

Morphometric and Histological Structure of Bulbus Oculi in Goats

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This study aimed to identify the morphometrical and histological structure of eyeball (*bulbus oculi*) in Abaza and Gurcu goats. The study analyzed ten pairs of *bulbus oculi* from Abaza and Gurcu goats (5F/5M), taking several morphometric measurements over the eyes using a digital caliper. The eyes observably possessed all recognized anatomical formations in both goat breeds. However, the lens was different in Gurcu goats. Analyses revealed that the *pupilla* dorso ventral diameter ($P<0.001^{***}$), lens dorso ventral diameter ($P<0.005^{**}$), and lens medio lateral diameter ($P<0.05^{*}$) parameters were all statistically significant in Abaza and Gurcu goats. Histologically, Abaza goats had narrow iris structures and dense muscle fibers, whereas Gurcu goats displayed wider and mostly connective tissue formation. Similarly, the choroid of Abaza goats had more dense connective tissue and a narrow structure, whereas the choroid of Gurcu goats retained less connective tissue and a larger layout.

Key words: eye anatomy, bulbus oculi, lens, goat

Introduction

A typical eye is a lens-containing specialized sensory organ [5]. The eye is also one of the complex sense organs, with an extreme sensitivity to external factors, light, and diseases [2, 28, 49], consisting of the optic nerve, eyeball (*bulbus oculi*), and accessory organs of the eye (*organa oculi accessoria*) [46]. The *bulbus oculi* develops from the optic vesicle, a protrusion of the brain, and the lens develops from the ectoderm adjacent to the optic vesicles. Cornea, however, forms through the induction of the lens sac [19, 53]. The macroanatomical and morphometric features of the eyeball differ between species and races. Cats have the largest eyeball compared to the body sizes of domestic mammals, followed by dogs, horses, and cattle [24]. The embowed anterior

and the posterior parts of the eyeball are called the *polus anterior* and *polus posterior*, respectively. The axis uniting these two parts from the outside from frontally to the back is called the axis bulbi externus (axis opticus), and the axis joining them from the inside is called the axis bulbi internus [24, 46].

From the past to the present, mainly anatomical, histological, and pathological studies have been conducted on eye tissues through modeling various animals. Some of these studies were performed in humans [18, 52], pigs [11, 26, 31], buffaloes [4], horses [43, 45], dogs [6, 40], sheep [12, 41], and bears [20, 36]. Only the eye is a specific sensory organ that pathologists routinely assess during pre-clinical general studies for the development of novel medicine. Therefore, it is crucial to ascertain and understand the typical histology of the eye tissue in all animal species [7, 51].

Materials and Methods

Ethical approval

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: 2022/067).

Animals

Accordingly, the study analyzed ten pairs of *bulbus oculi* from mature five Abaza and five Gurcu goats.

Anatomical findings

The eyeball from the skulls were removed from orbital cupping and isolated, taking several morphometric measurements over the dissected *bulbus oculi* using a digital caliper (**Figs. 1, 2**). After macro-anatomical and morphometric measurements, Abaza and Gurcu goat eyes were kept in a 10% formaldehyde solution for histological analyses.

Histological examination

Then, the tissues were paraffin blocked by processing tissues through the subsequent (standard) customary histological tissue solutions. By cutting 5 µm thick sections from the prepared blocks, Triple staining (Crossman's Modified Triple Staining) technique was used to analyze the tissues histologically.

Statistical analysis

Using the SPSS 20 program, the mean values and standard deviations of the obtained data and the correlation coefficients between these features were assessed. Taking the terms of Nomina Anatomica Veterinaria [30] as a basis, the study also utilized to photograph the images of *bulbus oculi* using the Nikon D700 digital camera.

Results

Morphometric Results

Figure 1 and **Figure 2** display the reference points taken from bulbus oculi and lens analyzed in Abaza and Gurcu goats. Correspondingly, **Table 1** and **Table 2** provide the mean and standard deviation values of the morphometric findings of the bulbus oculi and correlation analyses of these values, respectively. However, **Table 3** lists the comparative results of the morphometric measurements taken from *bulbus oculi* with other studies. Macro-anatomical analysis revealed that the bulbus oculi from Abaza goats demonstrated typical anatomical characteristics (**Fig. 1A**, **Fig. 2A**). However, the lens in Gurcu goats consisted of two nodes, although it visually displayed a typically similar structure (**Fig. 2B**). Analyses revealed that the pupilla dorso ventral (DV) diameter ($P<0.001^{***}$), lens DV diameter ($P<0.005^{**}$), and lens medio-lateral (ML) diameter ($P<0.05^{*}$) parameters were all statistically significant in Abaza and Gurcu goats.

Table 1. Mean values of morphometric measurements taken from *bulbus oculi* in Abaza and Gurcu goats (right/left)

Parameters (in mm)	Abaza right	Abaza left	Gurcu right	Gurcu left
BDV diameter	23,50±4,07	23,27±3,55	25,82±2,14	26,06±2,13
BML diameter	26,40±1,65	25,63±3,25	27,47±1,61	27,41±2,33
Axial diameter	27,21±1,79	25,77±2,43	27,39±3,86	27,98±1,73
Cornea DV diameter	10,30±2,01	10,55±2,04	11,13±1,03	11,55±1,62
Cornea ML diameter	17,42±1,64	17,30±3,68	16,85±1,17	16,96±1,10
Pupillary DV diameter	5,45±0,2	5,54±0,1	8,37±1,24	8,38±1,26
Pupillary ML	9,13±0,7	9,47±0,7	9,42±1,54	9,45±1,51
Lens DV diameter	10,31±0,3	10,33±0,4	11,92±1,28	11,44±0,99
Lens ML diameter	11,08±0,3	11,24±0,3	11,61±0,36	11,89±0,64
Lens Thickness	7,24±0,4	7,25±0,4	7,69±0,95	7,67±1,08

Table 2. Correlation analysis of parameters taken from *bulbus oculi*

Abaza→ Gurcu↓	DV diameter	ML diameter	Axial diameter	corDV	corML	PupiiDV	pupiiML	LensDV	lensML	Lens thickness
DVdiameter		,604	,207	,158	-,249	-,136	-,385	,759*	,162	,659*
MLdiameter	,720*		-,318	,207	,585	-,098	-,048	,662*	,398	,702*
Axial diameter	,758*	,711*		-,161	-,547	-,433	-,612	,143	-,596	,135
corDV	-,265	-,281	-,094		,221	,734*	,434	-,214	,280	-,176
corML	-,298	-,138	-,141	,556		,110	,406	,017	,377	,167
PupiiDV	,864**	,477	,556	-,583	-,336		,488	-,385	,449	-,551
pupiiML	-,559	-,370	-,312	,893**	,650*	-,814**		-,526	,642*	-,417
LensDV	-,243	,047	-,222	,331	,065	-,541	,562		,197	,919**
lensML	,456	-,080	-,015	,130	,012	,444	-,085	,009		,090
Lens thickness	,980**	,808**	,802**	-,313	-,297	,826**	-,549	-,148	,353	

Table 3. Statistical data of similar parameters in other studied species, * M and F stand for ‘Male’ and ‘Female,’ respectively

Parameters (in mm)	BML	BDV	CML	CDV	PDV	PML	Lens Thickness
Buffalo (12)	35,10±0,38	34,3±0,42					8,67±0,15
Camel					right 2,04±0,21 left 2,08±0,23		
Sahel Goat M*/right	8,12±0,53	7,17±0,46					
M/left	8,14±0,34	7,09±0,37					
F* /right	8,34±0,36	7,21±0,49					
F/left	8,20±0,44	7,13±0,52					
Wad M/right	8,16±0,74	6,82±0,70					
Wad M/left	7,70±0,67	6,88±0,87					
Wad F/right	8,50	7,43±0,35					
Wad F/left	8,47±0,39	7,39±0,27					
Red skoto M/right	8,07±0,15	6,97±0,19					
Red skoto M/left	7,87±0,29	7,17±0,03					
F/right	8,83±0,34	7,70±0,34					
F/left	8,70±0,31	7,53±0,21					
Barbary Sheep			25,05±2,18	17,95±1,68			9,44±0,33
Awassi sheep M/right		25,95±0,49	21,99±0,30	17,08±0,26	6,75±0,28	11,88±0,29	8,87±0,21
M/left		26,56±0,53	21,98±0,32	16,87±0,38	7,38±0,33	11,83±0,35	19,28±0,16
F/right		29,33±0,44	24,09±0,38	17,88±0,43	7,49±0,28	13,58±0,24	10,08±0,14
F/left		28,38±0,46	24,39±0,40	18,66±0,40	7,69±0,24	13,71±0,74	10,36±0,16

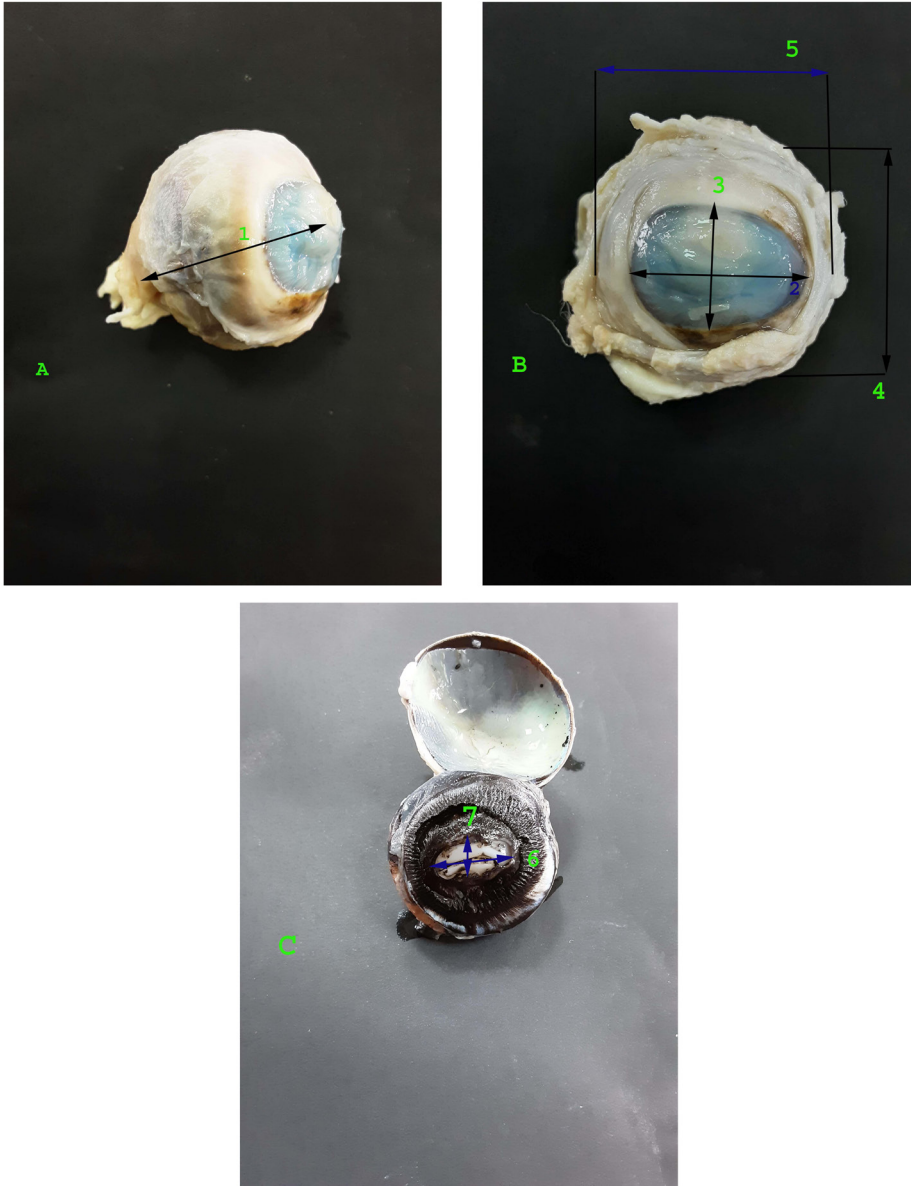


Fig. 1. Measuring points of samples taken over Bulbus oculi, (A, B, C): A: Axial length/diameter: Distance between Polus anterior and pole posterior (1), B: Medio-lateral (CML) diameter: Distance between temporal and nasal endpoints of the cornea (2), Dorso-ventral (CDV) diameter: Distance between the dorsal end of the cornea and endpoints in the ventral direction (3), Dorso-ventral (BDV) diameter: Distance between the endpoint of the bulbus oculi in the dorsal direction and its ventral endpoints (4), Medio-lateral (BML) diameter: Distance between temporal and nasal endpoints of bulbus oculi (5), C: Medio-lateral (PML) diameter: Distance between temporal and nasal endpoints of *pupilla* (6), Dorso-ventral (PDV) diameter: Distance between the endpoint of the *pupilla* in the dorsal direction and the endpoints in the ventral direction (7).

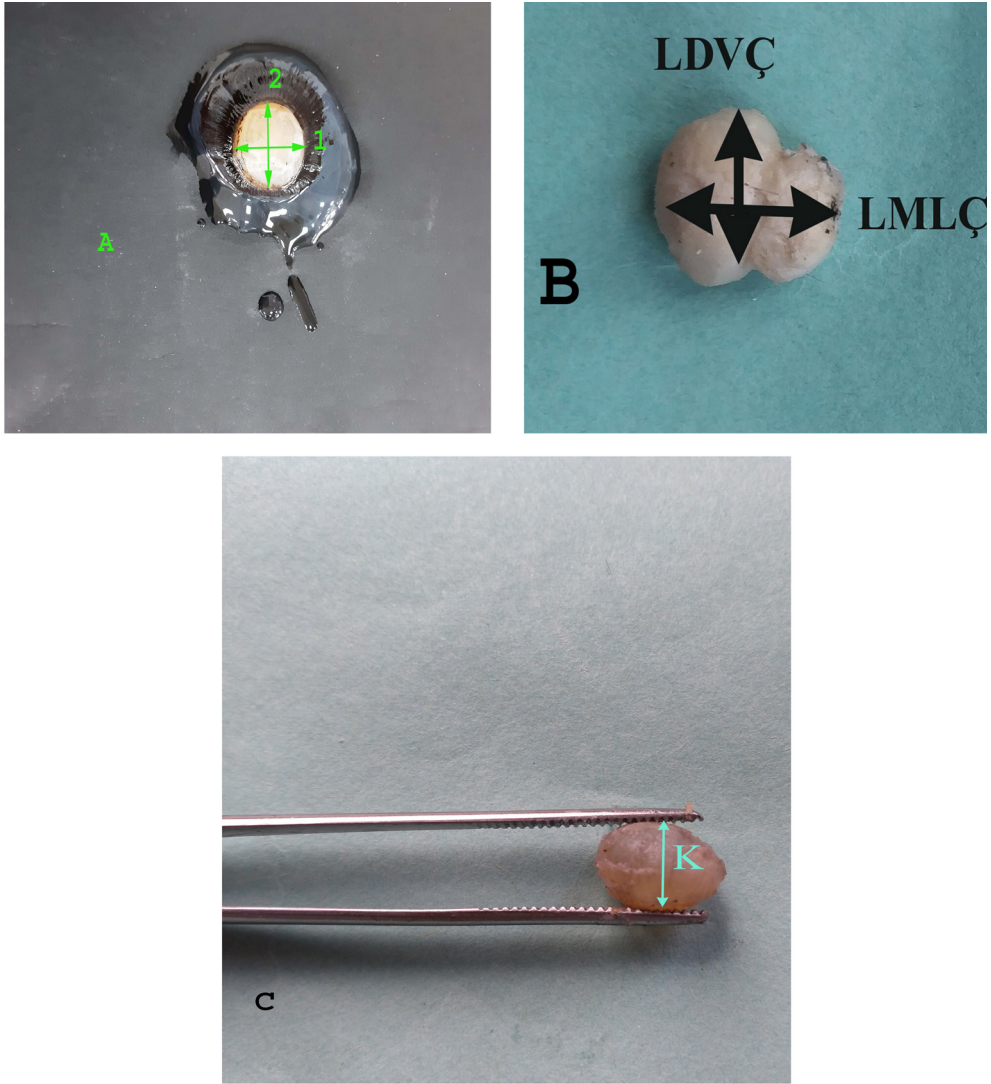


Fig. 2. (A, B, C): Lens measurement points **A:** Lens from Abaza goat, Dorso-ventral (LDV) diameter: Distance between the lens endpoint in the dorsal direction and the ventral direction (1), Medio-lateral (LML) diameter: Distance between the temporal and nasal endpoints of the lens (2), **B:** The same measurement points of the lens in Gurcu goats, **C:** Thickness: The distance from the middle point to the anterior and posterior endpoints of the lens.

Histological Results

Three main layers of the eyeball, the cornea and sclera (*from tunica fibrosa bulbi*), choroid, iris, *corpus ciliare* (*from tunica vasculosa bulbi*), and from *tunica interna bulbi* retina were studied histologically during the analysis of Abaza and Gurcu goats ocular structures.

Corneoscleral tegmental, sclera, and corneal structures the outer or fibrous layer were scrutinized. The sclera notably consisted of episcleral, *substantia propria*, and *lamina fusca* layers in both species. The propria of the sclera was explicitly composed of thick collagen fibers, and there were neural structures and blood vessels between the collagen fibers. The *lamina fusca* region on the opposite side of the choroid was composed of thinner collagen fibers than the *substantia propria*, and there were melanocytes between these fibers (Fig. 3A). Additionally, the distance between the *substantia propria* and episcleral layers of the sclera, known as Tenon's space

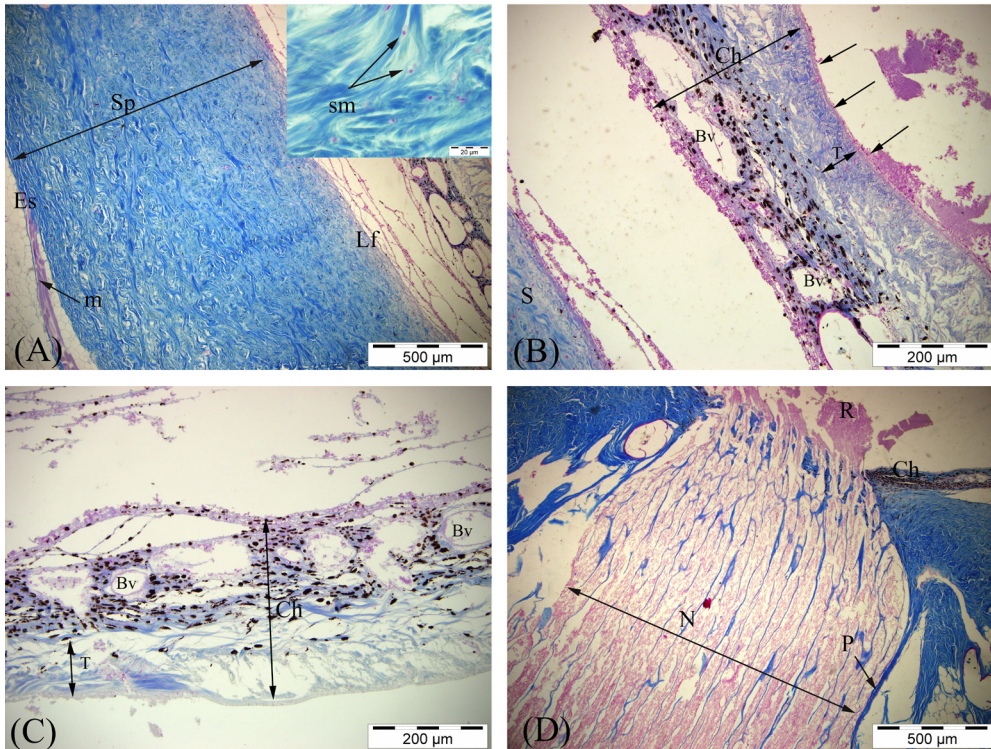


Fig. 3. Crossman's Modified Triple Staining of cross section of *bulbus oculi* A) Abaza goat sclera and choroid view, Ch: Choroid, Es: Episclera, Lf: Lamina fusca, m: muscle bundle in episclera, sm: smooth muscle, Sp: Substantia propria, S: Sclera, B) Abaza goat choroid, Bv: Blood vessel, Ch: Choroid, T: Tapetum fibrosum, Arrows: Retinal pigment epithelium, C) Gurcu goat choroid, Bv: Blood vessel, Ch: Choroid, T: Tapetum fibrosum, D) Ch: Choroid, R: Retina, S: Sclera, N: Optic nerve, P: Piameter, Triple stain.

(episcleral space), was identified. The cornea, the anterior segments of the eye, was identified to have a posterior surface consisting of a single-layered squamous epithelium, an anterior surface with stratified squamous non-keratinized epithelium, and a stroma in the middle. The stroma observably consisted of lamellar collagen fibers. Fontana clefts (the trabecular meshwork) and Schlemm's canal were explicitly definable in the corneoscleral region (Fig. 4A, B). In both Abaza and Gurcu goats, the choroid appeared to contain five layers: from the bottom *lamina basalis* (Bruch's

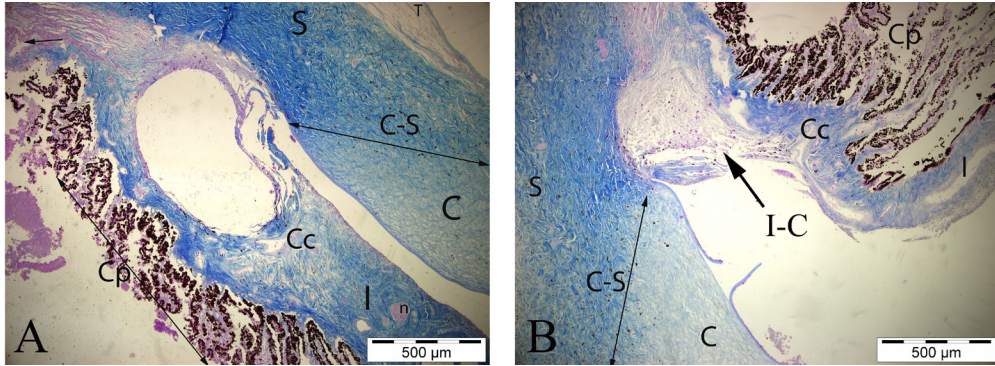


Fig. 4. Crossman's Modified Triple Staining of cross section of *bulbus oculi* A) Abaza Goat Corneoscleral Image, B) Gurcu Goat Corneoscleral Image, C: Cornea, Cc: Corpus ciliare, Cp: Ciliary process, I: Iris, I-C: Iridocorneal region, n: Neural degradation, C-S: Corneoscleral cross, S: Sclera, T: Trabecular network, Triple stain.

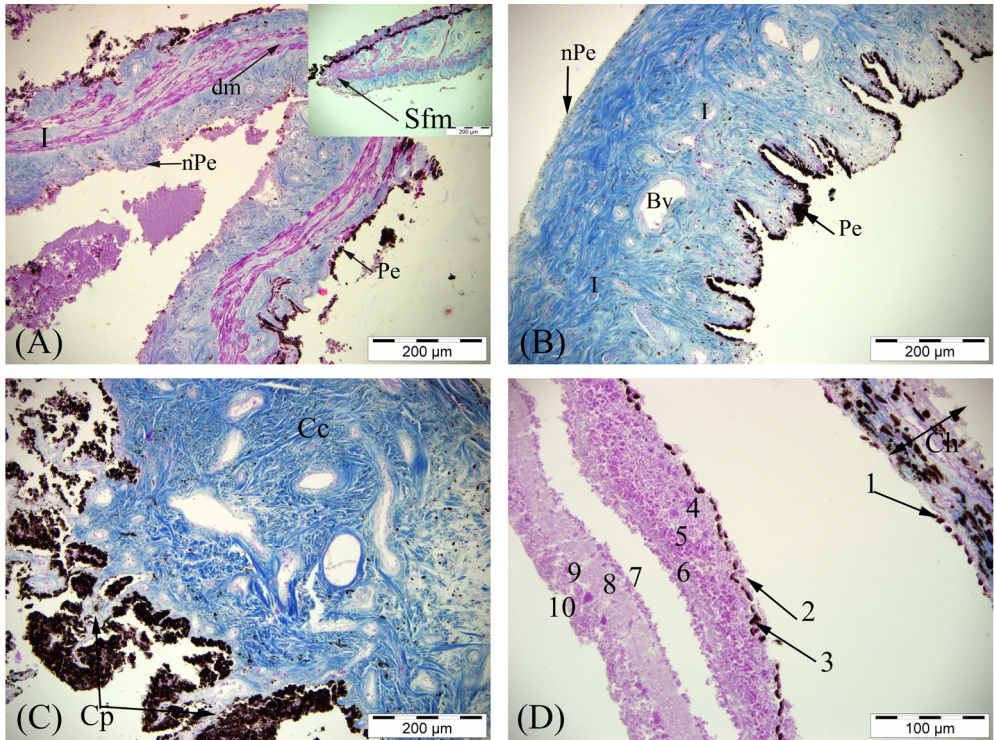


Fig. 5. Crossman's Modified Triple Staining of cross section of *bulbus oculi*. A) Abaza Goat Iris view, B) Gurcu Goat Iris view C) Gurcu Goat Corpus ciliare view, Bv: Blood vessel, Co: Cornea, I: Iris, Cc: Corpus ciliare, Cp: Ciliary process, dm: dilator muscle, nPe: Non-pigmented epithelium, Pe: Pigmented-epithelium, Sfm: Sphincter muscle, D) Retina, Ch: Choroid, 1: the pigmented layer of the iris, 2: rod and cone receptors layer, 3: *membrana limitans externa*, 4: outer nuclei layer, 5: outer plexiform layer, 6: inner nuclei layer, 7: inner plexiform layer, 8: ganglion cells layer, 9: neural fibers layer, 10: *membrana limitans interna*, Triple staining.

membrane), *lamina capillare*, *tapetum fibrosum*, *lamina vasculosa* and the *lamina suprachoroidea* (Fig. 3B, C). The *tapetum fibrosum*, nevertheless, observably consisted of wider collagen fibers and contained fibroblasts. The tapetum of the choroid in Abaza goats (Fig. 3B) was more orderly and firmer when compared to Gurcu goats (Fig. 3C). However, it might display vary depending on the age or gender. In both species, the choroid structure had explicitly dense pigment cells. The *processus ciliaris* and *corpus ciliare* structures rimmed by a bilayer epithelium (cubic) were observed between the choroid and iris layers of the *tunica vasculosa bulbi* region (Fig. 5C). There were also several distinctly identified tissues, including pigmented iris epithelium tissue the subsequent section of the corpus ciliare, neural plexuses in the dense collagen fibers of the stroma, numerous melanocytes, blood vessels, dilator, and sphincter muscle structures (Fig. 5A, B). The retinal structure of the *tunica interna bulbi* section from outside-in retained ten observable layers (Fig. 5D). In addition, the *nervus opticus* rimmed by the perineurium and epineurium was identified (Fig. 3D).

Discussion

In Sahel goats, the right medio-lateral (ML) diameter of bulbus oculi was reportedly 8.12 ± 0.53 mm and 8.34 ± 0.36 mm, in males and in females respectively, whereas the left ML diameter was 8.14 ± 0.34 mm in males and 8.20 ± 0.44 mm in females [33]. The same study reported the medio-lateral diameter of eyeball in two other goat breeds as follows in West African dwarf goats [33], the right and left diameters of mediolateral was 8.16 ± 0.74 mm, and 7.70 ± 0.67 mm in males, respectively; however, it was 8.50 ± 0.00 mm and 8.47 ± 0.39 mm for the right and left mediolateral diameters in females. In Red Sokoto goats [33], correspondingly, the right diameter of mediolateral was 8.07 ± 0.15 mm, in males and 8.83 ± 0.34 mm in females, whereas the left diameter of mediolateral was 7.87 ± 0.29 mm in males and 8.70 ± 0.31 mm, in females.

Considering the mentioned goat breeds above, the DV diameter of the both side (Right/Left) *bulbus oculi* for Sahel goats [33], were 7.17 ± 0.46 mm and 7.09 ± 0.37 mm, in males, whereas it was 7.21 ± 0.49 mm and 7.13 ± 0.52 mm, for the right and left *bulbus oculi* in females, respectively. Similarly, the right and left diameters of dorsoventral of the *bulbus oculi* in West African dwarf goats [33] were (6.82 ± 0.70) mm, and (6.88 ± 0.87) mm, in males; however, it was (7.43 ± 0.35) mm, and (7.39 ± 0.27) mm, in females, respectively. The right and left DV diameters, on the other hand, were measured as 6.97 ± 0.19 mm and 7.17 ± 0.03 mm among Red Sokoto male goats [33], whereas it was (7.70 ± 0.34) and (7.53 ± 0.21) mm, for the right and left *bulbus oculi* in females, respectively. Another study focusing on Awassi sheep [13] reportedly measured the dorsoventral diameter of the right and left eyeball as (25.95 ± 0.49) mm, and (26.56 ± 0.53) mm, in males, whereas it was (29.33 ± 0.44) mm and (28.38 ± 0.46) mm in females, respectively. Medio lateral diameter of the Awassi sheep [13], however, was (28.11 ± 0.42) mm and (27.50 ± 0.39) mm, for the right and left eyes in males, respectively [13]. A study on *Bubalus bubalus* also reported the medio-lateral diameter of the bulbus oculi as (35.10 ± 0.38) mm and the dorsoventral diameter as (34.3 ± 0.42) mm [50]. The current study, on the other hand, measured the DV diameter of *bulbus oculi* in Abaza goats as 23.50 ± 4.07 mm and 23.27 ± 3.55 mm on the right and left, whereas it was 25.82 ± 2.14 mm and 26.06 ± 2.13 mm for the right and left in Gurcu goats.

The mediolateral diameter of the cornea was 25.05 ± 2.18 mm, and the DV diameter was 17.95 ± 1.68 mm in Barbary sheep (*Ammotragus lervia*) [17]. The study on Awassi sheep [13], however, the medio-lateral diameter of the cornea was (21.99 ± 0.30) mm on the right side and, (21.98 ± 0.32) mm on the left side in males, whereas it was (24.09 ± 0.38) mm on the right and (24.09 ± 0.40) mm, on the left side in females. The same study documented that the dorso-ventral diameter of the cornea was (17.08 ± 0.26) mm, on the right and (16.87 ± 0.38) mm, on the left side in females; however, these measurements were 17.88 ± 0.43 mm, on the right and 18.66 ± 0.40 mm, on the left in females. Considering the Barbary sheep (*Ammotragus lervia*), Fornazari et al. [17], reported the lens thickness as (9.4 ± 0.33) mm. Verma et al., [50], measured a lens thickness of (8.67 ± 0.15) mm in buffalo (*Bubalus bubalus*). Similarly, the study on Awassi sheep reported that [13], mean lens thickness, was (8.87 ± 0.21) mm and (19.28 ± 0.16) mm, on the right and left side in males, whereas it was (10.08 ± 0.14) mm, and (10.36 ± 0.16) mm, on the right and left in females, respectively. The current study also measured the mean lens thickness in Abaza goats as 7.24 ± 0.4 on the right and 7.25 ± 0.4 mm, on the left. These measurements were comparable in Gurcu goats as the lens thickness on the right and left was 7.69 ± 0.95 and 7.67 ± 1.08 mm, respectively. The *pupilla* dorso ventral diameter ($P < 0.001^{***}$), lens dorso-ventral diameter ($P < 0.005^{**}$), and lens medio-lateral diameter ($P < 0.05^{*}$) parameters were all statistically significant in Abaza and Gurcu goats. However, the difference in lens thickness between the males and females in Awassi sheep [13], was statistically significant ($P < 0.001$).

Abuagla et al., [1], reported the *pupilla* diameter among camels as 2.04 ± 0.21 cm, and 2.08 ± 0.23 cm, on the right and left, respectively. The same literature documented that the DV diameter of the pupil in Awassi sheep [13], was 6.75 ± 0.28 mm, on the right and 7.38 ± 0.33 mm, on the left in males, whereas it was 7.49 ± 0.28 mm, on the right and 7.69 ± 0.24 mm, on the left in females. Additionally, they measured the ml diameter of the *pupilla* in Awassi sheep [13], as 11.88 ± 0.29 mm, on the right and 11.83 ± 0.35 mm, on the left in males; correspondingly, it was 13.58 ± 0.24 mm, on the right and 13.71 ± 0.24 mm, on the left in females. In Abaza goats, however, this study measured the DV and ML diameters of the pupilla as 5.45 ± 0.2 mm, and 9.13 ± 0.7 mm, on the right and 5.54 ± 0.1 mm, and 9.47 ± 0.7 mm, on the left, respectively. Similarly, the measurements for the same parameters in the Gurcu goats were 8.37 ± 1.24 mm, and 9.42 ± 1.54 mm, on the right and 8.38 ± 1.26 mm, and 9.45 ± 1.51 mm, on the left, respectively.

Tunica fibrosa bulbi (Sclera and cornea) is an outer section (Ross and Pawlina). Studies indicated that the sclera is composed of tightly packed collagen tissue in mammals, whereas some other vertebrates (such as geese and ducks) might additionally contain sclera structures such as cartilage or bone tissue [15, 34]. As in other mammalian creatures, the eye sclera of Abaza and Gurcu goats is histologically composed of three segments: Lamina fusca, stroma and episclera [21, 31], and these layers also contain collagen fibrils, with locally visible melanocytes and neural endings. Alternatively, the sclera part of the eyes in goats, buffalo, donkeys and cattle reportedly possessed bundles of smooth muscle cells [37]. In line with the literature, the current study also identified muscle cell bundles in the episclera of the goat's eye sclera.

The cornea is a transparent, densely innervated membrane that keep safe the eye from external traumas and foreign objects but contains no blood or lymphatic vessels. The structure of the corneas in several animals (donkeys, horses, wild and domesticated

ruminants, rodents, marine mammals, herbivores, pigs, bears, and primates) have reportedly been well-identified. In mammals, the cornea be composed of a non-keratinized stratified squamous epithelium in the upper section, Bowman's layers below the epithelium, stroma in the middle, Descemet's membrane and endothelium at the bottom [8, 20, 21, 23, 29, 31, 37]. The previous studies on the cornea of laboratory (experimental objects) and domestic animals reported no Bowman's layer in all species, albeit only primates might possess it [9]. The following studies, however, indicated that various mammalian species, including pigs [10], oxen [27], deer [38], bears [20], sheep [39], and dogs [35], reportedly possessed the Bowman layer. In line with the relevant literature, the current study also identified that the cornea of Abaza and Gurcu goats consisted of a well-located epithelial layer, a thin **Bowman's** swap, a stroma formed of wider collagen filaments, Descemet's membrane, and endothelium, as in other mammalian species. The current study additionally identified trabecular meshwork (Fontana slits) and Schlemm's canal structures in the corneasccleral region.

In most domesticated mammalian animals, the choroid is composed of 5 layers: basal layer (Bruch's membrane), capillary layer, tapetal regionand, vascular layer and the suprachoroidal [5, 47, 25]. Both invertebrates and vertebrates possess tapeta. In vertebrates, the deep retina or choroid contains the tapeta. Considering the choroidal tapeta structures, there are two classes: tapetum cellulosum and tapetum fibrosum. The tapetum fibrosum contains no cells and consists of tightly packed collagen fibrils [44]. Ollivier et al. [32], reported that tapetum lucidum is absent in red kangaroos, pigs, primates, squirrels and birds, whereas it is present in reptiles, fish, dogs, bats, cows, cats, sheep, crocodiles, goats, lemurs, and weasels. In Abaza and Gurcu goats, the tapetum fibrosum layer in the choroid, which retains numerous melanocytes and blood vessels, observably consists of collagen fibril bundles arranged regularly. Furthermore, it was devoid of melanocytes.

In Abaza and Gurcu goats, the epithelial layer of the *corpus ciliare* and *processeus ciliaris* is internally pigmented and resembles that of camels [22], bears [20, 36] and sheep [12]. Both goat species observably contained two epithelial layers, albeit the upper one is non-pigmented. Like other mammals, the current study also identified extensive blood vessels, dilator and sphincter muscle structures, a wide collagen tissue, and neural endings locally in the iris stroma of Abaza and Gurcu goats. Surprisingly, the iris of Abaza goats has well-developed dilator and sphincter muscles. Histologically, however, the retina consisted of ten layers, much like in other mammalian species [3, 12, 14, 16, 20, 42, 48].

Conclusion

The literature reviews revealed that studies referenced the *bulbus oculi* in some domestic and wild animals. Contrary to the current study, however, no study in the literature reported comparative anatomical and histological analyses. It is essential for clinical research to identify the species-specific traits of this anatomical, morphometric, and histological study on Abaza and Gurcu goats. Therefore, it is anticipated that the findings of this study will contribute significantly to the scientific fields where animal, human and three-dimensional experimental models can be used in the field of ophthalmology.

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