantly lower during early lactation compared with late pregnancy and parturition. Similar observations have been reported by Skrzypczak et al. (28), who described higher RBC and HGB values during late gestation. Likewise, Moretti et al. (19) and Tsiamadis et al. (30) reported elevated RBC, HGB, and HCT levels at calving compared with the lactation period. Acute stress responses occurring at parturition may temporarily preserve hematological values, but increases in plasma volume (hemodilution) and blood loss around calving are known to contribute to subsequent declines in RBC, HGB, and HCT (24). Elevated erythrocyte-related parameters during late gestation may be associated with increased oxygen demand required for fetal development. Expansion of blood volume during pregnancy and the resulting increase in vascular capacity may also contribute to higher HGB and MCH levels in late gestation (21). In the present study, MCHC, an indicator of hemoglobin concentration within erythrocytes was significantly higher during lactation than during pregnancy and parturition. Comparable findings were reported by Rafia et al. (26), who observed decreased RBC, HGB, and HCT but increased MCHC during early lactation, which suggests a post-calving tendency toward normocytic normochromic anemia, potentially associated with metabolic disturbances and nutritional deficiencies. RDW-CV values were higher during pregnancy compared with other periods in the current study. Increased RDW during late gestation has been linked to enhanced erythropoietic activity (11).

In the present study, leukocyte and neutrophil counts increased during the postpartum period, whereas lymphocyte and eosinophil counts were higher during pregnancy, and monocyte counts increased at parturition, but no significant differences were detected when periods were compared overall. Similar findings were reported by Oliveira et al. (22), who observed elevated lymphocyte and eosinophil counts during pregnancy and increased monocyte counts at calving. Nazifi et al. (20) also reported higher leukocyte and neutrophil counts during pregnancy, attributing these changes to gestational stress. Margarito Torres-Chablé et al. (17) documented increased neutrophil and monocyte counts at parturition and associated these findings with neutrophil migration toward the uterine lumen and the mammary gland. Basophil counts were lower during pregnancy compared with parturition in the current study. Pregnancy has been suggested to modulate immune regulation and leukocyte subpopulation dynamics (31). Therefore, the reduction in basophil numbers during pregnancy may reflect maternal immune adaptation. Lower ALY counts observed during lactation compared with pregnancy and parturition may be associated with attenuation of postpartum inflammatory responses and suppression of immune cell activity during metabolic adaptation.

Previous reports indicate that pregnant cows may exhibit significantly lower platelet counts compared with non-pregnant cows (21). Consistent with these findings, PLT values were higher in lactating cows than in pregnant animals in the present study. Platelet counts may be influenced by several physiological changes during pregnancy, and the increase in plasma volume supporting fetal development may contribute to reductions in PLT levels (1).

The monocyte-to-lymphocyte ratio (MLR), an index based on complete blood count (CBC) data, reflects changes in leukocyte distribution and serves as an indicator of the balance between systemic inflammation and the immune response. This study found that the ratio in question shows a significant increase during parturation. The parturition process is a complex period associated with physiological stress and the reorganization of immune responses, which may lead to changes in leukocyte subpopulations. Therefore, it is thought that MLR may provide supporting information for monitoring changes in leukocyte dynamics associated with parturition in dairy cows. No literature investigating the relationship between MLR and parturition was found.

In the present study, the higher platelet-to-lymphocyte ratio (PLR) observed during lactation compared with the other reproductive periods may be associated with the physiological and metabolic adaptations occurring during this period. Lactation is characterized by increased energy requirements and metabolic burden related to milk production, and alterations as well as adaptive regulations in immune system functions have been reported during this period. Therefore, the increased PLR values observed during lactation may be related to the metabolic burden imposed by milk production and the accompanying changes in immune responses (18).

Physiological changes in platelet production and related hematological indices may occur in dairy cows, particularly during the late stages of pregnancy. These changes have been reported to be part of the adaptive responses developing during the periparturient period. Data on mean platelet volume (MPV) in dairy cows are limited. In the present study, the higher MPV values observed during pregnancy may reflect alterations in platelet production and activation associated with the physiological adaptation process induced by pregnancy (18). MPV is a parameter reflecting platelet size, reactivity, and thrombopoiesis, but the available information regarding its physiological changes in pregnant dairy cows is still limited. In this study, the increase in MPV values observed during late pregnancy indicates an acceleration of platelet turnover. This likely reflects an increased release of larger and physiologically more active young platelets from the bone marrow into the systemic circulation. These changes represent a physiological adaptation mechanism – developed to compensate for peripheral consumption and optimize hemostatic readiness during late pregnancy – and suggest a state of increased platelet production and functional activation (18).

The present study has several limitations. The relatively small sample size and the inclusion of animals from a single farm limit the generalizability of the findings. In addition, the sampling schedule may not have fully captured acute physiological changes occurring during the early postpartum period. The absence of biochemical, metabolic, and inflammatory parameters, such as non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), calcium, cortisol, haptoglobin, fibrinogen, C-reactive protein, and cytokines, also limits the interpretation of the findings. Therefore, the current findings should be interpreted as preliminary and exploratory observations and should be validated by more comprehensive studies involving larger sample sizes, multiple farms, more frequent sampling, and additional metabolic and inflammatory biomarkers.

In conclusion, this study demonstrated that hematological parameters and CBC-derived inflammatory indices evaluated during pregnancy, parturition, and early lactation may provide supportive information for monitoring physiological adaptation processes associated with the transition period in dairy cattle. Given the limited sample size and the absence of direct metabolic and inflammatory validation, these findings should be interpreted as preliminary. Complete blood count parameters may be considered practical, low-cost, and easily accessible supportive tools in the assessment of transition-period changes. These findings, however, should be confirmed by further studies conducted with larger sample sizes, multiple farms, more frequent postpartum sampling, and additional metabolic and inflammatory biomarkers.

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