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The Effect of Age and Gender on Some Hematological Parameters and Erythrocyte Osmotic Fragility in Kangal Shepherd Dogs

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Abstract: This study was aimed to determine the effect of age and gender on physiological variations in some hematological parameters and erythrocyte osmotic fragility in Kangal Shepherd dogs. A total of 32 clinically healthy Kangal Shepherd dogs were taken into the study and divided into 4 groups in equal numbers (Adult male = 8, Adult female = 8, Senior male = 8, Senior female = 8). Blood samples from the cephalic vein were taken into vacuum tubes containing EDTA by the technique and values of erythrocyte osmotic fragility and complete blood count were determined. The main effect of age and gender with age x gender interaction has no significant effect on hematological parameters (P>0.05). The main effect of age and age x gender interaction of erythrocyte osmotic fragility was statistically significant and higher in the senior females at 0.7% and 0.9% (in addition gender) NaCl concentrations compared to the other groups (P<0.05). However, there was no statistical difference between the adult female and adult male and senior male group at 0.7% and 0.9% NaCl concentrations (P>0.05). The findings may be useful for veterinary clinical practice in establishing Kangal shepherd breed-specific reference ranges for some important hematological parameters.

Keywords: Age, Erythrocyte osmotic fragility, Gender, Hematology, Kangal shepherd dogs.

Kangal Çoban Köpeklerinde Yaş ve Cinsiyetin Bazı Hematolojik Parametreler ve Eritrosit Ozmotik Kırılganlık Üzerine Etkisi

Öz: Bu çalışmada, Kangal çoban köpeklerinde yaş ve cinsiyetin bazı hematolojik parametrelerdeki fizyolojik varyasyonlar ve eritrosit ozmotik kırılganlık üzerindeki etkisinin belirlenmesi amaçlanmıştır. Çalışmaya klinik olarak sağlıklı toplam 32 Kangal çoban köpeği alınmış ve Kangal çoban köpekleri eşit sayıda 4 gruba ayrılmıştır (Yetişkin erkek Kangal çoban köpekleri = 8, Yetişkin dişi Kangal çoban köpekleri= 8, Yaşlı erkek Kangal çoban köpekleri = 8, Yaşlı dişi Kangal çoban köpekleri = 8). Sefalik venden kan örnekleri, tekniğine uygun olarak EDTA içeren vakum tüplerine alındı ve eritrosit ozmotik kırılganlık ile tam kan sayımı değerleri belirlendi. Hematolojik parametreler üzerine yaş ve cinsiyetin ana etkisi ile yaş x cinsiyet etkileşiminin anlamlı bir etkisi yoktu (P>0.05). Eritrosit ozmotik kırılganlığında yaş x cinsiyet etkileşimi ile yaşın ana etkisi, diğer gruplara kıyasla %0.7 ve %0.9 (ek olarak cinsiyet) NaCl konsantrasyonlarında yaşlı dişilerde istatistiksel olarak anlamlı ve daha yüksekti (P<0.05). Bununla birlikte, yetişkin dişi köpekler ve yetişkin erkek köpekler ile yaşlı erkek köpek grubu arasında %0.7 ve %0.9 NaCl konsantrasyonlarda istatistiksel olarak bir fark bulunmadı (P>0.05). Elde edilen bulgular, bazı önemli hematolojik parametreler için Kangal çoban köpek ırkına spesifik referans aralıkları oluşturmada veteriner klinik uygulamalara yararlı olabilecektir.

Anahtar Kelimeler: Cinsiyet, Eritrosit ozmotik kırılganlık, Hematoloji, Kangal çoban köpekleri, Yaş.

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INTRODUCTION

Kangal Shepherd dog breed is kept in Turkey and many other countries throughout the world for the purpose of either protecting livestock or as watchdogs (1). Information about the physiological parameters of this dog breed is becoming increasingly important (2). In particular, hematological parameters are important indicators of the physiological and physiopathological conditions of animals (3). Mean values of adult male and female Kangal Shepherd dogs in hemogram analysis, respectively, have been revealed in different studies (4,5). The blood analysis values in animals may vary according to age, sex, climate, and environmental conditions (6,7).

Erythrocyte osmotic fragility refers to the sensitivity of the change in different osmotic pressures to which an erythrocyte is exposed and it may change in different conditions (8). Hemolysis is often recognized by the presence of free hemoglobin suspended in a medium originating from the rupture of red blood cells. Erythrocyte osmotic fragility (EOF) test is used to determine hemolysis caused by osmotic stress of red blood cells. The extent of osmotic stress depends on red blood cell volume, surface area and functional integrity of the cell membrane (9). Low osmotic resistance may lead to intravascular hemolysis, which can reduce the number of erythrocytes and cause anemia if not treated (10). For this reason, the EOF test is frequently used to diagnose hemolytic anemia and diagnose oxidative damage due to extensive destruction of RBCs (11). Osmotic fragility in animals tends to be affected by different factors. These include sheltering conditions, temperature, and season of the year (12). Erythrocyte osmotic fragility has been used as an indicator of oxidative stress in animals (13). The relationship between aging and osmotic fragility differs (14). In some studies, performed on elderly human and bovine erythrocytes, it has been shown that the osmotic fragility decreases as the erythrocyte in the circulation ages (15). However, Rifkind et al. (16) and Mosior and Gomulkiewicz (17) reported that osmotic fragility increases as human erythrocytes age in circulation.

Measurement of the hematological values can provide objective information about an animal's condition at the time of sampling and provides information about nutritional status, disease status or stress to which it is subjected (18). Specific reference ranges should be established for each animal species to compare hematological values and to ensure if the results are properly interpreted (19). Reference ranges that are defined as values of particular parameters in a healthy population, provide the baseline on which these measurements are interpreted. Well-structured reference intervals are a prerequisite for either screening or diagnostic tests to evaluate the health and disease in a particular population.

In the literature, we could not find any study comparing the hematological parameters and erythrocyte osmotic fragility in adult and senior Kangal shepherd dogs of different sex. The aim of this study is to investigate some hematological parameters and erythrocyte osmotic fragility values in different sexes of the adult and senior Kangal shepherd dogs.

MATERIALS and METHODS

The study was conducted with the permission of the Sivas Cumhuriyet University Animal Experiments Ethics Committee (confirmation number:65202830-050.04.04-506). This study was carried out in Kangal District Governorate Dog Breeding Farm (Altitude: 1533, Latitude: 39.233334, Longitude: 37.383331) in Kangal District of Sivas Province, in March 2021. Animals used in the study were housed in the same care and feeding conditions. All the animals included in the study were clinically healthy. The age range of the dogs used in the study were chosen with a bodyweight between

41.8 - 62.2±2 kg and classified as adults as adults aged 3-5 years, aged 6-7 years, geriatric ≥ 8 years old according to the literature (20).

The groups in the study are as follows:

1. Group 1 (Adult Male): 3-5 years old male Kangal Shepherd dogs (n=8)
2. Group 2 (Adult Female): Female Kangal Shepherd dogs between the ages of 3-5 (n = 8)
3. Group 3 (Senior Male): Male Kangal Shepherd dogs between the ages of 6-7 (n = 8)
4. Group 4 (Senior Female): 6-7 years old female Kangal Shepherd dogs (n = 8)

Blood samples were taken from the cephalic vein at the same time intervals (10.00-12.00) throughout the study and collected into Vacutainer tubes containing EDTA anticoagulant for hematological analysis and osmotic fragility. Hematological parameters in blood samples taken into tubes containing EDTA were analyzed with an automatic complete blood counting device (Mindray BC-2800). Hematological parameters included in the study were as follows: white blood cell (WBC), lymphocyte count (Lymph), monocyte count (Mon), granulocyte count (Gran), percentage of lymphocytes (Lymph%), monocyte percentage (Mon%), granulocyte percentage (Gran%), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean red blood cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and eosinophil percentage (Eos%). Osmotic fragility test was performed in different concentrations of NaCl (Merck, Germany) solutions, modified from the one previously described by Oyewale et al. (2009) (21). Briefly, varying concentrations of 1 mL of each NaCl test solution (0.0%, 0.1%, 0.3%, 0.5%, 0.7%, 0.9%) were prepared in each set of 6 centrifuge tubes with distilled water. 0.02 mL of blood was added to the test solution to each tube. Contents mixed and incubated for 30 minutes at room temperature. It was then centrifuged for 10 minutes at 3000 g. The obtained supernatants were placed on the empty ELISA plate and the hemoglobin content of the supernatant was determined spectrophotometrically at a wavelength of 540 nm in the ELISA reader. Distilled water was used blank. By taking the maximum hemolyzed tube value as 100%, percentage of hemolysis in each different NaCl added solutions were measured and the result was evaluated.

Statistical Analysis

Data were analyzed by two-way ANOVA in terms of the effects of age and gender. When age-by-gender interaction is significant, post-hoc multiple comparisons were made by Tukey's test with adjusted P values in GraphPad Prism 8.0.1. software (Graph Pad Software Inc., San Diego, CA, USA). Results were given as mean ± standard error. $P<0.05$ was considered significant.

RESULTS

Analyzed results of hematological parameters are shown in Table 1. The main effects of age and gender and age-by-gender interaction are not significant for the selected hematological parameters, which were Lymph, Mon, Gran, Lymph%, Mon%, Gran%, RBC, Hb, Hct, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, and Eos% ($P>0.05$).

Table 1. Change in total blood count results with age and gender in Kangal Shepherd dogs.
Tablo 1. Kangal Çoban köpeklerinde yaş ve cinsiyete göre toplam kan sayımı sonuçlarındaki değişim.

		3-5 years of age n=8 Mean ± S.E.M		6-9 years of age n=8 Mean ± S.E.M				
Haematological parameters	Unit	Adult Female Dog	Adult Male Dog	Senior Female Dog	Senior Male Dog	P (age)	P (sex)	P value (interaction)
WBC	$x10^9/L$	12.77±1.72	12.95±1.27	14.13±0.30	14.20±0.80	0.319	0.925	0.966
Lenf	$x10^9/L$	2.75±0.49	3.50±0.61	3.73±0.67	4.20±0.99	0.277	0.426	0.851
Mon	$x10^9/L$	0.57±0.06	0.60±0.07	0.67±0.09	0.68±0.05	0.239	0.808	0.903
Gran	$x10^9/L$	9.45±1.31	8.85±1.10	9.73±1.05	9.33±0.59	0.728	0.644	0.930
Lenf%	%	21.38±2.26	27.13±3.89	26.47±5.21	29.07±5.55	0.437	0.359	0.726
Mon%	%	4.48±0.38	4.70±0.36	4.97±0.78	4.82±0.21	0.486	0.924	0.677
Gran%	%	74.15±2.36	68.18±3.73	68.57±5.93	66.10±5.64	0.414	0.370	0.705
Eos%	%	1.92±0.22	2.32±0.90	1.53±0.58	1.47±0.21	0.294	0.768	0.692
RBC	$x10^{12}/L$	8.14±0.35	8.00±0.21	8.64±0.18	7.89±0.48	0.581	0.234	0.396
Hb	g/dl	18.80±0.70	18.77±0.69	19.90±0.35	18.58±1.15	0.601	0.436	0.453
Hct	%	67.42±2.35	65.57±1.23	67.77±0.95	65.02±3.12	0.964	0.331	0.847
MCV	fL	83.08±1.81	82.02±0.95	78.53±2.13	82.65±1.47	0.245	0.357	0.134
MCH	pg	23.07±0.16	23.40±0.39	22.97±0.76	23.47±0.40	0.970	0.354	0.835
MCHC	g/dl	27.85±0.58	28.55±0.61	29.30±0.15	28.45±0.43	0.217	0.887	0.161
RDW	%	11.85±0.39	12.03±0.35	12.23±0.23	11.07±0.33	0.435	0.188	0.083
PLT	$x10^9/L$	343.25±48.80	276.50±48.31	380.00±33.08	403.25±54.47	0.668	0.126	0.381
MPV	fL	9.10±0.31	9.05±0.18	9.36±0.03	8.97±0.29	0.712	0.402	0.514
PDW	%	15.92±0.08	16.37±0.16	16.10±0.15	16.67±0.39	0.346	0.057	0.800
PCT	%	0.31±0.04	0.25±0.05	0.36±0.03	0.35±0.04	0.077	0.482	0.455

WBC: White Blood Cell, Lenf: Lymphocyte, Mon: Monocyte, Gran: Granulocyte, Lenf%: Lymphocyte percentage, Mon%: Monocyte percentage, Gran%: Granulocyte percentage, RBC: Red Blood Cell, Hb: Hemoglobin, Hct: Haematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Blood Cell Distribution, PLT: Plateles, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Plateletcrits, Eos%: Eosinophil percentage, L: Liter, g/dL: gram/deciliter, %: percentage, fL: femtoliter, pg: picogram.

Osmotic fragility test results are shown in Table 2. The effect of age (P<0.05), but not gender (P>0.05), on osmotic fragility was significant at 0.7% NaCl concentration. In addition, a significant (P<0.05) interaction between age and gender was found for the same NaCl concentration. The post-hoc test for the main effect of age indicated that osmotic fragility at 0.7% NaCl concentration was significant between adult and senior female Kangal Shepherd dogs (P<0.05). Interestingly, there was no statistical difference between adult and senior females and adult and senior males at 0.7% NaCl concentration (P>0.05). The main effect of age and gender on osmotic fragility was significant at 0.9% NaCl concentration (P<0.05). Moreover, there was a significant interaction between age and gender at 0.9% NaCl concentration (P<0.05). Multiple comparisons revealed that osmotic fragility was significant at 0.9% NaCl concentration between senior females and other Kangal Shepherd dogs (P<0.05). There was no significant difference between the groups for other NaCl concentrations (P>0.05).

Table 2. Changes in erythrocyte osmotic fragility with age and gender in Kangal Shepherd dogs.
Tablo 2. Kangal Çoban Köpeklerinde yaş ve cinsiyet ile eritrosit ozmotik kırılganlığındaki değişiklikler.

Groups	NaCl concentrations					
	0.0 % n=8 Mean±S.E.M	0.1 % n=8 Mean±S.E.M	0.3 % n=8 Mean±S.E.M	0.5 % n=8 Mean±S.E.M	0.7 % n=8 Mean±S.E.M	0.9 % n=8 Mean±S.E.M
Adult Female Dog	100±0.00	97.89±1.10	94.85±0.89	27.36±4.71	1.72±0.14[a]	1.75±0.55[a]
Adult Male Dog	100±0.00	97.53±1.58	95.35±1.48	31.96±5.40	1.99±0.32[ab]	1.58±0.18[a]
Senior Female Dog	100±0.00	98.98±0.50	95.52±1.26	42.33±11.30	4.01±0.58[b]	4.48±0.76[b]
Senior Male Dog	100±0.00	98.63±0.54	94.92±0.96	25.28±1.40	2.08±0.67[ab]	1.51±0.38[a]
P value (age)	-	0.312	0.920	0.549	0.030	0.023
P value (sex)	-	0.738	0.967	0.373	0.110	0.010
P value (interaction)	-	0.999	0.651	0.133	0.040	0.018

[a,b:] Different letters in the column show the statistical difference between groups.

DISCUSSION and CONCLUSION

The need to improve pet dogs' well-being is an important aspect in the development of aging research. For this reason, it is necessary to develop and increase the perspective on the biological effects of aging in screening analyzes, which are generally carried out in the veterinary field. For this reason, in the present study, we aimed to define whole blood and osmotic fragility biomarkers, among the hematological parameters affected during the aging process, in male and female Kangal shepherd dogs of different ages. Aging is known as a continuous ontogenetic process that begins at birth and ends with the death of the individual (22). Numerous cellular and molecular parameters have been reported in mammals, including altered intercellular communication associated with aging, genomic imbalance, mitochondrial dysfunction, and epigenetic changes (23). Age classification in dogs is well established in the field of veterinary research (20). Detection of physiological changes in dogs with aging by complete blood count and serum biochemistry profiles has greater specificity and objectivity than routine physical exams (24). That is why it is common to use blood tests to identify a specific aging-related disease in older dogs. Changes in some hematological parameters, together with age and sex, have been previously reported in different dog breeds (25,26). A retrospective study by Lawrence et al. (26) investigated the effects of age and gender on hematological parameters in more than 6000 dogs in 75 different breeds. WBC and monocyte concentrations were lowest in the 4–6 years old age group, while they reported an increase in older and geriatric dogs. Lymphocyte concentrations gradually decreased with age, then increased marginally after 12 years of age. They reported that eosinophil concentrations peaked between 1 and 2 years of age and then decreased with age, reaching a value lower than the eosinophil concentration value of 1-year-old dogs. They reported that platelet concentrations gradually increased after two years of age. In addition, the same study group reported that gender has no effect on erythrocyte related parameters, but that leukocyte and subtypes and platelet concentrations vary according to gender in female dogs lower mean WBC, neutrophil, monocyte, and eosinophil concentrations have been reported than male dogs, but mean platelet concentrations were reported to be higher. Contrary to these findings, no difference was found between adult and senior male and female Kangal shepherd dogs in hematological parameters in our study. Consistent with the result we obtained in our study, Lee et al. (27) reported that most of the parameters were within normal limits in the results of complete blood count, and there was no significant difference between large breed young and old dogs for all parameters. When the effects of age and gender on hematological parameters in dogs of different breeds are evaluated, we estimated that environmental conditions affect hematological parameters in Kangal shepherd dogs.

There are studies in the literature that report the osmotic fragility of erythrocytes in animals is affected by physiological and pathological factors and shows differences between animal species (28,29). These reports show that the relationship between aging and osmotic fragility differs (30). Aging in animals changes physiological variables, and it is reported that the lipid composition of the erythrocyte membranes changes as the aging progresses and this condition affects the erythrocyte membrane fluidity (31). Increasing fragility in animals with age causes damage to the erythrocyte membrane by free oxygen radicals in the form of lipid peroxidation and protein degradation. However, the reduction of endogenous antioxidants with the disruption of the oxidant-antioxidant balance affects the erythrocyte membrane integrity and makes the erythrocytes more fragile and more vulnerable to damage (31,32). On the other hand, some authors have reported that the osmotic fragility of the erythrocyte that increases with aging is associated with a decrease in the number and activity of calcium-mediated potassium channels in the erythrocyte membrane (33). Also, it has been reported that erythrocyte osmotic fragility increases with aging in humans (16, 17, 32). Again, in poultry, the osmotic fragility of erythrocytes increases with age (34), but decreases with age in cattle (35) and ducks (36).

In the present study, osmotic fragility increased with aging in female Kangal shepherd dogs. This result is consistent with the results of the study conducted by Olayemi et al. (21). There are studies revealing the effects of gender on erythrocyte osmotic fragility. It has been reported that in cattle (37), birds (8,34) and sheep (38) erythrocyte osmotic fragility is higher in males than in females, and this may be related to the possible stabilizing effect of estrogen. Erythrocytes in dogs (21), African giant rats (39), Red Sokoto goats (40), and turkeys (30) are more fragile in females than in males. The effect of gender difference on erythrocyte osmotic fragility is thought to be caused by sex hormones. It has been reported that estrogen increases erythrocyte osmotic fragility, but androgens have no effect on osmotic membrane stability (35). Estrogen also causes changes in the cholesterol-phospholipid molar ratio in the membranes of erythrocytes (14). This indicates that estrogen also has the capacity to affect the physicochemical composition of cell membranes. In our study, both the effect of age and gender on erythrocyte osmotic fragility in Kangal shepherd dogs were examined for the first time so that the fragility of erythrocytes increased with aging in female Kangal shepherd dogs, and there was no change in erythrocyte fragility with aging in male Kangal shepherd dogs. This result is similar to the osmotic fragility findings obtained in Nigerian native dogs by Olayemi et al. (21). The results in our study showed that the effect of gender on the erythrocyte osmotic fragility might be depended on the variability of sex hormone receptors in the erythrocytes, so erythrocyte osmotic fragility changes depending on the presence and the continuity of the sex hormone effect.

As a result, this study examined the effect of age and gender on complete blood count and erythrocyte osmotic fragility in Kangal Shepherd dogs. No difference in complete blood count between adult and senior and between male and female dogs. However, with aging, osmotic fragility increased in senior female dogs. In conclusion, findings in the present study may advance the veterinary clinical practice to have and to use some breed-specific reference ranges for a number of important hematological parameters.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Koçkaya M., Özşensoy Y., İnsal B., 2019. Comparisons of some physiological and stress behavioral parameters of Kangal shepherd dogs with and without ankyloglossia in different environmental temperatures. Turk J Vet Anim Sci, 43, 314-322.
2. Koçkaya M., Ekici M., 2020. The effect of acute strenuous exercise on some physiological, blood and antioxidant system parameters in Kangal shepherd dogs with and without ankyloglossia. MAE Vet Fak Derg, 5, 100-105.
3. Insal B., Piskin İ., 2020. Determination of some coagulation parameters according to age and sex in Sivas Kangal dogs. Turk J Vet Anim Sci, 44, 214-219.
4. Durgun Z., Eksen M., Keskin E., 1993. Some hematological values in healthy Kangal and German wolf dogs. Selcuk Univ Vet Fak Derg, 9, 16-20.
5. Koçkaya M., 2019. Comparisons of some blood hematological levels and biochemical

parameters in pregnant and non-pregnant Kangal shepherd dogs. Int J Vet Sci Anim Husb, 4, 5-8.
6. Souza KL., Falbo MK., 2020. Effect of time and storage temperature on hematological parameters of healthy dogs. PUBVET, 14, 157-166.
7. Lee HS., Kim JH., 2020. The dog as an exercise science animal model: a review of physiological and hematological effects of exercise conditions. Phys Act Nutr, 24, 1-6.
8. Oyewale JO., Durotoye LA, 1988. Osmotic fragility of erythrocytes of two breeds of domestic fowl in the warm humid tropics. Lab Anim, 22, 250-254.
9. Lektib I., Bargaa R., Chakir Y., Belhouari A., Hammoumi A., El Khasmi M., 2016. Study of incubation conditions for erythrocytes osmotic fragility testing in dromedary camel (Camelus dromedarius). Int J Res Env Sci, 2, 22-32.
10. Wu SG., Jeng FR., Wei SY., Su CZ., Chung TC., Chang WJ., Chang HW., 1998. Red blood cell osmotic fragility in chronically hemodialyzed patients. Nephron, 78, 28-32.
11. Adenkola AY., Ayo JO., Sackey AKB., Adelaiye AB., 2010. Erythrocyte osmotic fragility of pigs administered ascorbic acid and transported by road for short term duration during the harmatan season. Afr J Biotechnol, 9, 226.
12. Oladele SB., Ayo JO., Ogundipe SO., Esieo KAN., 2003. Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern guinea savannah zone of Nigeria. Bull Anim Hlth Prod Afr, 51, 204-214.
13. Ambali SF., Abubakar AT., Shittu M., Yaqub LS., Kobo PI., Giwa A., 2010. Ameliorative effect of zinc on chlorpyrifos-induced erythrocyte fragility in Wistar rats. New York Sci J, 3, 117-122.
14. Igbokwe NA., Ojo NA., Igbokwe IO., 2016. Effects of sex and age on the osmotic stability of Sahel goat erythrocytes. Com Clin Path, 25, 15-22.
15. Mosior M., Gomulkıewicz J., 1988. Osmotic properties of bovine erythrocytes aged in vivo. Gen Physiol Biophys, 79, 73-79.
16. Rifkind JM., Araki K., Hadley EC., 1983. The relationship between the osmotic fragility of human erythrocytes and cell age. Arch Biochem Biophys, 222, 582-589.
17. Mosior M., Gomulkiewicz J., 1985. Effect of phosphate ions on osmotic properties of human and bovine erythrocytes-a relation between the state of glycolysis and critical cell-volume. Studia Biophysica, 107, 169-178.
18. Perez JM., Gonzalez FJ., Granados JE., Perez MC., Fandos P., Soriguer RC., Serrano E., 2003. Hematologic and biochemical reference intervals for Spanish ibex. J Wildl Dis, 39, 209-215.
19. Meyer DJ., Harvey JW. Veterinary laboratory medicine, 3rd edn. Saunders, St Louis, 2004.
20. Fortney WD., 2012. Implementing a successful senior/geriatric health care program for veterinarians, veterinary technicians, and office managers. Vet Clin North Am Small Anim Pract, 42, 823-834.
21. Olayemi FO., Azeez IO., Ogunyemi A., Ighagbon FO., 2009. Study on erythrocyte values of the Nigerian indigenous dog. Folia Veterinaria, 53, 65-67.
22. Ackermann M., Chao L., Bergstrom CT., Doebeli M., 2007. On the evolutionary origin of aging. Aging Cell, 6, 235-244.
23. Lopez-Otin C., Blasco MA., Partridge L., Serrano M., Kroemer G., 2013. The hallmarks of aging. Cell, 153, 1194-1217.
24. Metzger FL., Rebar AH., 2012. Clinical pathology interpretation in geriatric veterinary patients. Vet Clin North Am Small Anim Pract, 42, 615-629.
25. Harper EJ., Hackett RM., Wilkinson J., Heaton PR., 2003. Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers. J Am Vet Med Assoc, 223, 1436-1442.
26. Lawrence J., Chang YM., Szladovits B., Davison LJ., Garden OA., 2013. Breed-specific

hematological phenotypes in the dog: a natural resource for the genetic dissection of hematological parameters in a mammalian species. PLoS One, 8, e81288.
27. Lee SH., Kim JW., Lee BC., Oh HJ., 2020. Age-specific variations in hematological and biochemical parameters in middle-and large-sized of dogs. J Vet Sci, 21, e7.
28. Doğan A., 2020. Diyabetik ratlarda zakkum(nerium oleander L.) çiçeği etanolik liyofilize ekstresinin eritrosit frajilite, hematolojik ve antioksidan etkilerinin araştırılması. KSÜ Tarım ve Doğa Derg, 23, 1495-1502.
29. Pati S., Panda SK., Behera PC., Panda MR., 2017. Assessment of erythrocyte osmotic fragility in cattle due to haemoprotozoan diseases. Int J Sci Environ Technol, 6, 1560-1568.
30. Igbokwe NA., 2018. A review of the factors that influence erythrocyte osmotic fragility. Sokoto J of Vet Sci, 16, 1-23.
31. Marin MS., Fernandez A., Sanchez-Yaque J., Cabezas JA., Lianillo M., 1990. Changes in the phospholipid and fatty acid composittion in normal erythrocytes from sheep of different ages. Aminophospholoipid organisation in the membrane bilayer. Biochimie, 72, 745-750.
32. Kumar A., 2011. Biomedical studies on lipid peroxidation and erythrocyte fragility during the process of aging. Asian Pac J Trop Biomed, 1, 6-7.
33. Tiffert T., Daw N., Etzion Z., Bookchin RM., Lew VL., 2007. Age decline in the activity of the Ca2+-sensitive K+ channel of human red blood cells. J Gen Physiol, 129, 429-436.
34. Oyewale JO., 1988. Osmotic fragility of erythrocytes of guinea-fowls at 21 and 156 weeks of age. Vet Arhiv, 61, 49-56.
35. Basarab JA., Berg RT., Thompson JR., 1980. Erythrocyte fragility in double-muscled cattle. Can J Anim Sci, 60, 869-876.
36. Oyewale JO., Ajibade HA., 1990. Osmotic fragility of erythrocytes of the White Pekin duck. Vet Arhiv, 60, 91-100.
37. Olayemi FO., 2007. The effect of sex on the erythrocyte osmotic fragility of the Nigerian White Fulani and Ndama breeds of cattle. Top Vet 25, 106-111.
38. Durotoye LA., 1987. The effect of sex, pregnancy and lactation on the osmotic fragility of the West African dwarf sheep. Bull Anim Hlth Prod Afr, 35, 29-33.
39. Oyewale JO., Olayemi FO., Oke OA., 1998. Haematology of the wild adult African giant rat (Cricetomys gambianus, waterhouse). Vet Arhiv, 68, 91-99.
40. Habibu B., Kawu MU., Makun HJ., Aluwong T., Yaqub LS., Ahmad MS., Tauheed M., Buhari HU., 2014. Influence of sex, reproductive statusand foetal number on erythrocyte osmotic fragility, haematological and physiological parameters in goats during the hot-dry season. Vet Med, 59, 479-490.