

## A Morphological Study on Iridocorneal Angle and Ciliary Body of the Anatolian Shepherd Dogs (*Canis familiaris*)

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### Abstract

This study was carried out to determine morphological structures of Anatolian Shepherd dog eye to compare with other animals. This important sensory organ from five adult male dogs were investigated by light microscopic and scanning electron microscopic analyses. Anatolian Shepherd dog cornea is about 435 (center) to 501 (periphery)  $\mu\text{m}$  thick. The dense pigmentation observed in the anterior and posterior iridal epithelium. The ciliary cleft of the iridocorneal angle was not large and well-developed. The ciliary processes, another component of the ciliary body, formed the conspicuous bundles. In conclusion, it is considered that the description of the morphological properties of both the iridocorneal region and the ciliary body would contribute to the interpretation of the functional correlation, thus to future experimental studies to be conducted in this field.

**Keywords:** Dog, Eye, Iridocorneal angle, Ciliary body, Morphology

## Anadolu Çoban Köpeklerinde (*Canis familiaris*) Siliyer Cisim ve İridokorneal Açı Üzerine Morfolojik Bir Çalışma

### Özet

Bu çalışma diğer hayvanlarla karşılaştırma yapmak üzere Anadolu Çoban köpeği gözündeki morfolojik yapıları belirlemek amacıyla yapılmıştır. Beş yetişkin erkek köpekten elde edilen bu önemli duyu organı ışık ve taramalı elektron mikroskobu ile incelenmiştir. Anadolu Çoban köpeğinin korneası merkezde 435, periferde ise yaklaşık 501 mikron kalınlığındaydı. İrisin anterior ve posterior epitelinde yoğun pigmentasyon gözlemlendi. İridokorneal açının siliyer aralığı büyük ve çok gelişmiş değildi. Siliyer cismin başka bir bileşeni olan siliyer uzantılar belirgin şekilde demetler oluşturdu. Sonuç olarak, hem iridokorneal bölgenin hem de siliyer cismin morfolojik özelliklerinin tanımlanması fonksiyonel ilişkinin yorumlanmasına katkı sağlayarak daha sonra yapılacak deneysel çalışmalara da faydalı olabileceği düşünülmektedir.

**Anahtar sözcükler:** Köpek, Göz, İridokorneal açı, Siliyer cisim, Morfoloji

### INTRODUCTION

The iridocorneal angle, also referred to as the filtration or drainage angle, which is located on the anterior surface of the ciliary body, at the iridal base, in-between the borders of the cornea and sclera, and within the perimeter of the anterior chamber, is a structure responsible for the outflow of the aqueous humor from the anterior chamber of the eye <sup>[1]</sup>. In several researches, this structure has been reported to vary between animal species, depending on the adaptation of animals to terrestrial or aquatic life <sup>[2]</sup>.

The aqueous humor is a nutritious fluid, generated from the ultrafiltrate of the blood plasma in the micro-

circulation of the stroma of the ciliary body. This metabolically rich fluid serves as a nutritive supply for the avascular lens, cornea and trabecular meshwork. After being secreted by the non-pigmented epithelium located at the apical ends of the ciliary processes in the pars plicata, the aqueous humor enters the posterior chamber of the eye. Subsequently, it flows in-between the lens and pigmented epithelium of the iris, and passing rapidly through the pupillary opening, it enters the anterior chamber. The aqueous humor leaves the anterior chamber by two main drainage pathways, one which is conventional and referred to as the trabecular meshwork, and the other which is unconventional and referred to as the uveoscleral route. In the dog, the conventional route is mainly responsible



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for the drainage of the aqueous humor, and the uveoscleral route accounts for 10-15% of the outflow of the aqueous humor [3,4].

Several animal species have been used as a model in functional research. The anatomy of the outflow system of the aqueous humor has been studied extensively in multiple species, including horse [5], pig [6], and rabbits [7], and it has been aimed to investigate the conditions leading to the obstruction of the outflow of the aqueous fluid, which in general results in glaucoma in humans [2].

The interpretation of eye disorders requires knowledge of the normal eye structure and physiology. To the authors' knowledge, no literature report is available on the morphology of the eye in the Anatolian Shepherd dog. Thus, this study was aimed at obtaining detailed morphological images of the normal structure of this important sensory organ by light microscopy and ultra-structural analysis. The objective of this study was to demonstrate the structures involved in the outflow of the aqueous humor in the Anatolian Shepherd dog, which is known as a guard dog and is indigenous to Turkey. It was also aimed to make a comparison of the eye morphology with that of other mammalian species on the basis of a qualitative assessment with a view to determine the potential differences, and thereby, to contribute to future clinical studies. The detailed histological investigation of the iridocorneal angle is not only a novel approach to comparative research, but is also considered to contribute to the demonstration of anatomical associations in other animal models.

## MATERIAL and METHODS

### Animals

Five adult male Anatolian Shepherd dogs which died because of traumas such as traffic accidents and other disorders without eye problem were used in this study and the eyes were obtained from the clinics of the Dicle University Veterinary Medicine. The properties of animals used are presented in *Table 1*.

### Light Microscopy

The eyeballs were enucleated immediately before fixation. The samples were fixed in 10% neutral buffered formalin for 24 h and a small cut was made in the cornea to allow this solution to penetrate inside the eyeball to insure good fixation of the inner structures. After washed in running tap water for 24 h, the tissues were passed through a series of graded alcohols, methyl benzoate and benzole and were embedded in cross-section to long axis of body in paraffin. The 5 µm sections were cut from the paraffin blocks, mounted onto slides. The general histological structure of the eyeball was demonstrated by employing Crossman's triple staining method. The slides were examined by light microscopy and photographed using a Nikon Eclipse E400 (Nikon Instruments Inc.) microscope equipped with a digital camera (Nikon Coolpix 4500; Nikon Instruments Inc.).

### Scanning Electron Microscopy (SEM)

The eyeballs were dissected into smaller pieces, after enucleated, were washed in 0.1 M chilled phosphate buffer (pH 7.4), fixed in 2.5% glutaraldehyde for 6 h and again washed twice in 0.1 M phosphate buffer (pH 7.4). Secondary fixation was carried out in 1% osmium tetroxide for 1 h, and specimens were dehydrated by acetone. Then, they were critical point dried and coated with gold palladium. The specimens were observed and photographed under a scanning electron microscope (Leica-Leo S440, Cambridge, UK). The tissues were examined under a stereomicroscope (Nikon SMZ800; Nikon Instruments Inc., Melville, NY, USA) so as to determine their general anatomical features before examination under scanning electron microscopy. Terms are used in agreement with the *Nomina Anatomica Veterinaria* - 2012.

## RESULTS

### Cornea

It was determined that the cornea was composed of an outer epithelial layer, a stromal layer in the middle, and

**Table 1.** Animals used in this study

**Tablo 1.** Çalışmada kullanılan hayvanlar

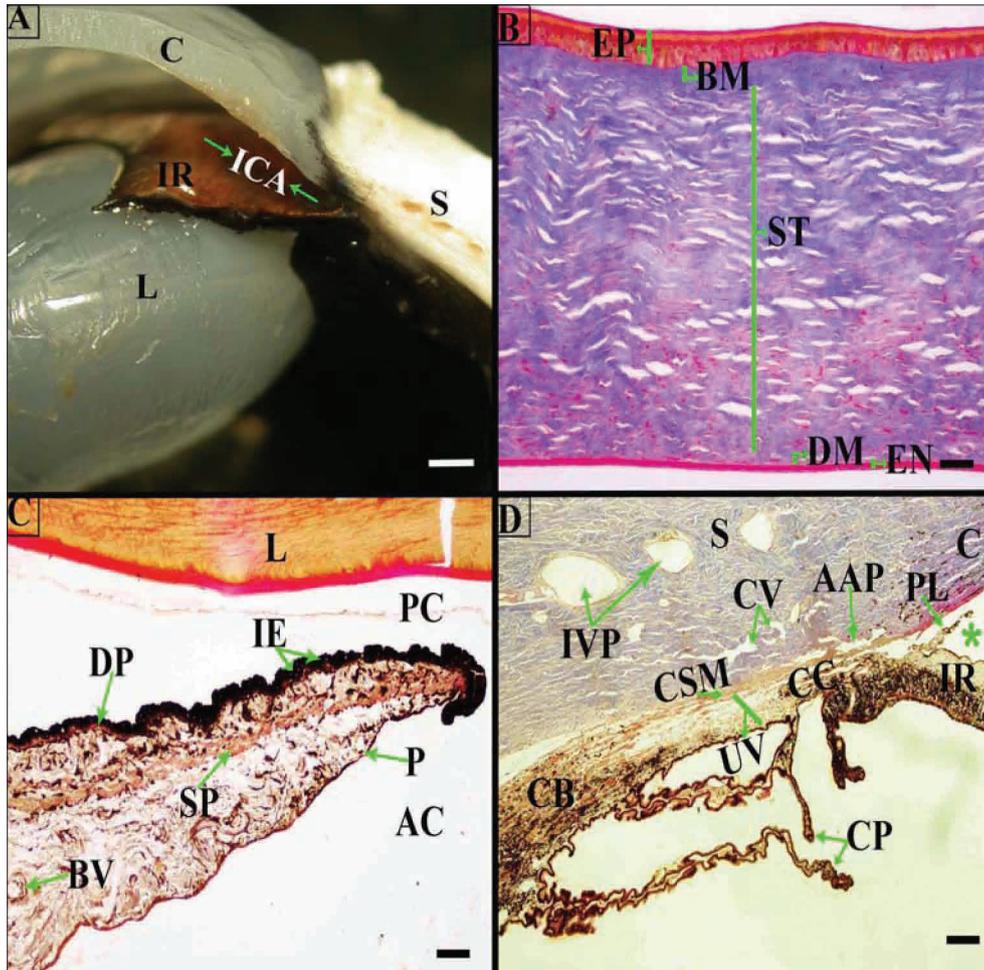
Clinical Login Information (Protocol Number)	Properties of Anatolian Shepherd Dogs			
	Age (years)	Sex	Weight (kg)	Clinical Status (Cause of Death)
09/15	1.5	Male	48	Paralysis due to traffic accident
10/24	1	Male	45	Paralysis of all foot and agony
11/181	2	Male	52	Paraplegia due to traffic accident
13/03	1.5	Male	50	L5 fracture due to traffic accident
13/14	2.5	Male	55	T12-T13 fracture due to traffic accident

an inner endothelial layer. The corneal thickness was measured as approximately  $435.20 \pm 14.99 \mu\text{m}$  in the centre and as  $501.43 \pm 16.81 \mu\text{m}$  in the periphery. The rather conspicuous basal membrane of the epithelium, which was approximately  $31.81 \pm 5.21 \mu\text{m}$  in thickness, underlay two or three layers of epithelial cells, the outer layer of which was highly flattened, and therefore, the cell borders and nuclei of which were hardly distinguished. The inconspicuous Bowman's membrane was determined to have an approximate thickness of  $6.60 \pm 1.29 \mu\text{m}$ , while the stroma, which was composed of multiple collagen fibrils organized in the form of lamellae, was determined

to have an approximate thickness of  $378.98 \pm 5.92 \mu\text{m}$ . The Descemet's membrane was of an approximate thickness of  $5.75 \pm 0.92 \mu\text{m}$ , and the endothelium lying beneath was composed of a single layer of squamous cells (Fig. 1 A,B).

### Iris

The iris was composed of abundant pigment cells, blood vessels and smooth muscle cells. The surface facing the posterior chamber was lined by two-layered pigmented epithelial cells, and in the periphery, this epithelium continued with ciliary epithelial cells. The stroma of the iris was composed of pigmented, vascular and loose



**Fig 1.** A- Anatomical view of the eye. C: cornea; S: sclera; L: lens; IR: iris; ICA: iridocorneal angle, B- Histological appearance of the cornea. EP: corneal epithelium; BM: bowman's membrane; ST: stroma; DM: descemet's membrane; EN: corneal endothelium, C- Histological appearance of the iris. L: lens; IE: iridal epithelium; DP: iris dilator muscle; SP: iris sphincter muscle; P: pigment cell; BV: blood vessel; PC: posterior chamber; AC: anterior chamber D- Histological appearance of the iridocorneal angle and ciliary body. IVP: intrascleral venous plexus; S: sclera; C: cornea; IR: iris; PL: pectinate ligament; AAP: angular aqueous plexus; CV: collecting veins; CC: ciliary cleft; CSM: corneoscleral meshwork; UV: uveal meshwork; CB: ciliary body; CP: ciliary processes; asterisk: iridocorneal angle. bar = 5 mm (A), 50  $\mu\text{m}$  (B, C) and 200  $\mu\text{m}$  (D)

**Şekil 1.** A- Gözün anatomik görünümü. C: kornea; S: sklera; L: lens; IR: iris; ICA: iridokorneal açı, B- Kornea'nın histolojik görünümü. EP: kornea epiteli; BM: bowman membranı; ST: stroma; DM: descemet membranı; EN: kornea endoteli, C- İris'in histolojik görünümü. L: lens; IE: iris epiteli; DP: irisin dilator kası; SP: irisin sphincter kası; P: pigment hücresi; BV: kan damarı; PC: arka kamara; AC: ön kamara, D- İridokorneal açı ve siliyer cismin histolojik görünümü. IVP: intraskleral venöz pleksus; S: sklera; C: kornea; IR: iris; PL: pektinat ligament; AAP: açılal aköz pleksus; CV: kolektör damarlar; CC: siliyer aralık; CSM: korneoskleral ağ; UV: uveal ağ; CB: siliyer cisim; CP: siliyer uzantılar; yıldız: iridokorneal açı. bar = 5 mm (A), 50  $\mu\text{m}$  (B, C) ve 200  $\mu\text{m}$  (D)

connective tissue. Furthermore, the stroma contained well-distributed and well-developed dilator and sphincter iridal muscles organized in bundles (Fig. 1 A,C,D).

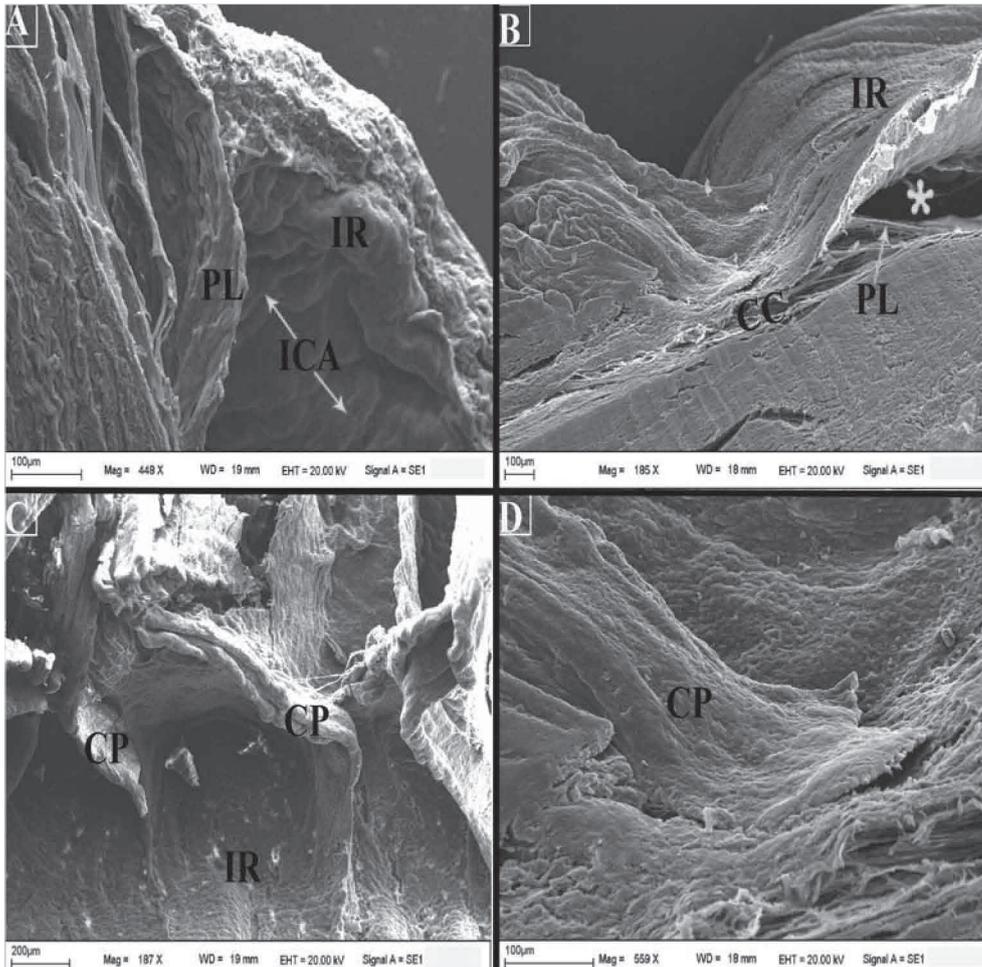
### Iridocorneal Angle

The iridocorneal angle (*angulus iridocornealis*) was located in-between the iridal base, the anterior aspect of the ciliary body and the inner surfaces of the cornea and sclera. This angle appeared to be relatively small. The iridocorneal angle was observed to be associated with ciliary cleft, pectinate ligament, angular aqueous plexus, uveal and corneoscleral meshwork (Fig. 1 D). The pectinate ligament, located peripheral to the anterior chamber, extended from the anterior surface of the root of the iris to the corneoscleral junction, and was observed as an isolated, fine fibre structure binding the two tissues (Fig. 2 A). The pectinate ligament was  $258.7 \pm 12.5 \mu\text{m}$  in length and  $51.6 \pm 11.3 \mu\text{m}$  in width. The partly discontinuous epithelial cell layer lining the anterior surface of the iris also covered this ligament. The cells forming this layer presented with pigmentation, and many collagen fibrils

extending in different directions were observed beneath the epithelium, which constituted the main skeleton of the ligament (Fig. 1 D).

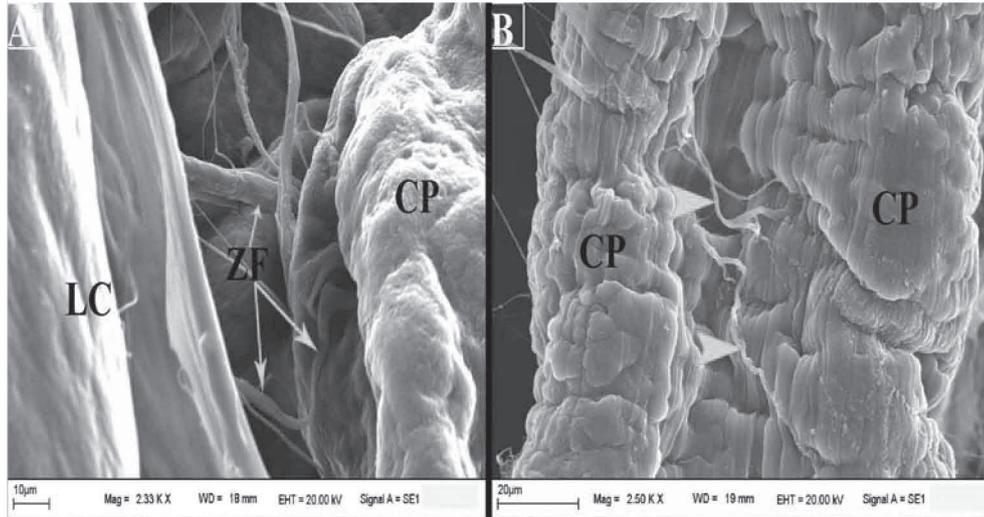
### Ciliary Cleft

The ciliary cleft was bordered by the pectinate ligament at the anterior aspect, the root of the iris and the ciliary body at the posterior aspect, and the corneal limbus at the exterior aspect (Fig. 2 B). In the iridocorneal angle, at which the corneal endothelium and the Descemet's membrane terminated, a dense trabecular meshwork existed, which was connected to the angular aqueous plexus. Two distinct regions were distinguished in this trabecular meshwork. The inner uveal meshwork, which originated from the anterior surface of the ciliary body and the root of the iris, was composed of loose trabecular plates. On the other hand, the corneoscleral meshwork, which originated from the sclera, was composed of leaf-like fine trabecular plates organized more tightly than that of the uveal meshwork. It was observed that, the trabecular plates forming both meshworks were made up of collagen fibrils (Fig. 1 D).



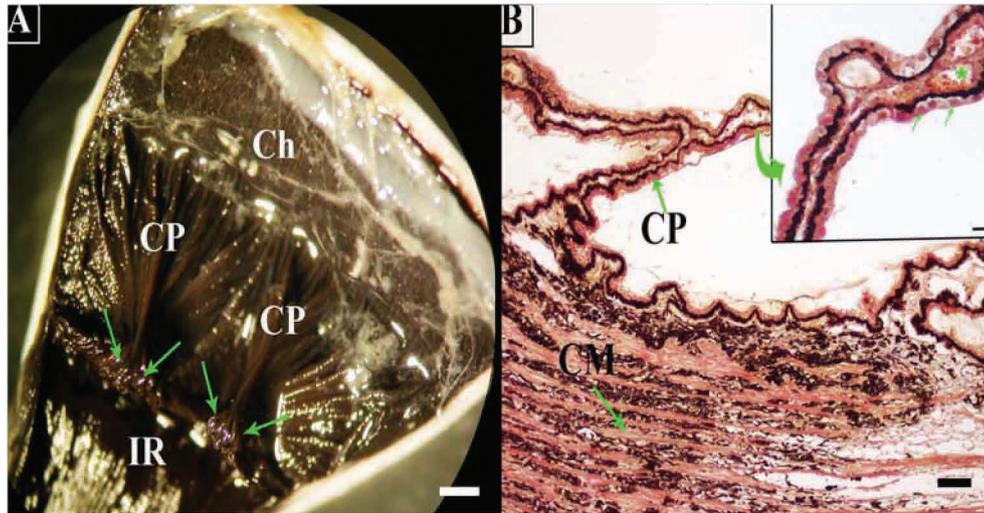
**Fig 2.** Scanning electron micro-graphs of the eye (A), (B), (C), (D). IR: iris; ICA: iridocorneal angle; PL: pectinate ligament; CP: ciliary processes; CC: ciliary cleft; asterisk: iridocorneal angle

**Şekil 2.** Gözün taramalı elektron fotoğrafı (A, B, C, D). IR: iris; ICA: iridokorneal açığı; PL: pektinat ligament; CP: siliyer uzantılar; CC: siliyer aralığı; yıldız: iridokorneal açığı



**Fig 3.** Scanning electron micrographs of the dog eye (A, B), showing ciliary processes (CP), zonular (ZF) and collagen (arrowheads) fibers. The zonular fibres are connected to the lens capsule (LC)

**Şekil 3.** Köpek gözündeki siliyer uzantılar (CP), zonüler (ZF) ve kolajen (okbaşları) liflerin taramalı elektron fotoğrafı (A, B). Zonüler lifler lens kapsülüne (LC) bağlanır



**Fig 4. A-** Anatomical view of the ciliary processes (CP) that form conspicuous bundles (arrows) at the sites of merge. Ch: choroidea; IR: iris, **B-** Histological appearance of the ciliary body. CP: ciliary processes; CM: ciliary muscle; asterisk: blood vessel; arrows: bilayered epithelium. bar=5 mm (A), 100 µm (B) and scale bar of overlapped figure is 25 µm

**Şekil 4. A-** Birleşme yerinde belirgin şekilde demetler (oklar) oluşturan siliyer uzantılarının (CP) anatomik görünümü. Ch: koroid; IR: iris, **B-** Ch: koroid; IR: iris, **B-** Siliyer cisminin histolojik görünümü. CP: siliyer uzantılar; CM: siliyer kas; yıldız: kan damarı; oklar: iki katmanlı epitel. bar = 5 mm (A), 100 µm (B) ve içindeki şeklin barı 25 µm

### Ciliary Body

The ciliary body extended from the root of the iris to the ora serrata and was composed of two major structures, namely, the ciliary processes and ciliary muscle (Fig. 1 D, 4 B). The ciliary processes originated with blunt ends at the posterior and extended anteriorly. While the ciliary processes were observed to be short and considerably close to each other at their origin, along their course to the iris their height was determined to increase progressively. Along their wavelike course, the ciliary processes were observed to merge at high points and form conspicuous

bundles at the sites of merge (Fig. 4 A), and the ciliary processes were reported to end separately (Fig. 2 C). Furthermore, the ciliary processes, which were initially column-like at their origin, progressively increased in height and acquired a leaf-like form particularly at the level of the anterior two-thirds of the ciliary body (Fig. 2 D). It was observed that while the posterior aspect of the ciliary processes lacked the zonular fibres (*lig. suspensorium lentis*), the anterior aspect supported the lens capsule by means of the zonular fibres (Fig. 3 A). The ciliary processes were composed of two-layered epithelial cells, which were non-pigmented in the inner layer and pigmented in the

outer layer (Fig. 4 B). The bodies of the ciliary processes were connected to each other with collagen fibrils, and these connections were observed to be denser at the posterior aspect (Fig. 3 B). Of the ciliary muscle forms, the radially organized bundles were relatively uncommon, while the well-developed meridionally arranged fibres were composed of smooth muscle cells tightly organized in bundles (Fig. 4 B).

## DISCUSSION

This study was aimed at the morphological investigation in the eye of the Anatolian Shepherd dogs. A moderately developed ciliary body musculature, well developed iridal muscles, small iridocorneal angle, poorly developed ciliary cleft, bundles formed by the ciliary processes, corneoscleral and uveal pathway for aqueous humor removal in Anatolian Shepherd dogs point out a marked morphological description.

The cornea, which supports the intraocular structures, refracts light owing to its convexity and allows the permeation of light owing to its transparency<sup>[8]</sup>. Similar to humans and non-human primates<sup>[9]</sup>, in the Anatolian Shepherd dog the corneal layers are finer in the centre, compared to the periphery. The thickness of the corneal epithelium was determined to be less in the Anatolian Shepherd dog, compared to humans<sup>[10]</sup>, and the corneal epithelium was ascertained to be composed of 2 to 3 layers, while in other domestic animals it has been reported to be composed of a greater number of layers<sup>[11]</sup>. While the Bowman's membrane, adjacent to the epithelium, is most evident in the cornea of humans and non-human primates, ruminants have been reported to possess a real-like Bowman membrane<sup>[11]</sup> and adult rabbits have been reported to lack the Bowman membrane<sup>[12]</sup>. In the present study, the Bowman membrane was inconspicuous beneath the epithelium and measured similar to that of humans<sup>[13]</sup>. The reports for the corneal stroma being composed of collagen lamellae extending parallel to the corneal surface and accounting for 90% of the total thickness of the cornea<sup>[11]</sup> have been confirmed for the dog in the present study. The thickness of the Descemet's membrane in the Anatolian Shepherd dog was found to be similar to that of humans<sup>[14]</sup>. The Descemet's membrane has been reported to serve as a semipermeable barrier for the transport of molecules between the corneal stroma and the anterior chamber of the eye, and to provide support to the posterior stroma<sup>[15]</sup>. The thickness of the corneal layers having been measured differently in various species suggests that this anatomical structure has species-specific features.

The main function of the iris is to regulate the amount of light entering into the eyeball<sup>[16]</sup>. To date, in all of the animal species investigated, the anterior surface of the iris has been determined to be lined by a monolayer of squamous epithelium, which is considered to be the continuation of

the corneal endothelium<sup>[17]</sup>. Furthermore, as described by Ham<sup>[18]</sup>, in the present study, at the posterior aspect, the iris was lined by two-layered pigmented epithelial cells, and the epithelium was undulated and in close contact with the dilator pupillae. While the iridal sphincter muscle has been reported to be composed of striated muscle in non-mammals and smooth muscle localized to the stroma in mammals, the localization of this muscle, which is indicated as mainly the central stroma in the horse<sup>[11]</sup>, has been ascertained as the posterior stroma in the Anatolian shepherd dog. On the other hand, the iridal dilator muscle, which has been reported to be composed of smooth muscle with an exception of striated muscle in birds<sup>[11]</sup>, was localized to the posterior iridal stroma in the present study. The dense pigmentation observed in the anterior and posterior iridal epithelium, apart from the iridal stroma, has been suggested to prevent the entry of light into the eyeball other than from the pupil<sup>[19]</sup>, and this opinion suggests this function of the iris to be fulfilled with the contraction of both muscles.

The iridocorneal angle plays a complementary role for fluid drainage in the eye. It was observed that, in the Anatolian Shepherd dog, the ciliary cleft of the iridocorneal angle was not large and well-developed as in the pig<sup>[6]</sup>. The ciliary cleft has been reported to be deep in small diurnal herbivores, and very well-developed in large diurnal herbivores<sup>[11]</sup>. Similarly, horses have been reported to have a wide ciliary cleft, supported by both the inner part of the trabecular meshwork and the pectinate ligament, which makes the collapse of the ciliary cleft nearly impossible and results in the occurrence of glaucoma in horses only rarely<sup>[5]</sup>. Generally, in animals, this structure facilitates the drainage of the aqueous humor, and it is claimed that the iridocorneal angle of primates and carnivores being small results from the enlargement of the ciliary body musculature<sup>[20]</sup>.

The pectinate ligament is composed of long strands that bind the anterior iridal base to the inner peripheral cornea. While in the present study the pectinate ligament was observed to have a fine and elongated structure, in the rabbit and pig it was observed to be shorter and thicker than in the domestic carnivores<sup>[21]</sup>. On the contrary, in the majority of ungulates, excluding the pig, the structure of the pectinate ligament ranges from moderate to thick<sup>[11]</sup>. Furthermore, reports indicate that the pectinate ligament resembles a fenestrated sheet in rabbits<sup>[21]</sup>, and horses<sup>[5]</sup>. Therefore, it could be said that the structure and shape of this ligament plays an important role in both the routing of the aqueous humor and the supporting of the iridal base.

The posterior aspect of the pectinate ligament is the region of the ciliary cleft and continues with the uveal and corneoscleral meshworks. While De Geest et al.<sup>[5]</sup> reported that the corneoscleral meshwork covered only the posterior angle of the ciliary cleft in the horse, McMennamin and Steptoe<sup>[6]</sup> reported that it occupied the

entire length of the outer wall of the ciliary cleft in the pig, which was also the case in the present study. Furthermore, in the present study, the uveal meshwork was observed to be associated with the corneoscleral meshwork, and it was concluded that these structures aided in maintaining the iridocorneal angle to prevent any collapse in the drainage of the aqueous humor in the eye. Research has demonstrated that rabbits<sup>[7]</sup> lack the canal of Schlemm, but possess a sinus structure to substitute for its function. Samuelson and Gelatt<sup>[22]</sup> reported that normal drainage of the aqueous humor from the anterior chamber of the canine eye is believed to occur mainly through collecting veins in the corneoscleral meshwork, which subsequently empty into the larger intrascleral venous plexus system and to a lesser degree posteriorly through the uveoscleral route. In addition the morphology of the angular drainage channels have been demonstrated to be similar among the rat, rabbit, ox, cat, and dog with the canal of Schlemm in the primate eye. Similarly, in the present study, it was ascertained that the angular aqueous plexus existed in the eye of the Anatolian Shepherd dog, and enabled the outflow of the aqueous humor from the anterior chamber.

The caudal part of the ciliary body contains the ciliary muscle, which is a smooth muscle that alters the shape of the lens for near and far vision<sup>[23]</sup>. The rhesus monkey has an anthropoid type of eye characterized by a very well-developed ciliary body musculature and small iridocorneal angle<sup>[11]</sup>. In general, in herbivores these muscles are not well-developed and are composed of only meridionally extending muscle fibres<sup>[6]</sup>. Dellmann and Collier<sup>[24]</sup> reported that, pigs possess only circular fibres, which predominate in the nasal region of the ciliary body. It has been suggested that in some aquatic mammals, which are devoid of these muscles, an increased vascularization of the ciliary processes constitutes an alternative mechanism for the accommodation of the lens<sup>[2]</sup>. In the present study, in agreement with previous study carried out in cats<sup>[25]</sup>, it was observed that these muscles were moderately developed and followed both a meridional and a radial course. The ciliary processes, another component of the ciliary body, generally do not exist in lower vertebrates<sup>[26]</sup>. Different from the results reported by Samuelson<sup>[26]</sup> suggesting the ciliary processes to be lined mostly by columnar epithelium in ungulates, the present study demonstrated that the ciliary processes were lined by a two-layered epithelium, composed of pigmented cuboidal epithelial cells in the outer layer and non-pigmented cuboidal epithelial cells in the inner layer. Thus, it can be suggested that the ciliary body plays a functional role in regulation, owing to its association with the iridocorneal angle, and that the structural differences between species are a result of adaptation to the environment.

The ciliary muscle is responsible for the optic accommodation mechanism. Its contractions move the ciliary processes and alter the tension of the zonular fibres.

The two-sided convexity of the lens is modified by this mechanism, which enables focusing in the visual space<sup>[27]</sup>. In previous research on mammals, the conspicuous bundles formed by the ciliary processes were not described in detail. As can be understood from the information given above, the accommodation of the lens depends on the transfiguration capability of the lens, and in the present study it was determined that the apical ends of the ciliary processes formed bundles and adhered tightly to the lens capsule. It is considered that this anatomical feature detected in the Anatolian Shepherd dog may have a strong impact on the accommodation mechanism.

Eye is one of important organs of the body and has many roles in relationship between animal and environment<sup>[28]</sup>. In conclusion, the course followed by the ciliary processes of the ciliary body in the Anatolian Shepherd dog is considered to be interesting. Furthermore, it is considered that the description of the morphological properties of both the iridocorneal region, in which the aqueous fluid is reabsorbed, and the ciliary body, in which the aqueous humor is secreted, would contribute to the interpretation of the functional correlation, thus to future experimental studies to be conducted in this field.

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