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Anatomical Study of Bulbus Oculi in Akkaraman Sheep*

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Abstract: With this investigation, the anatomy of the bulbus oculi was studied in 12 Akkaraman sheep. The bulbus oculi's specific morphological features of this breed were introduced in the study. In the border of the cornea and sclera, the limbus cornea ended at the point where it entered under the sclera. In the eyes examined, the existence of the rima cornealis sclera was not observed. Based on the observations made, the center of the cornea was thinner in comparison with its periphery. In Akkaraman sheep, it was determined that the cornea did not reach back to the iris and that an angle did not exist between them. Therefore, it is suggested anatomically, to name this an "iridoscleral angle" instead of an "iridocorneal angle". The diameter of spaces forming the schlemm canal was measured between 40 µm-170 µm. It is hoped that the research findings will provide a source for a sheep model to improve diagnosis and treatment methods in eye diseases and vision problems and to aid in future anatomical and clinical research.
Keywords: Anatomy, bulbus oculi, eyeball, iridocorneal angle, sheep

Akkaraman Koyununda Bulbus Oculi'nin Anatomik Incelemesi

Öz: Bu araştırma ile 12 adet Akkaraman koyununda bulbus oculi'nin anatomisi incelenmiştir. Çalışmada, bulbus oculi'nin bu ırka özgü morfolojik özelliklerine yer verilmiştir. Cornea ve sclera sınırında limbus cornea, sclera'nın altına girdiği noktada sonlandı. İncelenen gözlerde, rima cornealis sclera varlığı gözlenmedi. Yapılan gözlemlerde cornea'nın merkezi periferine göre daha inceydi. Akkaraman Koyununda, cornea'nın, iris'in gerisine kadar uzanmadığı ve aralarında bir açı oluşmadığı belirlendi. Bu nedenle buna "angulus iridocornealis" yerine "angulus iridoscleralis" isminin verilmesi önerilmektedir. SEM çalışmasında, schlemm kanalını oluşturan boşlukların çapının 40 µm-170 µm arasında olduğu ölçüldü. Araştırma bulgularının, göz hastalıkları ve görme problemlerinde tanı ve tedavi yöntemlerini iyileştirmek ve gelecekteki anatomik ve klinik araştırmalara yardımcı olmak için bir koyun modeli oluşturulmasına kaynak sağlaması beklenmektedir.
Anahtar kelimeler: Anatomi, bulbus oculi, gözküresi, iridocorneal açı, koyun

Introduction

The bulbus oculi in addition to being a bold spherical outline in mammals is slightly flattened in the anterio-posterior axis in sheep, pigs, horses, and cattle (Kirk, 2003; Prince et al., 1960). In a study on different goat breeds, it was observed that the eyeball circumference was larger in females of all breeds than in males (Olopade et al., 2005). In a study on Berber sheep, it was stated that the vertical and horizontal diameters of the cornea were 17.9 mm and 25 mm respectively, and the thickness of the cornea was 0.63 mm (Fornazari et al., 2006). There is a study, which reported the average thickness of the cornea was 0.85 mm in Marwari goats (Barhaiya et al., 2015). Corneal thickness varies by corneal region, animal species, and breeds, although in most domestic animals it is less than 1 mm (Barhaiya et al., 2015; Dellmann and Brown, 1976; Samuelson, 2013).

The sclera is composed of non-transparent fibrous tissue and, its function is to shape the eye (Dursun, 2008; Malkoç, 2006). The equator part of the sclera appears to be slightly thinner compared to the corneoscleral edge and posterior pole (Slatter, 2003). While the thickest part of the sclera is the area where the plexus venosus is present in the cat and dog, in the equus and ruminants it is polus posterior (Samuelson, 2013). In the sclera tissue, there is a trabecular meshwork canal made of loose connective tissue. This tissue is named the sinus venosus sclerae (schlemm's canal) (Dursun, 2008; Şaroğlu, 2013). A minimum of 50% of the segments of schlemm's canal lumen were divided into more than one lumen with many septae (Buskırk, 1989).

The corpus ciliare is a structure about the lens, which

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holds and accommodates it. There are formations named the plicae and orbicularis ciliares on the inner surface of the corpus ciliare. Bulges formed by these plicas coming together are named the processus ciliaris (Craigie, 1838). The corpus ciliare is held by a lens via fibrae zonulares (hanging ligaments) (Dursun, 2008; Curtis, 1983; Davanger, 1975; Orhan et al., 2009; Taşbaş, 1996).

Sheep are often used as a model to improve diagnosis and treatment methods of eye diseases and vision problems, as in research intended for the usage of sheep lenses in human cataract disease (Kayikcioğlu et al., 2004). It has been reported that various anterior segment surgical procedures can be studied in sheep eyes, from a study stating that the sheep eye is a practical choice in ophthalmic surgery applications (Mohammadi et al., 2011).

This study was aimed to exhibit the differences according to species, breed, gender, and left and right bulbus oculi with a detailed investigation of the bulbus oculi's anatomy in Akkaraman sheep.

Material and Methods

In this study 24 eyeballs were used, 6 were from females and 6 from males, for a total of 12 adult Akkaraman sheep. The Akkaraman is a native sheep breed of Turkey. After slaughter in a slaughterhouse, the eyes of the sheep were immediately removed. Sections were taken from different areas for stereomicroscope reviews after obtaining macroscopic measurements and qualifications of eyes stored in a 10% formaldehyde solution. The water displacement method (Archimedes' principle) was used to measure the volume of the lens. Measurements and investigations were performed with a dissection microscope (Olympus BX50), stereomicroscope (Zeiss 2000-C), and 0-150 mm Electronic Digital Caliper. Digital cameras (Canon 550D and Ucmos 10000 KPA) were used for the photographs. Image J and Toupview 3.7 programs were used to make measurements on the photographs.

Samples taken for use in SEM investigations were first fixed for 48 hours in 2.5% glutaraldehyde solution, according to the SEM procedures by Alan (Alan, 2011) and Onuk (Onuk et al., 2013). Then the tissues were washed twice with PBS (phosphate-buffered saline) solution for 10 mins. Afterwards, the tissues were passed through a sequential acetone series and the process of CPD (critical point dryer) was applied. Following the drying process, the tissues were coated with POLARON SC7620 Sputter Coater in gold-palladium to obtain electron microscopic images and they were visualized with a LEICA LEO 440 brand and model scanning electron microscope. In addition to sectioning, the crushing technique was also used in SEM imaging of lens. In this process, it was aimed to obtain a natural anatomical image of the fractured face by breaking the lens.

Shapiro-Wilks test was used to test the compliance of the data to normal distribution. The data was evaluated by descriptive statistics, chi square test and logistic regression analysis. The Mann-Whitney U test was used for statistical significance check of differences between males and females in terms of the parameters examined. For the significance level of the tests, $P<0.05$ and $P<0.01$ were accepted. IBM SPSS Statistics 22 sortware was used for statistical analyzes.

Results

The horizontal and vertical diameter of the bulbus oculi was observed to be larger in male animals than in females. In general, both diameters were detected to be larger in the right bulbus oculi than in the left (Table 1 J, K).

The cornea was dorso-ventral flattened and oval in shape with the lateral edge of the cornea found to be more convex (Figure 1 I). The mean horizontal diameter of the cornea was revealed to be 20.27 mm (♂ 20.07, ♀-20.4) (Figure 1 I-a). The average vertical diameter in the middle of the cornea length was 14.08 mm. (Figure 1 I-b). The height of the cornea at the highest point, in other words, the distance between the anterior facies of the iris and the posterior facies of the cornea, was detected to be 7 mm on average (Figure 1 II-h). The center of the cornea was thinner in comparison with the periphery (Table 1 C, D). When the posterior side of the cornea, was examined, the presence of polygonal-shaped endothelial areas were detected with both stereo microscopy and SEM images. The cornea, in the limbus, was ob-

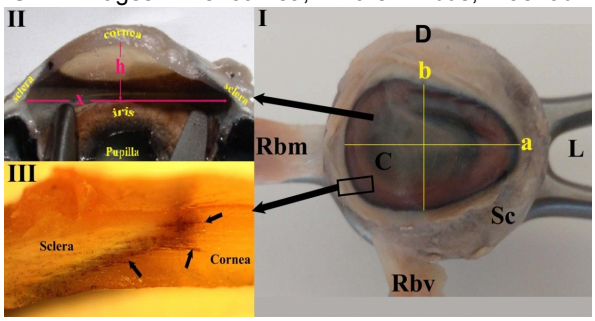

Figure 1. I-Akkaraman Sheep left eye bulbus oculi; D: Dorsal, L: Lateral, Rbm: Musculus rectus bulbi medialis, Rbv: Musculus rectus bulbi ventralis, C: cornea, Sc: sclera, a: horizontal diameter of the cornea, b: vertical diameter of the cornea. II-Akkaraman Sheep camera anterior bulbi; X: dorsoventral diameter of the camera anterior bulbi, h: distance of the central cornea to the iris. III-A cross-section of the limbus cornea from the marked area (Zeis 30X); Small black arrows: lamellar pigmentations extending to the limbus cornea.

Eye		Female	Male	
	N	Mean ± Std. Error	Mean ± Std.Error	P value
Right	A 6	0.87 ± 0.09	1.11± 0.15	0.240
	B 6	0.50 ± 0.03	0.52± 0.02	0.699
	C 6	0.73 ± 0.09	0.87± 0.03	0.240
	D 6	0.61 ± 0.08	0.83± 0.05	0.026
	E 6	14.31 ± 0.38	14.36± 0.54	0.937
	F 6	20.64 ± 0.28	20.06± 0.33	0.132
	G 6	7.97 ± 0.10	7.93± 0.13	0.937
	H 6	12.93 ± 0.07	12.28± 0.12	0.020
	I 6	12.92 ± 0.09	12.32± 0.06	0.020
	J 6	29.40 ± 0.36	28.11± 0.77	0.310
	K 6	29.74 ± 0.35	29.53± 0.48	0.818
Left	A 6	0.90 ± 0.13	1.00± 0.06	0.485
	B 6	0.47 ± 0.02	0.52± 0.03	0.310
	C 6	0.68 ± 0.08	0.84± 0.01	0.093
	D 6	0.60 ± 0.08	0.79± 0.02	0.041
	E 6	13.45 ± 0.33	14.23± 0.42	0.065
	F 6	20.31 ± 0.26	20.09± 0.26	0.937
	G 6	8.12 ± 0.27	7.82± 0.14	0.589
	H 6	12.71 ± 0.27	12.58± 0.11	0.589
	I 6	12.38 ± 0.19	12.685± 0.14	0.394
	J 6	28.43 ± 0.41	28.63± 0.34	0.699
	K 6	28.46 ± 1.32	29.62± 0.34	1.000

A: Sclera thickness at the thickest part, B: Thickness of the sclera in the limbus region, C: Cornea peripheral thickness, D: Cornea central thickness, E: Cornea's vertical diameter, F: The horizontal diameter of the cornea, G: Lens front-posterior diameter, H: Lens dorso-ventral diameter, I: Lens medio-lateral diameter, J: Bulbus oculi vertical diameter, K: Bulbus oculi horizontal diameter.

served to extend to the bottom of the sclera, getting thinner (Figure 1 II, III).

The mean thickness of the sclera was seen in Table 1: A and B. The thickness of the sclera in the equator area, which is the thinnest part.

In the SEM examinations, the presence of a schlemm canal, which had a fragmented lumen, was detected in the sclera tissue and in the area between the iris and sclera (Figure 2 white arrows). The diameters of the gaps forming the canal ranged from 40 µm to 170 µm.

Other measurements showed that the discus nevri

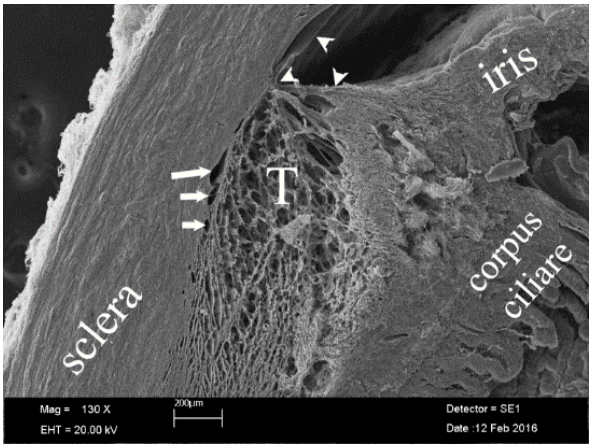

Figure 2. Angle between iris and sclera (SEM 130X). (White Arrows: Sinus venosus sclerae, White Arrow heads: epithelium posterius cornea, T: Trabecular meshwork)

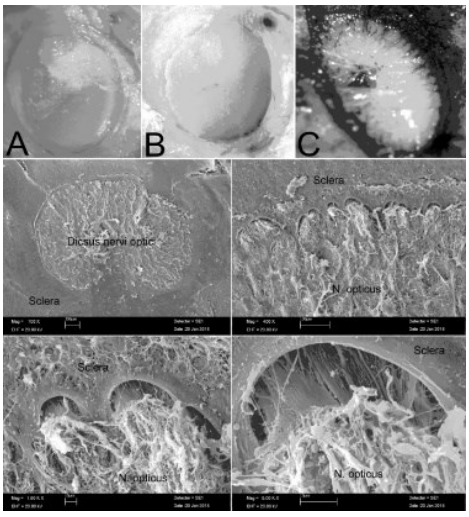

Figure 3. (Zeiss 40X). A: N. opticus immediately after exiting sclera (Zeiss 40X), B: N. opticus when exiting the sclera (Zeiss 40X), A: Discus nervi optici (Zeiss 50X). SEM: Discus nervi optici and area cribrosa sclera (SEM 100X, 400X, 1600X, and 5000X).

optici, which was located in the bulbus oculi in ventro lateral position near the central of the fundus, was found to be oval-shaped in size 1.5 x 2.2 mm (Figure 3 C). The nervus opticus, where it leaves the bulbus oculi, when viewed from the outside, namely where the sclera exits, was 2.7 x 3.4 mm in size, close to circular shape and monolithic (one piece-compact) (Figure 3 A).

On the cross-sectional surface of the iris, four layers were observed by SEM. The first anterior iris layer, the epithelium posterius cornea covering the posterior side of the cornea was completely covered by skipping to the anterior face of the iris at the iridocorneal angle (Figure 2 White arrow heads). The second layer observed on the cross-sectional face of the iris was the stroma layer with lamella structure. It was found that the stromal layer with the vascular layer reaches a thickness of 0.5 mm in places. The stroma was generally thicker in the periphery but thinned towards the pupilla. Besides this general structure of the stroma, it also had valley-like collapses and hill-like protuberances ranging from 4-7 mm. In the third and muscle layer of iris, the musculus dilatator pupilla was seen to be thinner than the sphincter muscle layer.

The third and fourth plica ciliaris were seen to be joined in the formation of a processus ciliaris (Figure 4 II). It was detected that there was an average of 250 ± 10 plica ciliaris in one eye in the count performed. When the counting process was performed at the border of the ora serrata in the examined eyes, the number of plicas reached up to 400. Fibrae zonulares, were detected to originate from different areas, back and between, of the plicae ciliares. It was seen in scanning electron microscopic images that the plicae ciliares were flat and packaged with a membrane layer and were curved and granular (Figure 4 V, VI). The metallic green-blue tapetal region was located at the caudal end of the choroidea and on the dorsal end of the discus nervi optici. Especially a metallic green 0.5-1 cm diameter area was observed in the upper part of the optical disc, while the surrounding parts were metallic blue.

A random oval circular network structure in the nontapetal areas was seen in SEM investigations of choroidea (Figure 5 I, II, III). 5-7 cornered polygonal fields and regular areas such as honeycomb were seen within the boundaries of the tapetum lucidum (Figure 5 IV, V, VI). The thickness of the choroidea varies by region, and in the tapetal region, it was reaching almost twice the thickness of the nontapetal region.

The facies posterior of all examined lenses were more convex than the facies anterior (Figure 6 I). The mean volume of the lens was detected as 0.57 cc. Fibers named fibrae zonulares were observed to ad-

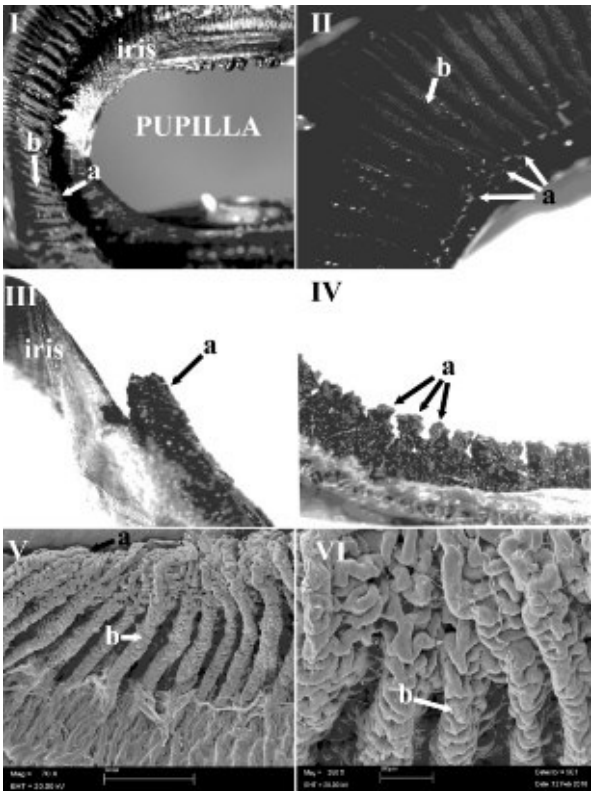

Figure 4. Iris and corpus ciliare view of; I, II, V, and VI: posterior, III: mediolateral, IV: anterior. a: processus ciliares, b: plicae ciliares, c: choroidea.

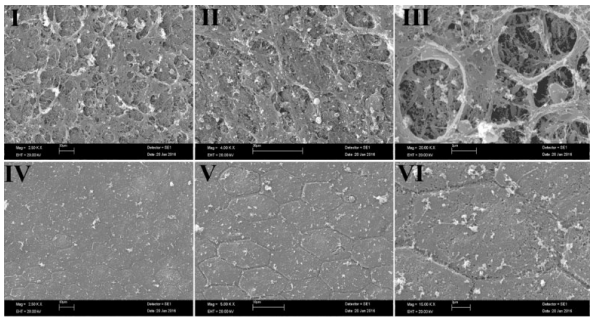

Figure 5. SEM images of the non-tapetal (I, II, III) and tapetal (IV, V, VI) of the Choroidea at different magnifications.

here all-around the capsula lentis at the level of the equator lentis (Figure 6 II). As shown in the figure, while a large part of the fibrae zonulares ends on the equator lentis, some of the fibers were attached anterior of equator and some posterior. The fibrae lentis seemed as flat hexagonal prismatic structures with a very regular and harmonious sequence (Figure 6 V, VI).

As seen in the measured values (Table 1), statistically significant differences were found between males and females in the right eye, the central thickness of

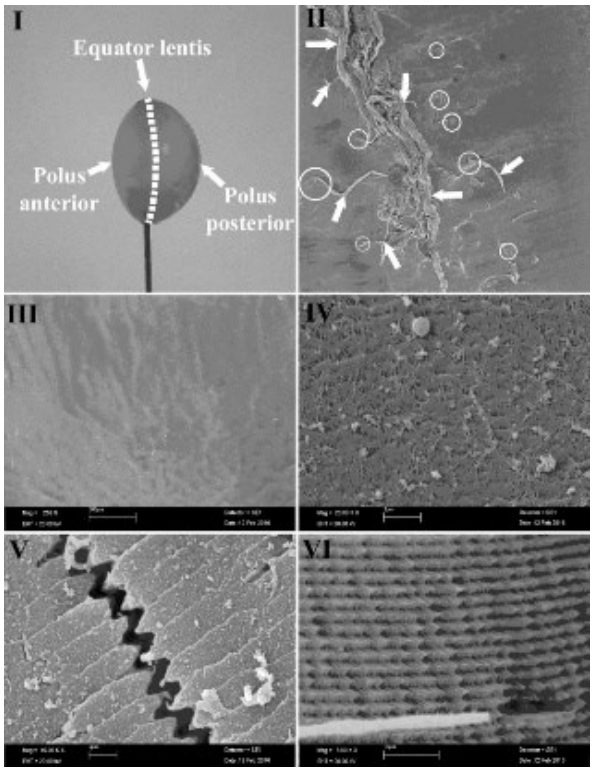

Figure 6. Lens in Akkaraman sheep. I. lens, II. Equator lentis (SEM 150X) White arrows: Fibrae zonulares, white circles: The adhesion sites of the fibrae zonulares to the capsula lentis, III.Capsula lentis (front side of the lens), IV. Capsula lentis (back side of the lens), V. Cross-sectional face of the lens and VI. fibrae lentis.

the cornea (D), the horizontal diameter of the lens (I) and the vertical diameter of the bulbus oculi (J), in the left eye, the central thickness of the cornea (D ($P<0.05$)).

Discussion and Conclusion

The bulbus oculi in Akkaraman sheep was found to be flattened in anterio-posterior direction as reported in the literature (Kirk, 2003; Prince et al., 1960; Olopade et al., 2005). In Akkaraman sheep, only the vertical diameter of the right bulbus oculi showed a statistical difference larger in females than males, consistent with the literature information (Olopade et al., 2005).

In many literature sources (Dursun, 2008; Malkoç, 2006; Taşbaş, 1996; Dyce et al., 2010) it was stated that the cornea is located in a groove formed by the sclera. Taşbaş (1996) elaborated this merging as the limbus cornea settling in a notch called rima cornealis sclera in the form of semilunar. Prince et al. (1960) reported that the sclera at the junction covers the cornea from the outside. In Akkaraman sheep, the limbus cornea was revealed to get thinner and go under the sclera in the corneoscleral junction, similar to the expression of Prince et al. (1960). The most important was the absence of a sulcus or rima at the junction of the sclera and cornea, as stated in the literature (Dursun, 2008; Malkoç, 2006; Taşbaş, 1996; Dyce et al., 2010).

Compared to corneal measurements reported by Prince et al. (1960) in sheep and by Fornazari et al. (2006) in Berber sheep, it was determined that the size of the cornea was smaller, but the thickness was larger in Akkaraman sheep. The numerical values obtained from the Akkaraman sheep in the cornea thickness measurements were closer to the numerical values reported in goats by Barhaiya et al. (2015). This recent literature also states that the cornea of the goat is thicker in the center than the periphery. In contrast to this finding, the center of the cornea in Akkaraman sheep was thinner in comparison with the outer parts. The periphery being thicker in the cornea of Akkaraman sheep's bulbus oculi was consistent with Prince et al. (1960), Dellmann and Brown (1976) and Slatter's (2003) expressions.

Slatter (2003) stated that in cats and dogs the equator of the sclera is thinner than the corneoscleral part and polus posterior. Samuelson (2013) reported that the thickest part of the sclera is where the plexus venosus is located in cats and dogs and the polus posterior sclera in equidae and ruminants. In Akkaraman sheep, the thickest part of the sclera was the polus posterior as Samuelson stated in equidae and ruminants.

It was seen that in Akkaraman sheep, the cornea did not extend to the iris. It was observed that there was no angle between the iris and the cornea and that the iris angled with the sclera. Therefore, it was thought that the term "angulus iridoscleralis" might be more accurate instead of the expression "angulus iridocornealis' in Nomina Anatomica Veterinaria (2012) indicating the region where the schlemm canal is also located The word "iridocorneal" can only express the epithelial layer (lamina posterius cornea) covering the posterior side of the cornea also covers the anterior side of the iris.

According to Samuelson (2013), 70 to 110 processus ciliaris are found in mammals, depending on the species. Orhan et al. (2009) reported that the number of processus ciliares was 131 and 2-3 plicae were combined to form a processus ciliaris. The number of processus ciliares in the Akkaraman sheep was found to be less than those reported in the literature. It was reported that the number of pilicae ciliares was 301. While the number of plicae ciliares in Akkaraman sheep was 250 ± 10 on average in the count performed at 1 mm distance from ora serrata, the number reached up to 400 when counting was car-

ried out on the ora serrata border. This suggests that the number of plicae ciliares will vary depending on where the counting was performed in corona ciliaris. In light of this information, we can say that a processus ciliaris in Akkaraman sheep is formed from the merging of more plicas than stated in the literature (Orhan et al., 2009).

In Akkaraman sheep, fibrae zonulares were found to reach out to the lens originated from the cristas and between plicae ciliares. However, Davanger (1975) stated that no fibrae zonulares enter or leave the ciliar valleys in the human eye. Although a study in the dog's eyes reported the presence of fibrae zonulares originated from the cristas of the processus ciliares, the fibras originated from the processus ciliares could not be seen in the Akkaraman sheep (Taşbaş, 1996).

Tapetum lucidum has been reported to be blue in ruminants Dursun (2008) and Dyce et al. (2010) and pale gold green and sometimes bluish in sheep, Craigie (1838). Tapetum lucidum in Akkaraman sheep was observed to have a metallic blue-green color.

Taşbaş (1996), it was reported that the length of the axis lentis in cattle is 11.5 mm, the transversal diameter of the lens is 16.2 mm and the volume of the lens is 1.1 cc. These values of the lens in Akkaraman sheep are 7.96 mm, 12.57 mm and 0.57 cc respectively.

The anatomy of bulbus oculi in Akkaraman sheep was examined in detail and the differences were revealed with this research. It is hoped that the detailed findings of this study will serve as a source for future anatomical and clinical investigations in the development of diagnostic and therapeutic methods for vision problems and eye diseases.

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