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Investigation of the Effect of Age and Gender on Hematologic Parameters in Neonatal Foals

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ABSTRACT

Background: The process of adaptation to life outside the uterus is known to be a critical period in neonatal foals. Several studies have shown that there are significant changes in the physiological parameters of foals during this period. In this study, the changes in hematologic parameters in thoroughbred foals (n=35) at postnatal days 1 and 4 and possible differences between genders were investigated.

Methods: This study consisted of blood samples obtained from female (n=11) and male (n=24) thoroughbred foals whose healthy pregnancy processes were followed and no pathology was observed at birth. Blood samples were collected on the 1st and 4th postnatal days. All foals included in the study (n=35) were randomly selected from among healthy individuals with IgG levels above 800 mg/dL, which were measured to determine the immune levels of the foals and did not show any signs of disease in clinical examinations. In this study, white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MONO), eosinophil (EOS), basophil (BASO), red blood cell (RBC), hemoglobin (Hb), hematocrit (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and red blood cell distribution width (RDW) were examined according to gender and age.

Result: It was determined that there were significant differences in hematologic parameters between different age groups of same genders. While there was a significant difference in neutrophil percentages only in the 4-day-old age group, there was no statistically significant difference in other parameters.

Key words: Age, Foal, Gender, Hematology, Immunity.

INTRODUCTION

Racehorse breeding is conducted according to the official breeding seasons established by the International Federation of Horseracing Authorities. During this limited period, with only one foal born each year, breeders must pay close attention to veterinary practices to ensure that their breeding mares conceive healthily and that the pregnancy proceeds without complications. The diagnosis and treatment of pathologies associated with conception problems, particularly endometritis, have been receiving increased attention in parallel with advancements in imaging techniques (Uçmak *et al.,* 2020; Virendra *et al.,* 2022). While studies on conception issues and diseases during pregnancy (Kurban and Uçmak, 2023; Uçmak *et al.,* 2024; Yendim *et al.,* 2025) have grown, research on the physiology and pathology of neonatal foals is also increasing.

Studies show that more than half of all live foal deaths occur within the first 24 hours, with a neonatal mortality rate of 5.8 and within the first 30 days (USDA-APHIS, 2015). In a healthy birth, foals typically achieve sternal recumbency within 5-10 minutes, develop a sucking reflex within 5-20 minutes *and* stand up within the first hour. In the first two hours, foals are expected to suckle colostrum, which boosts their immune system. During this process, the placenta is usually expelled within three hours (Koterba, 1990; Kurtz Filho *et al.,* 1997; Vaala, 2000; Pierce, 2003).

Colostrum plays a critical role in neonatal immunity and intestinal maturation (Kelly, 2003; Yaylak *et al.,* 2017). It contains various soluble and cellular components, such as immunoglobulins, hormones, growth factors, cytokines, lactoferrin and various enzymes, making it vital for the newborn foal's health (Zou *et al.,* 1998; Kelly, 2003). However, it is well-documented that neonatal foals exhibit variability in their hematologic parameters. These values tend to change significantly within the first 72 hours after birth (Sato *et al.,* 1979; Bauer *et al.,* 1984; Veronesi *et al.,* 2014; Duncan *et al.,* 2020). These hematological changes are closely associated with the foal's adaptation to extrauterine life; however, comprehensive investigations, particularly regarding the influence of gender differences on these parameters, remain limited.

This study aims to evaluate the complete blood count (CBC) parameters of purebred neonatal foals born in Türkiye

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within the first four days postpartum, with a specific focus on temporal and gender-related variations. It is hypothesized that key hematological indicators, including RBC, WBC and PLT counts, will exhibit significant changes over time and between genders. The investigation also considers the neonatal adaptation process and immune status, exploring their associations with age and gender. The findings are expected to offer reference data to support early-life health monitoring in foals and to contribute to the development of breed-specific hematological standards.

MATERIALS AND METHODS

All animal experiments were performed according to the following guidelines in compliance with the Local Ethics Committee of the Animal Experiments, Istanbul University-Cerrahpasa (Approval number: 2024/102). This study was conducted between January and May 2024 in the Northern Hemisphere at 41°N latitude. The farm where the animals used in the study were housed is located at 41°22′12″N latitude and 27° 51′ 58″E longitude. All analyses conducted in the study were performed in the on-site laboratory facilities of the farm.

In this study, foals of Thoroughbred mares (between the age of 4-17 years) with a body condition score of 5-6 (Body Condition Score Card; Kentucky Equine Research, USA), which were kept in a private stud, regularly complied with vaccination programmes and did not show any clinical signs of disease as a result of medical screening, were used.

During the official breeding season, breeding mares conceived by different stallions by natural insemination were kept in individual stables (3.5 m × 3.5 m) in the evenings and released to paddocks during the day (8-9 hours on average). Stallions were also kept in individual 3.5 m × 3.5 m stalls during the evenings and similarly turned out to paddocks during the day. They were fed 2.5-3 kg of mixed feed and roughage (alfalfa and meadow grass) three times a day (06:00, 15:00 and 23:00 hours) and received ad libitum fresh water. The mares were followed up until birth in terms of pregnancy pathologies, especially abortion and stillbirth and the foals of mares that had normal and healthy births with no pregnancy pathology were included in the study.

At the beginning of labour, the progress of each mare and the newborn foal was reported. In addition to the physical examination of the foal, neonatal adaptation (standing, first sucking time, meconium expulsion) was also evaluated. The foals of postpartum mares whose colostrum quality was evaluated by Brix refractometer and which produced high quality colostrum were included in the study. Inclusion criteria encompassed negative neonatal isoerythrolysis test results and confirmation of adequate passive transfer *via* serum IgG levels exceeding 800 mg/dL as determined by Immuno-chek G Foal IgG Quantitative assay (BioMetallics, Princeton, NJ, USA). The study group consisted of 35 foals fulfilling these criteria.

The first blood samples were taken from the foals on the 1st day (between 8-12 hours postnatal) and the second blood samples were taken from the jugular veins on the 4th day (between 72-80 hours postnatal) in aseptic conditions. All blood samples were collected in EDTA tubes (Vacuette, Greiner Bio-one North America, USA), transported to the laboratory in sample containers maintained at 4°C and stored at 4°C until analysis. Analyses were conducted within 10-15 minutes of blood collection. Complete blood counts were performed using a Blood Counting Device (CELL-DYN 3700, Abbott GmbH and Co. KG, Wiesbaden, Germany).

In this study, to minimize observer bias, samples were randomly numbered and analyses were performed using blinding methods during sample collection, hematological parameter measurement and data analysis. The parameters to be included in the study as a result of complete blood count are white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), hemoglobin (Hb), hematocrit (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and red blood cell distribution width (RDW). Hematological parameters were analysed in male and female foals to determine whether they differed according to gender and age of the foals.

A power analysis was performed using G-power 3.1.9.7 software (Heinrich-heine-universität Düsseldorf, Düsseldorf, Germany) to estimate the smallest sample size needed for the study. Calculations revealed that a total sample size of n = 32 would be sufficient to provide 90 and power with a 0.05 α-error probability and effect size 0.30. All statistical analyses were performed using the SPSS 23.0 program (SPSS 23.0, IBM, USA). Mean and standard error of the mean were given for all parameters. Whether the data showed normal distribution was analysed by the Shapiro-Wilk test. In the presence of normal distribution, homogeneity of variance was checked by Levene's test and comparison between the groups was performed by independent sample t-test. In the absence of normal distribution, the Mann-whitney U test was preferred. With these analyses, the effect of gender on hematological parameters was statistically tested. Significance was accepted as $p<0.05$.

RESULTS AND DISCUSSION

The mean body weights of female foals (n=11) and male foals (n=24) used in the study were 51.54±6.33 kg and 52.16±6.03 kg, respectively. The mean age of the thoroughbred mares were 10.71±0.47 years. The mean complete blood count values of neonatal foals and their significance were given in Table 1. There was no significant difference between genders in WBC, lymphocyte, monocyte, eosinophil, basophil, RBC, Hb, PCV, MCV, MCH, MCHC, PLT, RDW values for each age group. Significance was found only in neutrophil values of 4 day age group between

genders (p=0.03), female foals had higher neutrophil values compared to male foals.

In female foals, the values of RBC (p<0.01), Hb (p<0.001), PCV (p<0.01), RDW (p<0.01), PLT (p<0.05), monocytes (p<0.05) and eosinophils (p<0.05) were significantly higher in the 1-day-old group compared to the 4-day-old group (Fig 1A). No significant differences were observed between these age groups in terms of WBC, lymphocyte, basophil, MCV, MCH and MCHC values (p>0.05). Similarly, in male foals, although no significant differences were found between the 1-day and 4-day age groups for WBC (p<0.001), neutrophils (p<0.001), monocytes (p<0.001), eosinophils (p<0.01), basophils (p<0.001), RBC (p<0.001), Hb (p<0.001) *and* PCV (p<0.001), the values of MCV (p<0.01), RDW (p<0.01) and PLT (p<0.001) were significantly higher in the 1-day-old group compared to the 4-day-old group (Fig 1B). No statistically significant differences were observed in lymphocyte, MCH and MCHC values between the two age groups in male foals (p>0.05).

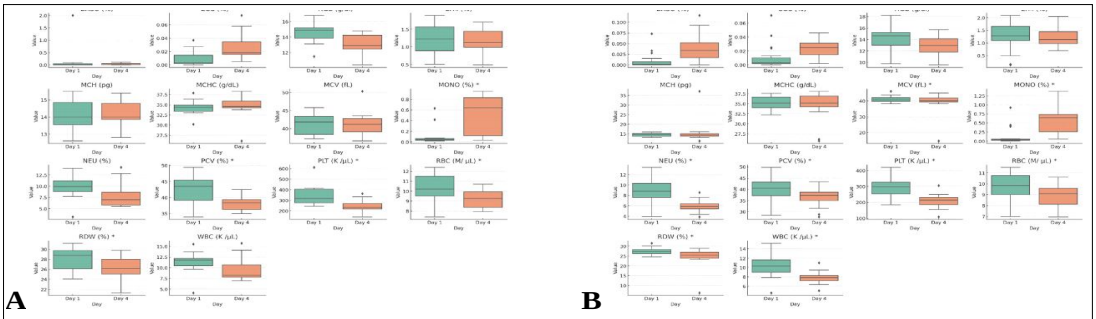
Age-related changes in hematological parameters have been reported during the early weeks of life (Aoki and Ishii, 2012; Aoki *et al.,* 2013; Cruz *et al.,* 2015). Several studies have attributed the decline in RBC counts in foals to increased haemodilution (Becht and Semrad, 1985; Aoki and Ishii, 2012; Uluisik *et al.,* 2013; Duncan *et al.,* 2020). RBC, Hb and PCV values decrease rapidly within the first 12-24 hours after birth and approach the lower limit of adult reference values by one year of age (Jain, 1986; Harvey, 1990; Grondin *et al.,* 2007). In the present study, RBC (p<0.001), Hb (p<0.001) and PCV (p<0.001) values in 1-day-old male foals were significantly higher than those in 4-day-old foals, consistent with previous findings (Uluisik *et al.,* 2013; Duncan *et al.,* 2020). Similarly, in female foals, RBC (p<0.01), Hb (p<0.001) and PCV (p<0.01) values were significantly higher in the 1-day group compared to the 4-day group. No significant differences were observed in WBC, neutrophil, lymphocyte and basophil counts between the age groups in female foals (p>0.05).

Satué *et al.* (2012) stated that the elevated RBC, Hb and PCV values in neonatal foals are primarily due to the blood transferred through the umbilical cord at birth, indicating that this transfer directly influences hematological parameters. Mean corpuscular hemoglobin concentration (MCHC) levels are known to remain stable after birth (Harvey, 1990) and in line with previous findings (Satué *et al.,* 2012; Uluisik *et al.,* 2013; Duncan *et al.,* 2020), our study found no significant differences in MCH and MCHC values between genders (p>0.05). Although MCV values are initially high in neonatal foals, they gradually decline and reach their lowest levels around 3-5 months of age (Jain, 1986; Harvey, 1990). This decline has been attributed to both reduced serum iron levels during growth (Harvey, 1990) and the maturation of erythrocytes in early life (Satué *et al.,* 2012). Consistent with previous reports (Uluisik *et al.,* 2013; Duncan *et al.,* 2020), MCV values in 1-day-old male foals were significantly higher than those in the 4-day-old group (p<0.01). Red cell distribution width (RDW), which reflects the variation in erythrocyte size relative to mean cell volume (MCV), is a useful parameter for assessing erythrocyte morphology and detecting anaemia types in veterinary medicine (Salvagno *et al.,* 2015; Hodges and Christopher, 2011; Scalco *et al.,* 2023). Scalco *et al.* (2023) evaluated RDW values in neonatal foals of different breeds within the first 24 hours of life and reported no significant differences between clinically healthy and at-risk groups. However, their study did not address gender-based or time-dependent variations in RDW. In contrast, our study revealed a significant gender difference in RDW values and RDW was significantly higher in the 1-day age group than in the 4-day group in both female (p<0.01) and male foals (p<0.01).

Table 1: Mean complete blood count values in neonatal thoroughbred foals (n=35) (Mean±Standart error).

Parameters	Female (n=11)		Male (n=25)	
	1 day age	4 day age	1 day age	4 day age
WBC (K/µL)	11.20±0.82	9.73±0.83	10.39±0.44^{x}	7.77±0.23^{y}
Neutrophil (%)	9.74±0.8	7.96±0.86	8.99±0.41^{x}	5.92 0.20^{y}
Lymphocyte (%)	1.23±0.13	1.19±0.11	1.28±0.11	1.25±0.07
Monocyte (%)	0.13±0.06^{x}	0.53±0.10^{y}	0.12±0.04^{x}	0.54±0.07^{y}
Eosinophil	0.01±0.00^{x}	0.03±0.01^{y}	0.01±0.00^{x}	0.02±0.00^{y}
Basophil (%)	0.20±0.17	0.04±0.01	0.01±0.00^{x}	0.04±0.01^{y}
RBC (M/ µL)	10.28±0.44^{x}	9.27±0.27^{y}	9.77±0.23^{x}	8.95±0.19^{y}
Hb (g/dl)	14.61±0.44^{x}	13.15±0.38^{y}	14.14±0.39^{x}	12.84±0.34^{y}
PCV (%)	42.59±1.37^{x}	38.10±0.65^{y}	40.02±0.97^{x}	36.38±0.77^{y}
MCV (fL)	41.35±0.86	41.40±1.07	41.01±0.38^{x}	39.68±1.13^{y}
MCH (pg)	14.12±0.28	14.20±0.23	14.47±0.17	15.28±0.95
MCHC (g/dL)	34.26±0.57	34.55±0.92	35.29± 0.32	34.82±0.62
RDW (%)	28.05±0.66^{x}	26.31±0.68^{y}	27.38±0.34^{x}	24.93±0.87^{y}
PLT (K/µL)	347.18±31.02^{x}	248.36±17.90^{y}	296.54±11.78^{x}	207.33±7.99^{y}

^{x,y} : Different letters in the same row indicate the significance P<0.05.



(A: Female foals; B: Male folas). *: Different letters in the same row indicate the significance P<0.05.

Fig 1: Relationship between 1 day old age group and 4 days old age group complete blood count values.

Similarly, Aoki and Ishii (2012) noted that platelet (PLT) counts are elevated in early neonatal life but tend to decline in the following days, with a subsequent increase at around two weeks of age. Duncan *et al.* (2020) also observed a decrease in PLT values between the early hours postpartum. In alignment with these findings, our study showed significantly higher PLT values in 1-day-old foals compared to 4-day-olds for both sexes (female foals: $p<0.05$; male foals: $p<0.001$), although no significant gender-based difference was detected.

Adrenocortical activity affects various physiological systems throughout life. The significant increase in cortisol secretion at birth has a profound impact on fetal development (Liggins, 1976). The elevated WBC levels observed in the early neonatal period are believed to be associated with this cortisol surge (Sgorbini *et al.,* 2013). Duncan *et al.* (2020) emphasized that WBC levels are time-dependent, with higher values during the initial hours of life that gradually decline over time. Similarly, Sgorbini *et al.* (2013) reported low WBC levels immediately after birth in donkey foals, followed by a gradual increase. These variations may be attributed to differences in breed or husbandry practices. Even under similar environmental conditions, individual responses to pathogens may differ (Duncan *et al.,* 2020).

In our study, WBC ($p<0.001$), neutrophil ($p<0.001$), monocyte ($p<0.001$), eosinophil ($p<0.01$) and basophil ($p<0.001$) counts in male foals were significantly higher on day 1 compared to day 4. Previous studies have shown that neutrophil levels increase in response to cortisol postnatally and then gradually decline to adult levels by 4-6 months of age. Davidson and Smith (1954) identified nonspecific appendages 'Davidson bodies' on neutrophil drumsticks, which reflect gender-related differences. The distribution of these structures is thought to be influenced not only by sex but also by hormones, granulocyte metabolism, cell proliferation and age (Brahimi *et al.,* 2013; Verma and Adinarayan, 2017).

In human medicine, such gender-related differences in neutrophil morphology are even used in sex determination (Miknienė and Ivanauskaitė, 2018). Furthermore, studies have shown that females are born with a more efficient immune system than males (Klein and Flanagan, 2016; De Toda *et al.,* 2023). Given the limited number of studies specifically addressing neonatal foals, we hypothesize that the observed differences may, similar to findings in human medicine, reflect sex-related variations in neutrophil morphology and function, as well as the generally more robust immune competency reported in females compared to males. Lymphopenia has been associated with immunodeficiency in foals (Axon and Palmer, 2008). Since only foals with adequate immune status (IgG>800 mg/dL) were included in our study, no significant differences in lymphocyte counts were detected between age groups or genders ($p>0.05$).

Monocytes, eosinophils and basophils-key components of the innate immune system-are either absent or present in very low numbers in fetal and neonatal foals (Jeffcott *et al.,* 1982; Harvey *et al.,* 1984; Harvey, 1990; Axon and Palmer, 2008; Uluisik *et al.,* 2013). In our study, female foals exhibited significantly lower monocyte ($p<0.05$) and eosinophil ($p<0.05$) levels on day 1 compared to day 4. Similarly, male foals showed lower levels of monocyte ($p<0.001$), eosinophil ($p<0.01$) and basophil ($p<0.001$) on day 1. Previous literature also supports the influence of both age and gender on these hematological parameters (Harvey *et al.,* 1984; Uluisik *et al.,* 2013).

CONCLUSION

Conducted on blood samples from two different age groups of thoroughbred neonatal foals, the study aimed to explore the relationship between hematological values, age and gender and contribute to establishing reference ranges for such foals. Consistent with many previous studies, significant differences were found between age groups, though no significant differences were observed between genders. However, it is believed that potential gender differences could be more clearly identified in studies with larger sample sizes. Additionally, some hematological parameters in our study differed from those reported in earlier research, particularly the WBC values. These differences are likely due to the sampling of foals after colostrum intake, when passive immunity levels are sufficient. In conclusion, this study marks an important step toward better understanding the complex hematological profile of neonatal foals and will serve as a foundation for future research.

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Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author is responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The author declares that there are no conflicts of interest related to the publication of this article. No funding or sponsorship influenced the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

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