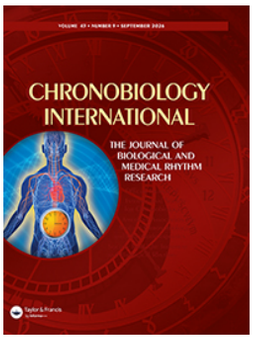

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Seasonal modulation of platelet indices in mares

Katiuska Satué[a], Deborah La Fauci[b], Rocío Colomer-Selva[a], Cristina Cravana[b], and Esterina Fazio[b]

[a]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, CEU-Cardenal Herrera University, Valencia, Spain; [b]Department of Veterinary Sciences, Veterinary Physiology Unit, Polo Universitario Annunziata, Messina, Italy

ABSTRACT

Platelet indices provide valuable information on platelet activation, heterogeneity, and production kinetics, yet their physiological variability remains poorly characterized in horses. Although seasonal changes in platelet count have been described, the seasonal modulation of derived platelet indices in mares remains poorly understood. The aim of this study was to evaluate the seasonal variation of platelet count and derived platelet indices in clinically healthy mares from the Valencia region (Spain), and to assess their interrelationships. Forty clinically healthy Spanish Purebred mares (6–12 y) were monitored over one year under natural environmental conditions. Monthly blood samples were collected and analyzed for platelet count (PLT) and derived indices using an automated hematology analyzer. Seasonal effects were evaluated using non-parametric repeated-measures analysis. Significant seasonal variation was observed in most platelet indices ($p < 0.05$), while PCT and aggregation index remained unchanged. Platelet count was lowest in autumn, whereas indices reflecting platelet size and heterogeneity increased during both warm and cold seasons. Correlation analysis revealed consistent associations among platelet size, mass, and heterogeneity, as well as inverse relationships between platelet density and activation-related variables. These findings demonstrate a clear seasonal modulation of platelet dynamics in mares, likely reflecting adaptive responses to environmental conditions. The results highlight the importance of considering seasonal variation when interpreting platelet indices and support the need for season-adjusted reference intervals in clinical practice.

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Platelet indexes; mares; seasonal variations

Introduction

Beyond their classical involvement in hemostasis, platelets in horses are increasingly recognized as active participants in inflammation, immune responses, thrombosis, and tissue repair. Their quantitative and morphological characteristics—captured through routinely available platelet indices such as plateletcrit (PCT), mean platelet volume (MPV), platelet large cell ratio (P-LCR), platelet distribution width (PDW), mean platelet component (MPC), mean platelet mass (MPM), platelet component distribution width (PCDW), and platelet mass distribution width (PMDW)—provide valuable insights into platelet activation status, heterogeneity, and bone marrow production kinetics. Notably, MPC has been validated as a biomarker of platelet activation in both foals and adult horses (Segura et al. 2007), and increasing evidence shows that platelet aggregation dynamically mirrors inflammatory–like states in the species (Arfuso et al. 2024). In equine medicine, these indices have shown clinical potential for diagnosis, prognosis, and monitoring of systemic inflammatory, infectious, endocrine, and coagulopathic disorders, where alterations in PLT size, density, or mass may accompany disease progression. However, despite their diagnostic value, the physiological behavior of equine PLT indices—and the environmental factors that modulate them—remains poorly characterized. Their routine use in horses is still limited because fundamental aspects of their variability, reference patterns, and influencing factors have not been fully established. This gap is particularly relevant in the context of seasonal physiology, which affects numerous hematologic parameters in mammals but has never been explored in detail for derived platelet indices in horses.

Seasonal variation is a well-recognized driver of physiological change, influencing temperature-dependent metabolism, photoperiod-driven endocrine rhythms, nutritional balance, and pathogen exposure. These factors can modulate thrombopoiesis and produce

CONTACT Katiuska Satué ksatue@uchceu.es Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, CEU-Cardenal Herrera University, Tirant Lo Blanc, 7, Alfara Del Patriarca, Valencia 46115, Spain.

characteristic seasonal PLT patterns across species (Giri et al. 2017; Nathawat et al. 2023; Nimra et al. 2023; Kausar et al. 2025). This is especially relevant in horses, whose environmental exposure, management, and exercise conditions vary markedly throughout the year (Satué et al. 2014; Deniz et al. 2024; Czech et al. 2025).

Studies investigating the seasonal influence on hematological parameters in horses remain limited, and existing literature reveals notable variability in PLT behavior. In Shetland ponies, modest but significant seasonal changes in PLT have been reported, while MPV remained unaffected (Shawaf et al. 2018). Seasonal fluctuations in PLT have also been described in broodmares (Satué et al. 2014), and Aragona et al. (2023) documented both circadian rhythmicity and seasonal variation in equine PLT, underscoring the sensitivity of platelet dynamics to environmental conditions. However, findings across equine studies are inconsistent. In mixed-breed horses, PLT and related hematological variables appear stable throughout the year (Dmoch et al. 2008), and similar stability has been observed in Noma and traction horses, where only PLT was measured (Souza et al. 2018; Ono et al. 2021). In recreational horses, Andriichuk and Tkachenko (2015) reported seasonal variation in hematological parameters, including PLT, while Zakari et al. (2015) described comparable patterns in donkeys. Notably, all these studies evaluated only conventional hematologic variables and did not include derived platelet indices. Together, this body of work illustrates that previous research in equids has focused almost exclusively on platelet count, leaving the seasonal behavior of detailed morphometric and functional platelet indices largely unexplored.

Taking together, current evidence suggests that some equine populations experience measurable seasonal modulation of thrombopoiesis, whereas others maintain stable platelet profiles throughout the year. Importantly, most studies have examined only platelet count (PLT), and none has assessed the seasonal behavior of derived platelet indices—such as MPV, PDW, PCT, P-LCR, MPC, MPM, PCDW, or PMDW—in horses. Moreover, no work to date has specifically examined these indices in broodmares. This represents a critical gap in understanding how seasonality may influence platelet mass, heterogeneity, and activation dynamics in adult female horses. We hypothesized that platelet indices in broodmares—whose seasonal patterns have never been characterized—would show distinct and predictable fluctuations across seasons, consistent with environmentally driven modulation of thrombopoiesis.

Environmental conditions are increasingly influenced by global climate change, leading to greater variability in temperature and seasonal patterns. These changes may have important implications for equine physiology, particularly in regions with marked climatic fluctuations. In this context, understanding how environmental factors affect hematological parameters is essential for the accurate interpretation of laboratory results and may contribute to explaining seasonal variability in platelet indices in mares from the Valencia region (Spain).

The aim of this study was to evaluate the seasonal behavior of PLT indices in mares, providing the first comprehensive description of their physiological modulation throughout the year.

Material and Methods

Animals

This descriptive longitudinal study was conducted during the 2020 and 2021 breeding seasons at four stud farms located within the Valencia region (Valencian Community, Spain) (39°25′33.60″ N, 0°46′30.00″ W).

All procedures complied with Spanish animal welfare legislation (Royal Decree 37/2014). The Animal Ethics Committee at CEU-Cardenal Herrera University determined that formal approval was not required, as sampling formed part of routine clinical reproductive evaluation (CEEA 22/01). Written informed consent was obtained from all owners.

The study population consisted of 40 healthy Spanish Purebred mares, aged 6–12 y. Inclusion criteria required that animals were appropriately vaccinated and dewormed, showed no reproductive abnormalities or systemic disease on physical examination, had no recent history of illness, and had not received medication capable of altering platelet number, activation or morphology during the three months preceding blood sampling (e.g. non-steroidal anti-inflammatory drugs, corticosteroids, anticoagulants, antiplatelet agents, immunosuppressive drugs, or treatments affecting bone marrow function). Body condition score (BCS) was assessed at the beginning of the study using the Henneke BCS system (Henneke et al. 1983), and all mares presented a BCS between 5 and 6, indicating moderate to moderately fleshy condition.

All animals were managed under similar husbandry conditions. Mares were housed in individual stalls but had unrestricted, year-round access to outdoor paddocks, and were fed with mixed grains and alfalfa hay in amounts adjusted to their nutritional requirements. Concentrate was provided in two daily meals of 4–6 kg, and fiber was supplied as 2–3 kg of alfalfa hay and straw. Water was available *ad libitum*, and mares had free access to mineral blocks. All handling procedures were

carried out calmly to minimize stress during sampling and routine reproductive examinations.

Seasonal Grouping of Data

To assess seasonal variation in platelet indices, data were grouped according to climatic seasons adapted to the environmental conditions of Valencia (Spain), based on official meteorological records provided by AEMET (Agencia Estatal de Meteorología, Comunidad Valenciana). Environmental variables, including temperature, relative humidity, and photoperiod, were obtained from these records (Table 1). This approach reflects the actual environmental conditions experienced by mares and provides a physiologically relevant framework for interpreting hematologic changes.

Based on the monthly meteorological records, average seasonal temperature, relative humidity, and photoperiod were 12.0°C, 69.7% and 6 h 33 min in winter; 19.8°C, 64.3% and 8 h 44 min in spring; 25.5°C, 57.3% and 11 h 22 min in summer; and 18.7°C, 69.7%, and 7 h 17 min in autumn, respectively.

Four climate-based seasons were defined according to the meteorological patterns of the Valencia region (Spain):

- Winter (15 December–15 March): Corresponds to the coolest and most humid part of the year, with the lowest ambient temperatures and a higher incidence of respiratory and inflammatory challenges.
- Spring (15 March–31 May): A warm transitional period marked by rising temperatures, increasing photoperiod and relatively stable environmental conditions, generally associated with favorable physiological status.
- Summer (1 June–15 September): Characterized by sustained heat and elevated humidity, frequently exceeding 30–35°C. These conditions impose marked thermal strain and increased metabolic demand for thermoregulation.
- Autumn (15 September–15 December): A warm-humid transition phase with progressively decreasing temperatures. Environmental conditions remain variable, with lingering heat and increased humidity.

Table 1. Monthly meteorological parameters (humidity, temperature, and daylight hours) characterizing the Mediterranean climate of Valencia and supporting seasonal grouping.

	Relative Humidity (%)	Mean Temperature (°C)	Hours of Light (h: min:sec)
January	76	9.9	06:15:02
February	68	13.50	07:10:07
March	59	15.20	08:16:12
April	69	19.70	07:25:00
May	65	24.40	10:31:36
June	57	24.00	11:33:24
July	57	26.40	11:32:24
August	58	26.20	11:01:36
September	64	22.70	08:10:24
October	76	20.40	06:01:48
November	69	13.00	07:38:48
December	65	12.60	06:15:02

This climate-adapted seasonal grouping reflects the environmental conditions experienced by broodmares in the Valencia region (Spain) and provides a physiologically relevant framework for interpreting seasonal changes in PLT indices.

Blood Samples Collection and Analysis

Blood sampling was performed following a standardized protocol to minimize handling-related variability. Mares were calmly approached, accustomed to routine manipulation, and manually restrained with a halter and lead rope without sedation. All procedures were conducted in a quiet environment to avoid stress-inducing stimuli. Blood samples were obtained once per month, providing three samples per season for each mare (12 samples per mare throughout the study period).

All samples were collected by an experienced operator under consistent conditions and handled carefully to avoid platelet activation. Samples were processed promptly after collection, and the time between sampling and analysis was minimized and kept constant (within 60 min) to limit pre-analytical variability.

A total of 480 blood samples were collected, ensuring a balanced sampling design across seasons, with three samples per season for each mare.

All farms operated under comparable management and environmental conditions. No significant differences were observed between farms for any of the studied platelet parameters ($p > 0.05$).

To control circadian influences, all blood samples were obtained between 08:00 and 10:00h throughout the study period. Jugular venipuncture was performed using a 20-gauge sterile needle and a vacuum collection system. The skin was cleaned with 70% alcohol prior to puncture, and excessive negative pressure or prolonged venous occlusion were avoided to prevent artefactual platelet activation. Blood was collected into K_2-EDTA tubes (2–3 mL per sample), which were gently inverted 8–10 times immediately after collection to ensure proper anticoagulation and prevent platelet aggregation. Samples showing clotting, hemolysis, or visible aggregates were discarded and recollected. All samples were kept at room temperature (18–22°C), protected from direct sunlight, and processed within 2 h of

venipuncture to avoid artefactual changes in platelet morphology and volume.

All animals were sampled before feeding and without prior exercise to ensure consistent metabolic and physiological conditions across seasons.

PLT indices were measured in whole blood using an ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostics Inc.).

For interpretative clarity, analytical variables were grouped into two categories:

- **Quantitative PLT parameters**: platelet count (PLT; 10^3/μL), plateletcrit (PCT; %), mean platelet volume (MPV; fL), large platelets (P-LCR; 10^3/μL), and platelet clump count (AGREG; count).
- **Morphological, mass-related, or heterogeneity indices**: platelet distribution width (PDW; %), mean platelet component (MPC; g/dL), mean platelet mass (MPM; pg), platelet component distribution width (PCDW; g/dL), and platelet mass distribution width (PMDW; %).

MPC reflects the internal refractive index or protein density of circulating platelets, whereas PCDW describes the dispersion of MPC values. MPM represents the estimated mean platelet mass, and PMDW quantifies the variability within the platelet mass distribution. These parameters are specific to the ADVIA analytical platform and offer detailed information on platelet composition and heterogeneity. Their analytical performance in horses—including MPC, MPM, and PCDW—has been previously validated (Giordano et al. 2008; Bauer et al. 2011).

To assess hydration status and exclude potential hemoconcentration effects on platelet indices, packed cell volume (PCV) was determined using an ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostics Inc.), while total plasma protein (TPP), albumin (ALB), and blood urea nitrogen (BUN) concentrations were measured using a Spin 200 biochemical analyzer (Spinreact, Tarragona, Spain).

Statistical Analysis

Normality was assessed using the Shapiro–Wilk test. Most variables (PLT, MPV, PDW, P-LCR, MPC, MPM, PCDW, PMDW, and AGREG) did not follow a normal distribution. Although some variables met normality assumptions, non-parametric methods were consistently applied to all analyses to ensure methodological uniformity and robustness, given the repeated-measures design and the non-normal distribution observed in most variables. Seasonal differences were evaluated using the Friedman test for repeated measures, followed—when significant—by Wilcoxon signed-rank pairwise comparisons with Bonferroni correction. Correlations among variables were analyzed using Spearman's rho. Descriptive data are presented as median and interquartile range (IQR) for statistical interpretation, whereas mean ± standard deviation (SD) was used exclusively for graphical representation. Statistical significance was set at $p < 0.05$.

Results

PLT counts were significantly higher in spring, summer, and winter compared with autumn ($p < 0.05$), while no other pairwise differences were detected (Figure 1).

PCT remained stable across seasons—0.32 ± 0.13, 0.34 ± 0.12, 0.34 ± 0.15, and 0.3 ± 0.10—showing broad overlap and no identifiable seasonal pattern. MPV was significantly higher in autumn and winter than in summer ($p < 0.05$; Figure 2). Autumn showed significantly higher P-LCR than spring, summer, and winter ($p < 0.05$; Figure 3). MPC was highest in spring and showed a progressive and significant decline across the remaining seasons, with lower values in autumn and winter ($p < 0.05$; Figure 4). PDW was significantly higher in summer and winter compared to spring and autumn ($p < 0.05$; Figure 5). MPM was lowest in summer compared to spring, autumn, and winter ($p < 0.05$; Figure 6). PCDW showed the lowest values in spring compared to summer, autumn, and winter ($p < 0.05$; Figure 7). PMDW showed the lowest values in spring compared to summer, autumn, and winter ($p < 0.05$; Figure 8).

AGREG showed a progressive upward trend across seasons, with the lowest values observed in spring, followed by higher levels in summer, sustained elevations in

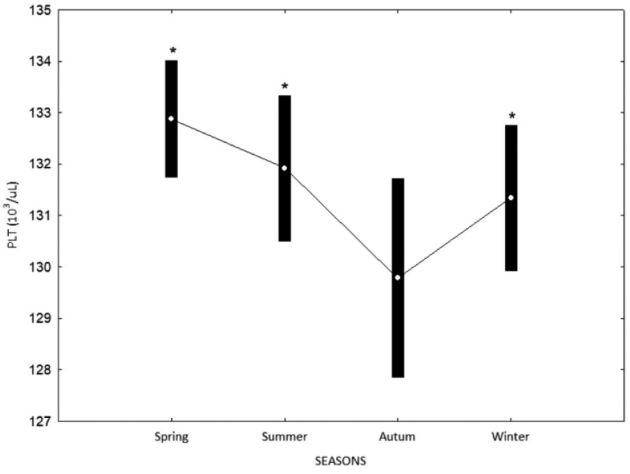

Figure 1. Seasonal variation in platelet (PLT) count. Values are mean ± SE. Asterisks indicate significant differences vs. autum (*p < 0.05*).

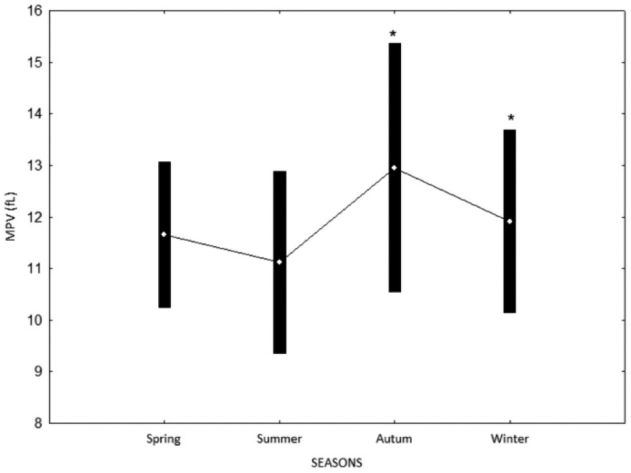

Figure 2. Seasonal variation in mean platelet volume (MPV). Values are mean ± SE. Asterisks indicate significant differences vs Summer ($p < 0.05$).

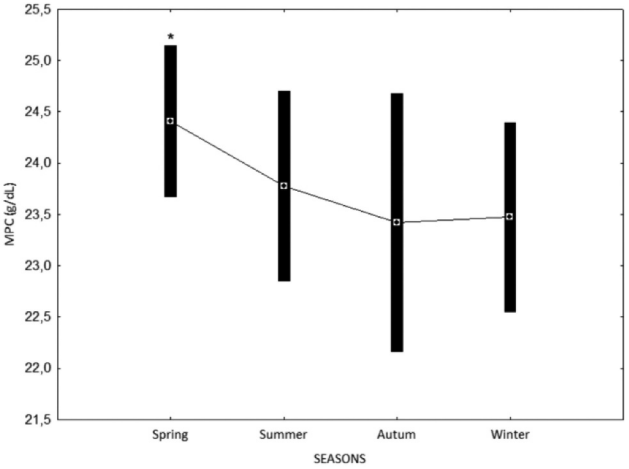

Figure 4. Seasonal variation in mean platelet component (MPC). Values are mean ± SE. Asterisks indicate significant differences vs other seasons ($p < 0.05$).

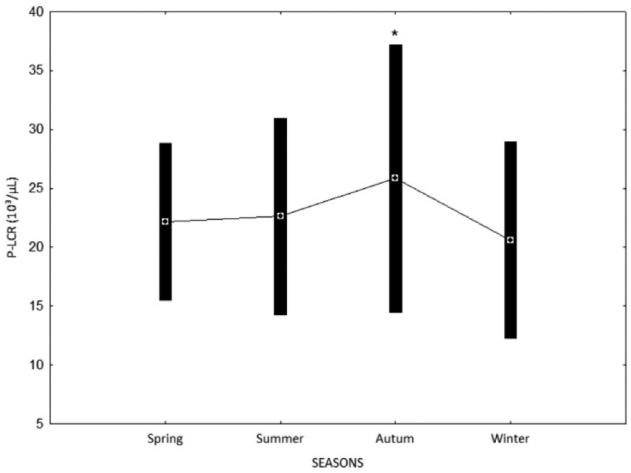

Figure 3. Seasonal variation in platelet large-cell ratio (P-LCR). Values are mean ± SE. Asterisks indicate significant differences vs other seasons ($p < 0.05$).

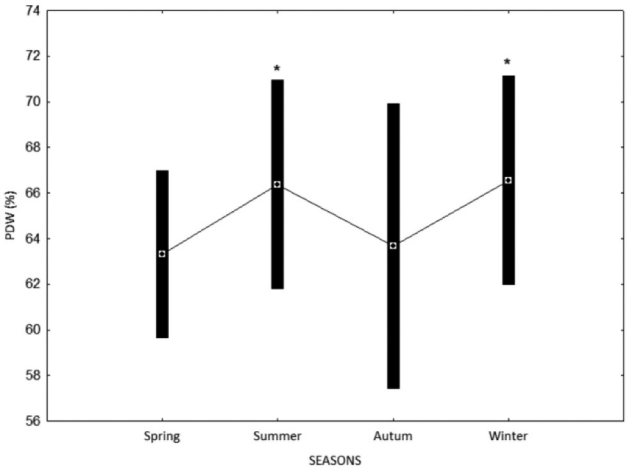

Figure 5. Seasonal variation in platelet distribution width (PDW). Values are mean ± SE. Asterisks indicate significant differences vs spring and autum ($p < 0.05$).

autumn, and the highest values recorded in winter, although these differences were not statistically significant.

Correlation analysis revealed several significant associations among platelet indices (Table 2). Indices of intraplatelet heterogeneity showed strong positive correlations with season and with each other. Positive relationships were also observed between platelet size and mass-related parameters, indicating that larger platelets tend to have greater mass. In contrast, negative associations were identified between platelet concentration and heterogeneity-related indices, consistent with reduced internal density in activated platelets. Additionally, positive relationships were found between the proportion of large platelets and overall platelet mass.

Hydration-related parameters (PCV, TPP, ALB, and BUN) showed mild but statistically significant seasonal variations, with slightly lower values observed in summer and higher values in autumn and winter. This pattern is consistent with a relative hemodilution during warmer months rather than dehydration. However, all values remained within physiological ranges and were not considered clinically relevant. These findings suggest that seasonal changes in hydration status were minimal and unlikely to have significantly influenced platelet indices (Table 3).

Discussion

The present study demonstrates that platelet indices in broodmares exhibit clear seasonal variation, generating distinct hematological profiles across winter, spring, summer, and autumn. The observed fluctuations in

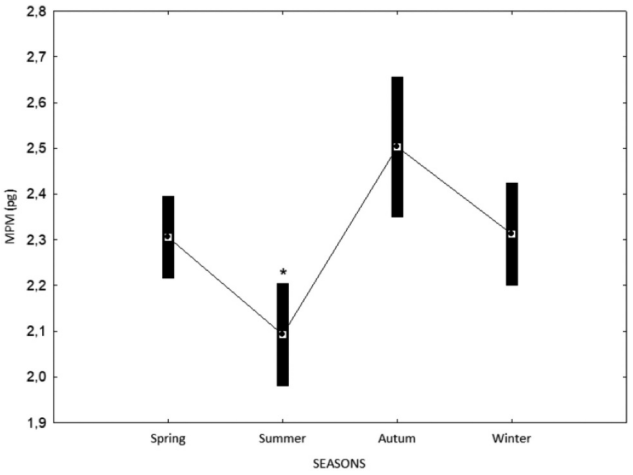

Figure 6. Seasonal variation in mean platelet mass (MPM). Values are mean ± SE. Asterisks indicate significant differences vs other seasons ($p < 0.05$).

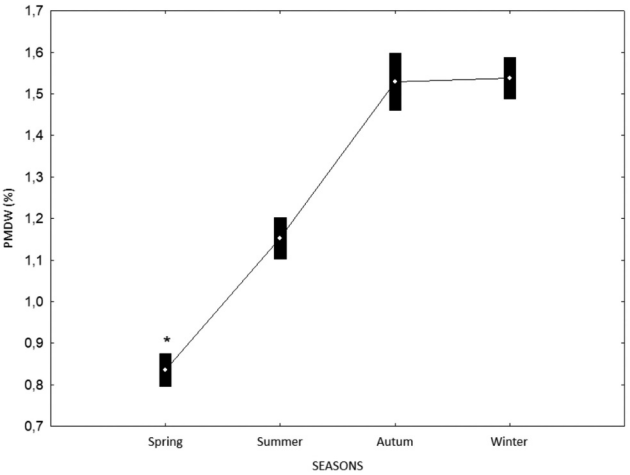

Figure 8. Seasonal variation in platelet mass distribution width (PMDW). Values are mean ± SE. Asterisks indicate significant differences vs other seasons ($p < 0.05$).

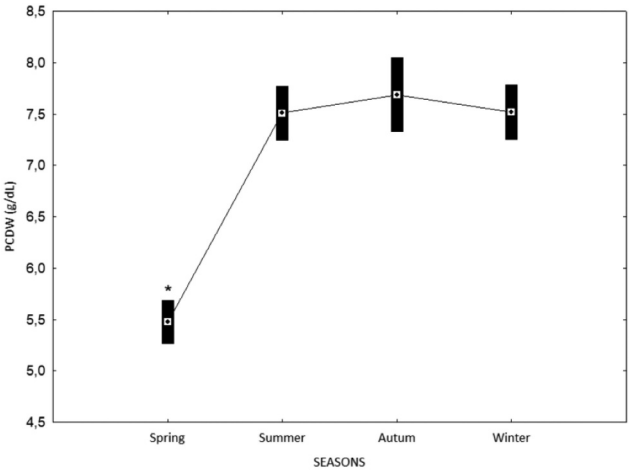

Figure 7. Seasonal variation in platelet component distribution width (PCDW). Values are mean ± SE. Asterisks indicate significant differences vs other seasons ($p < 0.05$).

PLT, PCT, MPV, PDW, P-LCR, MPC, MPM, PCDW, PMDW, and AGREG indicate that platelet physiology in mares undergoes consistent, multidimensional modulation throughout the year, encompassing changes in platelet number, circulating mass, size distribution, internal density, and aggregation behavior. Collectively, these patterns highlight the marked sensitivity of equine thrombopoiesis to environmental conditions and complement previous observations in horses and other mammalian species.

Although data were grouped by season to evaluate overall trends, some degree of intra-seasonal variability between early and late months may occur, likely reflecting short-term fluctuations in environmental conditions such as temperature and photoperiod.

Seasonal and monthly variations in physiological and biochemical parameters have also been described in horses, including changes in trace elements associated with nutritional and environmental factors (Or et al. 2004), further supporting the influence of seasonal conditions on equine physiology.

Winter: Mild Inflammatory Activation and Increased Platelet Heterogeneity

During winter, mares exhibited higher PLT accompanied by increases in MPV, PDW, PCDW, PMDW, P-LCR, and AGREG. Although a slight decrease in platelet

Table 2. Spearman correlation coefficients (ρ) between platelet count (PLT) and derived platelet indices across seasons.

	PCT (%)	MVP (fL)	P-LCR (10^3/μL)	AGREG (count)	SEASON	MPC (g/dL)	PCDW (g/dL)	MPM (pg)	PMDW (%)
PLT (103/mL)	−0,20	−0,28	−0,24	−0,17	−0,21	0,25	−0,11	−0,18	−0,25
PCT (%)		−0,05	0,71	0,18	−0,03	0,05	0,07	−0,10	−0,06
MVP (fL)			0,46	−0,20	0,05	−0,53	0,05	**0,63**	0,12
P-LCR (10^3/μL)				0,05	−0,01	−0,16	0,09	0,35	0,02
AGREG (count)					0,08	0,03	0,08	−0,09	0,07
SEASON						−0,16	**0,68**	0,09	**0,89**
MPC (g/dL)							−0,08	−0,32	−0,22
PCDW (g/dL)								−0,05	**0,67**
MPM (pg)									0,17

Table 3. Seasonal variation (mean ± SD) of hydration-related parameters, including packed cell volume (PCV), total protein (TPP), albumin (ALB), and blood urea nitrogen (BUN), in mares. Different superscript letters denote significant differences among seasons ($p < 0.05$).

Parameter	Spring	Summer	Autumn	Winter
PCV (%)	42.5 ± 1.6	41.8 ± 1.5	43.2 ± 1.7	43.3 ± 1.8
TPP (g/dL)	7.20 ± 0.06^{ab}	7.10 ± 0.07^{a}	7.30 ± 0.05^{bc}	7.40 ± 0.06^{c}
ALB (g/dL)	3.15 ± 0.03^{ab}	3.10 ± 0.03^{a}	3.18 ± 0.02^{b}	3.22 ± 0.03^{b}
BUN (mg/dl)	10.0 ± 0.3^{a}	9.8 ± 0.3^{a}	10.4 ± 0.2^{b}	10.7 ± 0.3^{c}

count might be expected under low-grade chronic inflammation, PLT did not decrease in this study, suggesting that winter-associated inflammatory activation manifested primarily through morphological changes (MPV, PDW, P-LCR) rather than through a reduction in total platelet number. This pattern is indicative of low-grade inflammatory activation and enhanced platelet turnover, leading to the release of larger, younger, and more heterogeneous platelets into circulation. Seasonal increases in respiratory, uterine, and integumentary infections during the colder months are well recognized in horses and other domestic mammals, where they contribute to cytokine-driven megakaryocytic stimulation and subsequent alterations in thrombopoiesis. The combined rise in platelet size–related indices (MPV, PDW, P-LCR) and density–distribution parameters (PCDW, PMDW), together with augmented aggregation (AGREG), aligns with the characteristic hematologic response to inflammatory cytokines and accelerated megakaryocytic maturation described across species. In horses, platelet activation and aggregability have been shown to mirror inflammatory-like states (Arfuso et al. 2024), and winter is commonly associated with higher infectious pressure and subclinical respiratory or gastrointestinal disease, providing a plausible biological framework for the observed winter profile.

The mild reduction in MPC further supports the presence of increased platelet activation, since activated platelets typically undergo granular recondensation and a consequent decrease in internal refractive index, leading to lower MPC values (Segura et al. 2007). This pattern is consistent with a state of low-grade systemic activation, in which inflammatory mediators accelerate megakaryocytic activity and promote the release of larger, less dense, and more metabolically active platelets. Together with the concurrent increases in MPV, PDW, P-LCR, PCDW, and PMDW, the winter profile suggests that broodmares experience a subtle but physiologically meaningful immunological load during colder months, sufficient to alter platelet morphology, density, and aggregation behavior.

Comparable seasonal effects on thrombopoiesis have been reported in humans and ruminants, where winter is associated with measurable hematologic adjustments linked to increased infectious pressure and inflammatory tone. In humans, large population-based studies have shown consistent winter-associated changes in platelet parameters, including elevated platelet counts and shifts in thrombopoietic activity (Gallerani et al. 2013; Liu and Taioli 2015). Similarly, dairy cattle living under cold-desert conditions show significant winter-related increases in platelet production and hematologic activity (Giri et al. 2017). Although the direction of change in PLT count varies among species, the overarching pattern—greater platelet activation and altered thrombopoiesis during colder periods—is broadly conserved. The present findings in mares therefore align well with the interspecies evidence, reinforcing the view that winter imposes additional metabolic and immunological challenges capable of modulating platelet dynamics across mammals.

Spring: Hematologic Stability and Assumed Physiological Baseline

Spring represented the most hematological stable period in broodmares. During this season, PLT values remained within the upper range observed across the year, while PDW, PCDW, PMDW, and P-LCR showed minimal variation. MPV, PCT, MPM, and AGREG exhibited only small fluctuations, indicating a largely steady platelet profile. The combination of consistently high PLT and low variability in derived indices suggests that spring imposes minimal inflammatory or metabolic strain. The environmental conditions characteristic of the Valencia region during spring, including moderate temperatures and increasing photoperiod, may contribute to maintaining hematological homeostasis.

This seasonal pattern contrasts with findings from horses kept in stabled management or in colder climates, where slower post-winter recovery and reduced exposure to natural environmental variation often result in little or no spring increase in PLT (Dmoch et al. 2008; Ono et al. 2021; Souza et al. 2018, Czech et al. 2025; Deniz et al. 2024).

Overall, the stability observed in our broodmares aligns with previous work identifying spring as

a physiologically balanced period in horses, characterized by reduced metabolic and inflammatory demand (Satué et al. 2014; Shawaf et al. 2018; Aragona et al. 2023). This is further supported by studies showing minimal seasonal variation in PLT count or related hematologic parameters under mild environmental conditions (Dmoch et al. 2008; Souza et al. 2018; Ono et al. 2021). In Shetland ponies, only modest shifts in PLT have been described, with MPV remaining largely unchanged (Shawaf et al. 2018), and previous work in broodmares reported spring as one of the most stable hematologic periods, although PLT indices were not assessed (Satué et al. 2014). Recent evidence of seasonal signatures and circadian rhythmicity in equine PLT dynamics (Aragona et al. 2023) further supports the interpretation of spring as a homeostatic phase with minimal hematologic activation.

Taken together, the steadiness of PLT indices in spring likely reflects a baseline hematopoietic and immunometabolic state, largely unperturbed by environmental or infectious stressors. This season therefore provides a valuable reference point for interpreting PLT indices in broodmares and may represent the most appropriate period for establishing physiologically grounded reference intervals.

Summer: Heat-Related Stress and Enhanced Platelet Activation

Summer elicited the most pronounced alterations in PLT indices, with marked increases in MPV, PDW, P-LCR, PCDW, PMDW, and AGREG, together with a clear decrease in MPC. However, in contrast to what might be expected under severe heat stress, PLT did not decrease and instead remained within the higher range observed across the year. This indicates that, although heat induced evident platelet activation and morphological remodeling, such activation did not result in a reduction in circulating platelet number. The PLT profile therefore remained characteristic of thermal strain, despite the absence of a PLT count decline.

High ambient temperatures reduce evaporative efficiency and relative dehydration is known to increase blood viscosity, oxidative load, and endothelial activation—conditions that stimulate megakaryocytic output and promote the release of larger, younger, and less dense platelets into circulation. The concurrent fall in MPC is particularly indicative of activation, as PLTs undergoing degranulation and metabolic engagement display a reduced internal refractive index, a response well documented in equine inflammatory and stress-related states (Segura et al. 2007).

The combination of higher MPV, PDW, P-LCR, and PMDW corresponds with the classic hematologic signature of thermal strain, in which oxidative and inflammatory pathways converge to enhance PLT heterogeneity, turnover, and pro-aggregatory potential (Iba et al. 2022). Experimental work in horses demonstrates that heat burden amplifies oxidative stress and disrupts vascular and endothelial function, closely paralleling the changes identified in broodmares during summer (Kang et al. 2023). Recent findings also indicate that equine PLT are highly responsive to metabolic stressors, exhibiting circadian and seasonal fluctuations in activation-related parameters (Aragona et al. 2023; Ehrmann et al. 2021).

Findings in other species reinforce this interpretation. In humans, summer heat exposure has been associated with increased PLT activation markers, oxidative stress, and alterations in hemostatic balance, even when PLT remains stable or decreases slightly (Crawford et al. 2003; Liu and Taioli 2015). Dairy cattle and buffaloes under hot-humid or high-temperature environments commonly show reduced PLT and increased indices of oxidative stress and vascular activation, patterns that mirror the elevated platelet size indices observed in mares during summer (Enculescu et al. 2017). Small ruminants such as goats exhibit heat-associated thrombopoietic shifts, including increased MPV and enhanced PLT reactivity (Nathawat et al. 2023).

Altogether, the summer profile observed in broodmares represents a physiologically coherent response to the combined effects of thermal burden, dehydration tendency, oxidative imbalance, and endothelial stimulation. These conditions produced a consistent pattern of increased platelet size, heterogeneity, activation, and aggregation behavior, while PLT itself remained high. The magnitude of these changes underscores the considerable sensitivity of equine platelet physiology to environmental heat stress and highlights the importance of accounting for season when interpreting platelet indices in broodmares.

Autumn: Residual Subclinical Inflammation and Transitional Physiology

Autumn produced a moderate but consistent pattern of platelet changes, characterized by the lowest PLT values of the year together with slight increases in MPV, PDW, P-LCR, PCDW, PMDW, and AGREG, while MPC showed a modest decline. The reduction in PLT, although mild, is consistent with the transitional nature of this season and may reflect the gradual normalization of thrombopoietic activity following summer heat stress, alongside shifting metabolic and nutritional conditions.

The moderate increases observed in platelet size- and heterogeneity-related indices (MPV, PDW, P-LCR, PCDW, and PMDW) suggest a low-grade rise in platelet turnover and mild activation, compatible with residual oxidative and inflammatory effects from the summer period. The concurrent decline in MPC supports this interpretation, as reduced internal platelet density is a recognized feature of partially activated or degranulated platelets (Segura et al. 2007). Although the specific triggers may vary, transitional seasons typically involve moderate immunometabolic adjustments, and the pattern detected here fits a subtle systemic activation that is less intense than in winter or summer.

Direct characterization of autumnal thrombopoiesis in equids is limited, but available evidence provides indirect support for this interpretation. Studies in mixed-breed and native horses report minimal or only modest seasonal variation in PLT during transitional periods (Dmoch et al. 2008; Souza et al. 2018; Ono et al. 2021), indicating that autumn generally exerts limited hematological influence unless additional stressors are present. Broodmares examined in late transitional months have similarly shown stable or only slightly altered platelet indices (Satué et al. 2014), which agrees with the mild deviations observed in the present study. Furthermore, recent work demonstrates seasonal and circadian rhythmicity in equine platelet behavior (Aragona et al. 2023) reinforces the concept that seasons of transition impose moderate, rather than extreme, physiological challenge.

Comparisons with non-equine species further support the biological plausibility of this autumn profile. In goats and cattle, transitional periods are commonly associated with modest increases in immune activity and subtle hematologic adjustments as animals shift between summer and winter metabolic states (Giri et al. 2017; Nathawat et al. 2023). In humans, autumn often coincides with rising inflammatory markers and preparatory immune activation ahead of winter, along with mild changes in platelet reactivity and aggregation (Crawford et al. 2003; Liu and Taioli 2015). These interspecies parallels suggest that the mild increases in platelet heterogeneity and activation observed during autumn represent a conserved adaptive response to seasonal transition.

Taken together, the autumn profile in mares reflects a coherent pattern of low-grade activation and moderate thrombopoietic remodeling—subtle enough to distinguish it from the higher physiological demands of winter and summer, yet sufficiently consistent to demonstrate that seasonal transition itself exerts a measurable influence on platelet phenotype.

Overall Interpretation and Contextualization of Seasonal Findings

Seasonal changes in hydration-related parameters may partially contribute to hematological variability. In the present study, the slightly lower values observed during summer are consistent with a relative hemodilution under warmer conditions rather than dehydration. However, as all parameters remain within physiological ranges, these variations are unlikely to have significantly influenced the platelet indices observed.

Although all farms were located within the same geographical region, potential differences in management, feeding practices, or microenvironmental conditions cannot be completely excluded and may have influenced the results.

The observed relationships between platelet count and platelet indices are consistent with established physiological patterns in which platelet number, size, and function are dynamically interrelated. Variations in platelet count are often accompanied by adaptive changes reflecting the regulation of thrombopoiesis. In this context, platelet number, size, and mass have been shown to be closely linked variables, and may exhibit inverse relationships across species, supporting the existence of compensatory mechanisms in platelet production and turnover (Boudreaux and Ebbe 1998; Kaushansky 2009).

Our findings indicate that seasonal modulation of platelet physiology in horses is strongly influenced by environmental context, management conditions, breed-related traits, and local climate. Broodmares living predominantly outdoors are continuously exposed to natural thermal fluctuations, variable pasture quality, changes in humidity, and shifting pathogen pressure—factors capable of modulating thrombopoiesis and platelet activation. In contrast, horses maintained under controlled stable conditions are subjected to more stable microenvironments, reduced thermal load, and lower exposure to environmental stressors, which may attenuate or obscure seasonal hematologic variation. These contextual differences may explain why some equine studies report clear seasonal patterns in platelet indices (Satué et al. 2014; Shawaf et al. 2018; Aragona et al. 2023), whereas others describe minimal or absent fluctuations (Dmoch et al. 2008; Souza et al. 2018; Ono et al. 2021). Additionally, breed-specific adaptability, differences in metabolic efficiency, and variability in thermoregulatory capacity may further influence susceptibility to seasonal changes. Collectively, these findings suggest that discrepancies among studies are not contradictory but instead reflect differences in environmental exposure and management systems, underscoring the

importance of considering ecological context when interpreting platelet indices in horses.

The seasonal fluctuations identified in this study have clear clinical relevance. The consistent variation observed in PLT, MPV, PDW, P LCR, MPC, and related indices suggests that reference intervals in broodmares may require seasonal adjustment to avoid misinterpreting physiological changes—particularly those occurring during summer heat or winter infectious peaks—as pathological. The marked sensitivity of these indices to environmental conditions highlights their potential as low-cost, readily accessible biomarkers of subclinical inflammation, metabolic strain, and environmental stress, providing clinicians with a practical tool for detecting early systemic alterations in mares managed under natural conditions. Because summer and winter produced the most pronounced deviations in platelet morphology, activation markers, and aggregation behavior, season should be considered an important confounding factor when evaluating thrombopoietic activity or monitoring hematologic trends.

The platelet patterns observed across seasons were consistent with the meteorological conditions recorded in Valencia. Spring was characterized by moderate temperatures, increasing photoperiod, and decreasing humidity, conditions that favor physiological homeostasis and correspond with the hematological stability observed during this season. Summer was marked by sustained high temperatures, long daylight hours, and low humidity, consistent with the increased platelet size, heterogeneity, and activation markers associated with thermal stress. Autumn, defined by elevated humidity and gradually declining temperatures, paralleled the mild but persistent activation profile observed during this transitional phase. Finally, winter represented the coldest and most humid period, consistent with the increase in platelet activation indices and the subtle inflammatory influence detected in broodmares. Together, these meteorological patterns provide a coherent physiological framework supporting the seasonal dynamics of platelet indices described in this study.

Correlation analysis further reinforced these findings. Indices of intraplatelet heterogeneity (PCDW and PMDW) showed the strongest positive associations with season and with each other, indicating that platelet structural variability increased under conditions of greater environmental stress. MPV correlated positively with MPM, suggesting that larger platelets tend to possess greater mass. Conversely, MPC showed negative associations with heterogeneity-related indices, particularly PCDW, consistent with reduced internal density in activated or partially degranulated platelets (Theuerkauf et al. 2022). P LCR also showed positive associations with MPM, linking the proportion of large platelets with overall platelet mass. Collectively, these relationships support a coordinated seasonal modulation of platelet size, mass, density, and heterogeneity, in agreement with the activation patterns described across seasons.

This study has several limitations. First, the population consisted exclusively of clinically healthy broodmares maintained under similar outdoor conditions, which may limit extrapolation to other breeds, management systems, or physiological states. Second, although platelet indices are sensitive indicators of systemic activation, they provide only indirect information on thrombopoiesis and platelet function, as direct assessments of oxidative stress, endothelial activation, or cytokine profiles were not included. Third, seasonal influences were evaluated under real-world field conditions, where environmental and management factors may act as unmeasured confounders. In addition, although major health conditions and drug administration were controlled, other potential influences on platelet indices, such as minor trauma, stress, exercise, or subclinical inflammation, were not specifically monitored and therefore cannot be completely excluded. Finally, functional platelet assays or bone marrow evaluations were not performed, which could have provided additional mechanistic insight into the observed changes. Despite these limitations, the consistency of the trends observed across platelet indices supports the biological relevance of the seasonal modulation described in this study.

Conclusion

This study demonstrates that mare platelet physiology is markedly shaped by seasonal environmental conditions. Winter and summer produced the greatest alterations in platelet morphology, mass, and density—consistent with infectious pressure and heat-associated metabolic strain—while spring represented the most hematological stable period. Derived platelet indices, particularly MPV, PDW, P-LCR, MPC, PCDW, and PMDW, proved more sensitive to seasonal modulation than total platelet count alone.

These findings highlight the importance of considering season as a relevant biological variable when interpreting platelet indices in mares. The pronounced responsiveness of platelet morphology-related markers underscores their value as accessible indicators of subtle systemic activation and environmental stress. Differences from previous equine studies likely reflect variation in climate, management, and breed

adaptability, reinforcing the need to interpret hematologic data within its ecological context.

Overall, this work demonstrates that seasonal physiology plays a significant role in shaping platelet dynamics in mares and supports the development of seasonally informed reference intervals to improve clinical interpretation in reproductively active animals.

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Author Contributions

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