











Characterization of nonpathological intrascleral cartilage in the domestic sheep (*Ovis aries*)¹

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ABSTRACT.- Teodoro T.G.W., Campos M.E.S.T., Silva L.A., Watanabe T.T.N., Alves C.E.F., Laufer-Amorim R., Wouters F. & Wouters A.T.B. 2023. **Characterization of nonpathological intrascleral cartilage in the domestic sheep (*Ovis aries*).** *Pesquisa Veterinária Brasileira* 43:e07142, 2023. Setor de Patologia Veterinária, Departamento de Medicina Veterinária, Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras, Campus Universitário, Cx. Postal 3037, Lavras, MG 37200-900, Brazil. E-mail: angelica.wouters@ufla.br

Birds, cartilaginous and teleost fish, reptiles, and some amphibians have intrascleral cartilage and/or bone; however, these are rarely reported in therian mammals. This study aimed to investigate and characterize a nonpathological formation of cartilage in the posterior sclera of sheep macroscopically, histologically, and by immunohistochemical exam (IHC). Ninety eyes from 45 domestic sheep were collected, underwent gross examination, fixed in formalin, and embedded in paraffin for the microscopical assessment. Sections with histological shreds of cartilage were selected to perform IHC to confirm the presence of cartilage. Intrascleral cartilage was detected in 60 eyeballs (66.66%) from 37 sheep (82.22%). A slight whitish thickening was grossly seen in the posterior sclera. The histologic exam revealed a few scattered, isolated chondrocytes to larger aggregates of cartilaginous islands in the posterior sclera. Eighteen (30%) of 60 eyeballs revealed marked anti-collagen type II immunolabeling. The development of cartilaginous structures in the eyes is considered rare in mammalian animals. The high occurrence of intrascleral cartilage in the examined sheep eyes suggests that this finding corresponds to an anatomical component of sheep sclera, despite the age, breed, or body condition.

INDEX TERMS: Ophthalmology, ocular anatomy, ocular cartilage, sheep, *Ovis aries*.

RESUMO.- [Caracterização de cartilagem intraescleral não patológica em ovinos (*Ovis aries*).] Aves, peixes cartilaginosos e teleostes e répteis, bem como alguns anfíbios possuem cartilagem e/ou osso intraescleral, contudo, isso é raramente

descrito em marsupiais. O objetivo deste trabalho foi investigar e caracterizar a formação não patológica de cartilagem hialina na esclera posterior de ovinos, por exame macroscópico, histológico e imuno-histoquímico. Olhos de 45 ovinos foram coletados, submetidos a exame macroscópico, fixados em formalina a 10% tamponada e incluídos em parafina para o exame histológico. Amostras com evidência de formação cartilaginosa ao exame histológico foram selecionadas para a realização da técnica de imuno-histoquímica para confirmação. Cartilagem intraescleral foi detectada em 60 amostras oculares (66,66%) de 37 ovinos (82,22%), caracterizada na avaliação macroscópica como discretos espessamentos esbranquiçados na esclera posterior. O exame histológico revelou formações que variaram de condrocitos isolados e dispersos a grandes agregados, formando ilhas cartilaginosas na esclera. Dezoito (30%) de 60 bulbos oculares mostraram imunomarcção anti-colágeno tipo II. O desenvolvimento de estruturas cartilaginosas nos bulbos oculares é considerado

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raro em mamíferos. A identificação de cartilagem intraescleral em 66% dos olhos ovinos avaliados no estudo indica que a formação de cartilagem corresponde a um componente anatômico normal na esclera de ovinos, independente de idade, raça ou condição corporal.

TERMOS DE INDEXAÇÃO: Oftalmologia, anatomia ocular, cartilagem ocular, ovinos, *Ovis aries*.

INTRODUCTION

The eyes are sensory organs responsible for capturing visual stimuli and the transmission to the brain, where transduction of these signs occurs. Eyeballs or *Bulbus oculi* have a spherical shape with distinct layers of photosensitive tissues (Vaughan 2002). The histopathologic examination is considered complex and specific due to the various particularities of its tissue composition (Wilcock & Njaa 2015). The eyeball is composed of three layers with a concentric arrangement. The outer layer is formed by the sclera and the cornea; the middle layer has the iris, choroid, and ciliary body; and the inner layer is composed of the retina (Maggs 2007, Samuelson 2013).

The sclera is a component of the fibrous tunic of the eyeball and consists of densely packed connective tissue with flat bundles predominantly composed of collagen type I and lower proportions of collagen type III (Watson & Young 2004, Trier 2005). The opaque appearance of the sclera is due to the variation in the thickness of the collagen fibrils (Trier 2005). The scleral thickness varies among species and in the different portions of the eyeball (Samuelson 2013). It offers protection for the delicate inner ocular structures and is a site of insertion for tendons from extraocular muscles, therefore, enabling eyeball movements (Trier 2005), and playing an important role in determining the eye shape and resistance (Foster & De la Maza 1994).

Some animal species have intrascleral cartilage or bone, or even both. Birds, for example, have well-developed ossicles and cartilage in all the scleral extension (Lima et al. 2009, Carvalho et al. 2018), some cartilaginous fish species have scleral cartilage. In reptiles, the presence of cartilage or bone in the eyes is variable (Fran-Odendaal & Vickaryous 2006, Samuelson 2013). These structures are associated with the preservation of the eye shape when exposed to different environmental conditions such as water pressure or high altitude and facilitate visual accommodation (Lima et al. 2009).

The *tapetum lucidum* is a structure that may be located immediately within the cells of the retinal epithelium or in the choroid adjacent to the retina and varies depending on the species. In dogs and cats, it is located in the superior fundus portion of the eye (Lesiuk & Braekevelt 1983, Braekevelt 1990). This is a biologic reflector system that works in low light conditions, important for giving a second opportunity to a photon-photoreceptor stimulation, responsible for the phenomenon of “eye-shines” in some animals. (Braekevelt 1990, Ollivier et al. 2004).

Therefore, the purpose of this study was to describe and characterize the presence of intrascleral cartilage as a normal component of sheep eyes by macroscopic and microscopic assessment, supported by immunohistochemistry (IHC) findings.

MATERIALS AND METHODS

Animals. This study was performed following the “Universidade Federal de Lavras” (UFLA) Ethics Committee on Animal Use (protocol No. 044/2018). Overall, 20 eyes from 10 sheep submitted for *post mortem* examination at the Veterinary Pathology Sector of UFLA, Lavras, State of Minas Gerais, Brazil, and 70 eyes from 35 sheep at a slaughterhouse in the inland São Paulo State were collected.

Eye inspection and tissue preparation. All ninety collected eyes underwent gross and histologic examination. Both eyes and the respective optic nerve were extracted from the orbit and fixed in a 10% buffered formalin solution. Formalin injection into the vitreous chamber (up to 2.0ml) improved the retinal fixation. The eyelid was kept only in the right eye to distinguish the right from the left eye. The remaining periocular tissues were removed with a scissor and trimming blades. The cornea and sclera were carefully examined for any gross lesions. After fixation of at least 48 hours, all eyeballs were trimmed using sagittal sections, which included a 5mm distance from the optic nerve at the posterior aspect of the bulb. Both right and left eyes were sectioned. The obtained sections with the optic nerve were routinely processed for histology, paraffin-embedded, sectioned at 3µm, and stained with hematoxylin and eosin (HE) for microscopic examination. All the structures of the eye; cornea, uvea, retina, lens, optic nerve, and sclera were examined by two veterinary anatomic pathologists.

Immunohistochemistry. The sections with any evidence of intrascleral cartilaginous or matrix at the HE-stained histological exam were selected for the IHC. The IHC protocol was adapted from Smith et al. (2011). The eyes were sectioned at 4µm. For antigen retrieval, the sections were incubated in 1% pepsin solution in deionized water (pH 1.8) for 30 minutes at 50°C, followed by 30 minutes at 37°C in Dako Envision Peroxidase-blocking reagent, and protein block with Novocastra protein Block (Leica). Primary monoclonal antibody anti-collagen type II alpha 1 chain (Origene, Maryland, EUA) at 1:500 dilution was used, and sections were incubated for 18 hours in a humid chamber at 5°C. The immunolabeling was done with Dako Envision-Dual-link HRP-system, revealed by diaminobenzidine (DAB), and counterstained with Harris hematoxylin.

RESULTS

Cartilaginous formations were first detected in the sclera of a sheep during the histologic examination of the eyes in a study of ocular diseases in necropsied animals at the Veterinary Pathology Service of UFLA. After this first histological finding of cartilaginous structures in the posterior sclera, all the received sheep had their eyes gross and histologically examined for the presence of cartilage in the posterior sclera.

Ten sheep from different farms of the Southern Minas Gerais State were received at the Veterinary Pathology Service of the UFLA for postmortem examination. They were diagnosed with haemonchosis (2), copper toxicosis (1), monensin toxicosis (1), suppurative spondylitis with medullar compression (1), aborted fetuses (2), and stillbirth (1), tetanus (1), and inconclusive case (1). All animals were deemed free of any ocular disease and/or any systemic disease with possible ocular involvement. Additional 70 eyes from 35 healthy 10-12 months-old Santa Inês lambs were obtained from one slaughterhouse in Southern São Paulo State as supplementary material to improve the investigation about intrascleral cartilage in sheep.

Ninety eyes were examined from forty-five sheep of different ages, sex, and breeds (Table 1). The studied population included aborted fetuses to 5-year-old years old sheep, with an average age of 14.6 months for females and 10.8 months for males. The sheep belonged to the breeds Santa Inês (84.5%), Dorper (2.2%) or crossbreed (13.3%); 39 (86.67%) animals were male, and six (13.33%) female.



Fig.1. Characterization of nonpathological intrascleral cartilage in the domestic sheep. Gross finding. Note the thickening (arrow) at the posterior sclera of an eye from a male 10-12 months old Santa Inês sheep. Sheep No. 18.

At the gross examination, a slight white thickness was observed at the posterior aspect of the eyeball, dorsal to the optic nerve (Fig.1) in sheep diagnosed with scleral cartilaginous structure at microscopy (Table 2). The microscopic exam revealed individual to little groups of chondrocytes containing a basophilic cytoplasm surrounded by a scarce basophilic cartilaginous matrix (25 animals) to aggregates of chondrocytes forming islands of cartilage (12 animals) at the posterior aspect of the sclera (Fig.2-3). Additionally, a homogeneous basophilic matrix was predominately surrounding the islands. The younger lambs (sheep 5, 6, 7, and 9) had a basophilic amorphous material throughout the inner sclera. A strong anti-collagen type II immunolabeling occurred in the cartilaginous matrix surrounding the chondrocytes. It was associated with multifocal to coalescing nests of chondrocytes in eighteen from sixty eyeballs (Fig.4-7, Table 2).

DISCUSSION

Our study herein reports intrascleral cartilaginous formation that, to our knowledge, has yet to be described affecting domestic sheep. Hyaline cartilage in the sclera is a common finding in several vertebrate animals, such as birds (Lima et al. 2009, Samuelson 2013, Carvalho et al. 2018), reptiles, and fish (Fran-Odendaal & Vickaryous 2006, Samuelson 2013). In species of reptiles and fish that have cartilage in the sclera, the connective tissue of the sclera is reinforced by the cartilage development (Fran-Odendaal & Vickaryous 2006). In birds like chickens, studies about scleral cartilage development proved that the retinal pigmented epithelium induces the formation of the scleral cartilage (Thompson et al. 2010). In mammals, this cartilage formation

Table 1. Data from the sheep (*Ovis aries*) of eye collection for characterization of nonpathological intrascleral cartilage

Source of the animals	Sheep	Age	Sex	Breed
Veterinary Pathology Sector, UFLA, Lavras Minas Gerais State	1	2 years	Female	Santa Inês
	2	14 months	Male	Dorper X Santa Inês
	3	5 years	Female	Santa Inês crossbred
	4	2 months	Female	Dorper crossbred
	5	Aborted fetus	Female	Santa Inês
	6	Aborted fetus	Female	Santa Inês
	7	2 weeks	Male	Dorper X Santa Inês
	8	2 months	Female	Santa Inês crossbred
	9	Stillbirth	Male	Dorper
	10	2 years	Male	Dorper crossbred
Slaughterhouse Inland São Paulo State	11-45 ^a	10-12 months	Male	Santa Inês

UFLA = Universidade Federal de Lavras; ^a n=35.

Table 2. Identification of scleral cartilage on histological and immunohistochemical exam of sheep (*Ovis aries*)

Presence of cartilage	Occurrence	
	Sheep	Globes
Hyaline matrix/Cartilaginous evident on HE stained histology	37/45 (82.22%)	60/90 (66.66%)
Cartilaginous highlighted on immunohistochemistry	14/37 (37.83%)	18/60 (30%)

HE = hematoxylin and eosin.

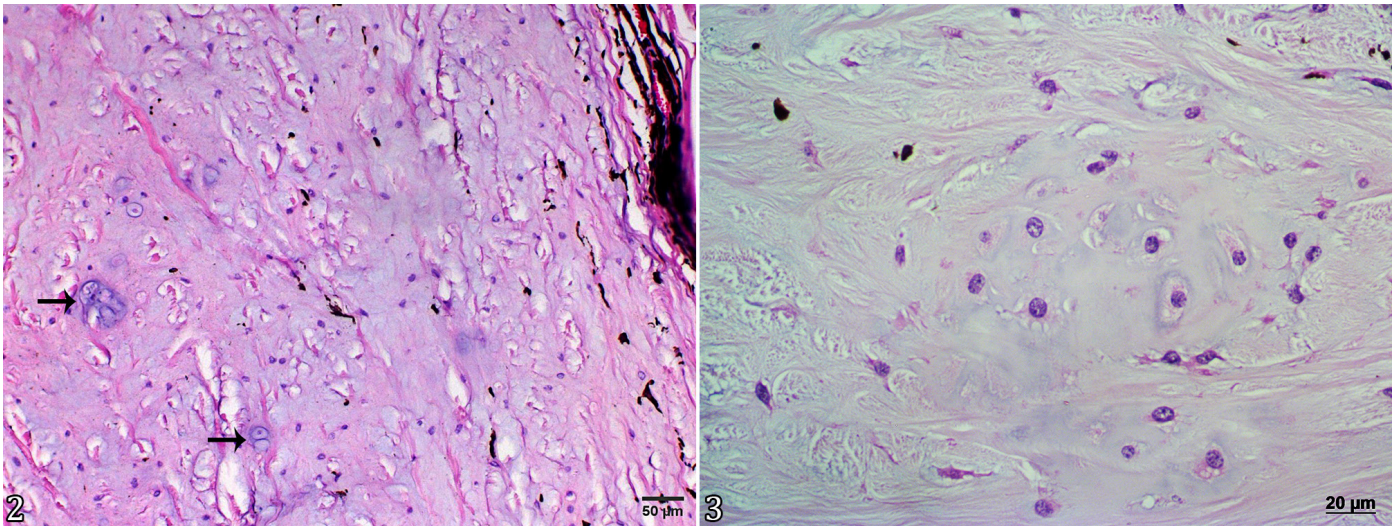


Fig.2-3. Characterization of nonpathological intrascleral cartilage in the domestic sheep. Histological findings on sheep sclera. (2) Little groups of chondrocytes (arrows) surrounded by a basophilic matrix in Sheep No. 4. HE, obj.20x. (3) Collection of chondrocytes surrounded by a basophilic matrix in the center of the image, Sheep No. 29. HE, obj.40x.

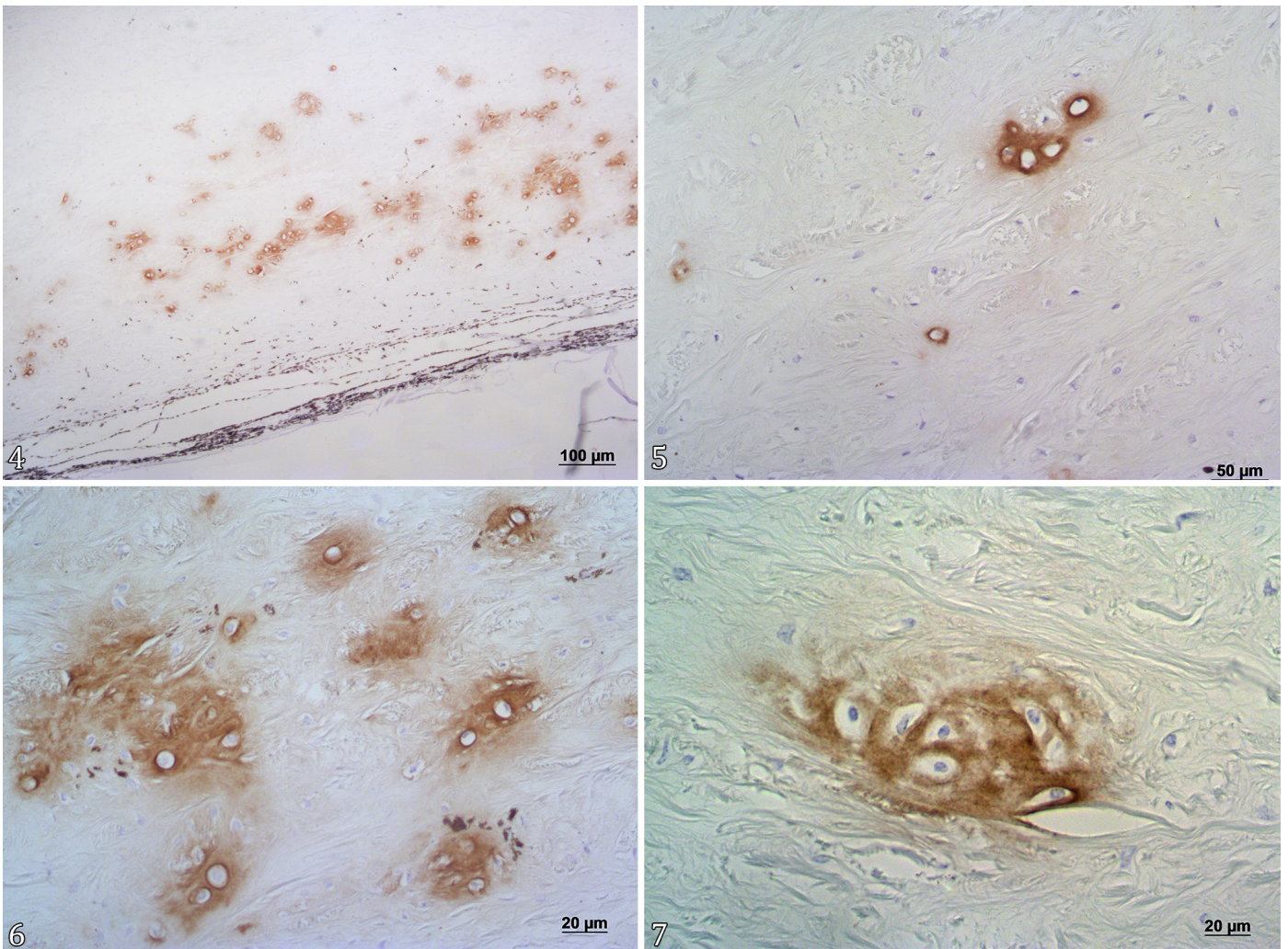


Fig.4-7. Characterization of nonpathological intrascleral cartilage in the domestic sheep. Scleral cartilage was hallmarkd by anti-collagen II immunolabeling, Dako Envision-Dual-link HRP-system, revealed by DAB. (4) Note chondrocyte collections in labeled (in brown) cartilaginous matrix in the sclera of sheep No. 18. IHC, obj.10x. (5) Isolated small groups of chondrocytes, sheep No. 1. IHC, obj.20x. (6) Sheep No. 18. IHC, obj.40x. (7) Chondrocyte island, Sheep No. 29, IHC, obj. 40x.

is uncommon, with descriptions in monotremes (Thompson et al. 2010) and domestic goats (Tusler et al. 2017). The human sclera maintains the expression of cartilage-associated genes, which represents a chondrogenic potential (Seko et al. 2008).

Responses of the ocular tissues to injuries can be degenerative, inflammatory, or reparative and vary according to the nature and duration of the injury. Various cell types of eye tunics respond to aggression with adaptation. This can be characterized by atrophy, hyperplasia, hypertrophy, metaplasia, or dysplasia (Dubielzig et al. 2010). There were reports of cartilaginous metaplasia in different portions of the eye related to some pathological conditions, for instance, in intraocular neoplastic processes in dogs, cats (Attali-Soussay et al. 2001), and horses (Baptiste & Grahn 2000). Dubielzig et al. (2010) described areas of chondromatous differentiation or only cartilaginous differentiation in cases of ocular post-traumatic sarcoma in cats. These authors also describe foci of myxomatous, cartilaginous, or osseous metaplasia as a characteristic feature present in about 90% of canine orbital meningiomas. The present study aimed to characterize intrascleral cartilage in domestic sheep without ocular disease. In this report, the intrascleral cartilage was detected in 60 eyes, including slaughtered healthy sheep, showing an incidence of 66.66%. The intrascleral cartilage in sheep has been reported in five individuals in a control group of a study that evaluated the effects of oral inoculation with scrapie (Smith et al. 2011). Those authors concluded that the formation of intrascleral cartilage occurred due to a metaplastic process with an unknown clinical significance. It was suggested that scleral cartilaginous metaplasia might not be rare in sheep nor associated with breed predisposition.

Our analysis included male and female sheep from different breeds and ages. The histological finding of intrascleral cartilage ranged from the deposition of a hyaline matrix within scattered, isolated chondrocytes to multifocal to coalescing islands of hyaline cartilage in two months old or older animals. These formations were observed at the dorsal posterior portion of the eye, immediately behind the tapetal fundus. Anti-collagen type II immunoreaction was observed in 18/60 eyes, representing a prevalence near 30%. We had immunolabeling problems in some eyes, which were attributed to failures in the fixation and/or processing of these eye samples. Interestingly, the cartilaginous formation appeared to be less developed in animals less than two weeks old, such as fetuses and stillbirths.

Sheep have a *tapetum lucidum* fibrosum, essentially a horizontal strip at the dorsal portion of the eye, that reflects greenish-blue with a lower edge at the point of entry on the optic nerve (Ollivier et al. 2004). *Tapetum lucidum* is an ocular structure that can be found in vertebrates and invertebrates. In vertebrates, it is commonly located in the choroid or deep retina, like in some reptile species (Schwab et al. 2002). The absence of *tapetum lucidum* is seen in animals with diurnal habits, like primates and birds (Ollivier et al. 2004). The cartilage in the present study was detected mostly at the dorsal portion of the posterior sclera. Hence, it is speculated that the scleral cartilage development may be associated with *tapetum lucidum* as a support structure.

The anti-collagen type II antibody used for the immunohistochemical exam showed strong labeling of cartilaginous components in the sheep's eyes. It is explained by the fact that type II collagen is the major component of the cartilage matrix (Lian et al. 2019).

CONCLUSION

Based on these findings, the occurrence of intrascleral cartilage in domestic sheep should be considered a nonpathological structure, particularly seen at the dorsal posterior aspect of the eye. Further studies are warranted to determine the embryologic formation and physiologic function of this intrascleral cartilage.

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Conflict of interest statement.- The authors declare having no conflicts of interest.

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