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HISTOLOGY, HISTOCHEMISTRY AND ELECTRON MICROSCOPIC STUDIES ON LENS OF DOMESTIC PIG (*SUS SCROFA DOMESTICUS*)

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ABSTRACT

Lens is an important refractive structure to focus the light on the photosensitive layer of the retina. The present study was contemplated to study the histology, histochemistry and ultrastructure of the lens of six adult pigs. Lens capsule was an interrupted, homogenous, translucent layer that completely enclosed the lens fibres. It showed intense positive reaction for glycogen by McManus' PAS method and also showed weak to moderate reaction to the Alcian-blue staining for acidic mucosubstances. The newly formed lens fibres in the cortex were thick and appeared as long elongated fibres and hexagonal shape in cross sections. They were interlocked together by ball and socket type of joints at each of their hexagonal edge. Integrity of the lens fibres was lost and less recognizable towards the nucleus. Cortical lens fibres showed uniformly distributed granular cytoplasm and cell organelles were not observed in the inner cortex and nucleus of the lens.

Key words: Electron microscopy, Histology, Histochemistry, Lens, Lens capsule, Pig.

Introduction

The lens is a biconvex, avascular, transparent, multicellular syncytial structure enclosed in a delicate capsule of basement membrane, held in position by zonular fibres that arise from the ciliary body. Anteriorly it faces the posterior surface of the iris and posteriorly, contact with the vitreous body. Lens is an important refractive structure to focus the light on the photoreceptors cells of the retina. Although cornea is a major refractive structure but only lens has the power to adjust the focal length by changing the shape through the action of ciliary smooth muscles, lens capsule and zonular fibres (Gelatt, 2007). The lens is ectodermal in origin which develops from the evagination of the surface epithelium of the lens vesicles. At birth, only cuboidal lenticular epithelium is present towards anterior surface of the lens which gradually becomes squamous with the advancement of the age. Swine is considered to be one of the major animal species used in translational research, surgical models, ophthalmic research and procedural trainings and is increasingly being used as an alternative to the dog or monkey as the choice of non-rodent species (Swindle *et al*., 2012). The literature available pertaining to the anatomy and histology of the normal lens of pig is extremely scarce and the basic knowledge may be useful for further research in the field of experimental cataract surgery (Sugiura*et al*., 1999; Mekada*et al*., 1999; Shentu*et al*., 2009) and other ophthalmic research and thus the present study was contemplated to study the histology, histochemistry and ultrastructure of lens of the pigs.

Materials and methods

The present study was conducted on six adult pigs of either sex, of local mixed breed at Department of Veterinary Anatomy, College of Veterinary Sciences, Lala Lajpat Rai University of

Veterinary and Animal Sciences, Hisar, India. The whole eye balls procured from local slaughter house immediately after slaughter of pigs and immersed in the Davidson's fluid (Latendresse *et al*., 2002) after a long incision of 8-10 mm given at the limbus. After immersion in Davidson's fluid, the cornea and lens became white and opaque. The eye ball cut into two equal halves and again immersed in fixatives for next 24 hours. Lens having anterior and posterior surfaces were further fixed in these fixatives for next 24 hours. The other samples of lens were also fixed separately in 10% neutral buffer formalin for 15–30 minutes. Removal of fixative from the tissues was achieved by washing for 6-8 hours in running tap water followed by dehydration in ascending grades of ethanol starting from 50% ethanol for 50 minutes, overnight in 70% ethanol and followed by 50 minutes each in 90% ethanol, absolute ethanol I and II. Clearing was done in benzene I and II, giving 50 minutes each. Infiltration by paraffin wax with a melting point of 58–60°C giving three paraffin baths for 50 minutes each and then prepared the blocks. The sections of 5 µm were stained by routine Harris' haematoxylin and eosin stain for general histomorphological examination, Gomori's stain for reticular fibres, Weigert's method for elastic fibres (Luna, 1968), Crossman's trichrome stain for collagen fibres (Crossman, 1937). In addition, selected sections were processed for the histochemical demonstration of mucopolysaccharides using McManus' PAS method, periodic-acid-Schiff-Alcian blue (PAS-AB), Alcian blue (AB) at pH 2.5, colloidal iron method and Mayer's mucicarmine method (Luna, 1968).

For scanning and transmission electron-microscopy, fresh lens samples collected immediately after slaughter were fixed in Karnovsky's fluid for 8-12 hours after thorough washing with chilled 0.2 M phosphate buffer (pH 7.4). The tissues were again washed twice with 0.2 M phosphate buffer and rest of the procedure was carried out at Sophisticated Analytical Instrumentation Facility, AIIMS, New Delhi. For scanning electron microscopy, dehydration of the tissues was achieved by acetone and then critical point drying was done by liquid carbon dioxide at 1100 psi and 31.5°C by using critical point dryer (Mfd. by Electron Microscopy Science). The dried tissues were firmly earthed to brass stubs by painting a stripe of silver paint and gold coating of the tissue was done by sputter coating devise (Agar sputter coater/Quorum) and kept in vacuum chamber for a while and viewed in a scanning electron- microscope (Zeiss EVO-18). For transmission electron-microscopy, primary fixation was done in Karnovsky's fluid and secondarily fixed in 2% osmium tetraoxide for one hour and processed to make resin blocks. The ultrathin sections (70–80 nm) were taken on copper grids and stained with uranyl acetate and lead citrate and then viewed under transmission electron-microscope (Technai G2).

Results and discussion

Histology and histochemistry

The lens was a completely avascular, transparent biconvex structure formed by lens fibres which was enclosed in a lens capsule (*Capsule lentis*) (Fig. 1) as described earlier (Prince *et al*., 1960), and in goat (Barhaiya, 2014). It was suspended between the posterior surface of the iris and anterior face of the vitreous chamber through zonular fibres (*Fibrae zonulares*) which arose from the ciliary body and attached to the lens capsule at lens equator (*Aequator lentis*) (Tomar and Bansal, 2017). Lens capsule was an interrupted, homogenous, translucent modified basement membrane that completely enclosed the lens fibres (*Fibrae lentis*) which might have various functions like involvement in visual accommodation (Krag and Andreassen, 2003), protection from infectious disease of various origins (Beyer *et al*., 1984), and cell signalling (Danysh and Duncan, 2009). It was thicker

on the anterior surface than the posterior surface as observed in domestic animals (Atia *et al*., 2011; Gelatt, 2007). It showed intense positive reaction for glycogen (Fig. 2) by McManus' PAS method (Barhaiya, 2014; Hogan *et al.*, 1971) and also showed weak to moderate reaction to the Alcian-blue staining (Fig. 3) for acidic mucosubstances whereas it did not show any reaction to the colloidal iron for strongly sulphated acidic mucosubstances and Mayer's mucicarmine for mucin.

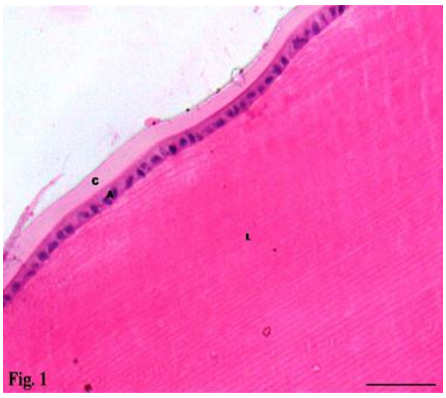

Figure 1: Photomicrograph of the anterior surface of the lens (L) showing capsule (c) and anterior epithelium (A). H. E. X 100 (Bar 100µm)

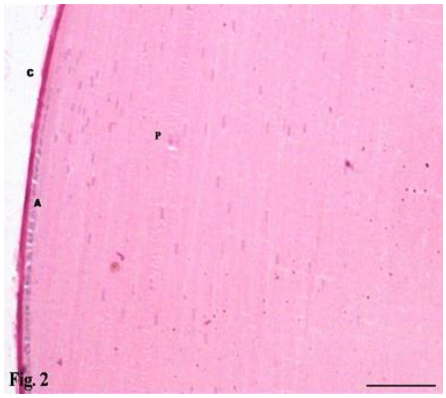

Figure 2: Photomicrograph of the lens at equator showing presence of proliferative zone in cortex (P), PAS positive lens capsule (C) and anterior epithelium (A).McManus' PAS X 100 (Bar 100µm)

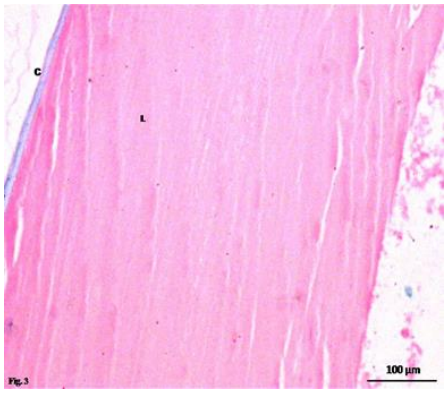

Figure 3: Photomicrograph of the lens showing Alcianophilic reaction in the capsule (C).PAS-AB X 100 (Bar 100µm)

Anterior epithelium (*Epithelium lentis*) was observed only towards the anterior surface (*Facies anterior lentis*) of the lens and at the equator (*Aequator lentis*), it was having single row of high cuboidal to squamous cells (Figs. 1, 2) as described in domestic animals and primates (Tomar and Bansal, 2017; Murphy *et al*., 2012; Atia *et al*., 2011; Cogan, 1962). These cells contained round to oval nuclei at center. At the equator, a zone of mitotic activity was observed (Fig. 2) in which the newly formed lens cells shifted towards the anterior epithelium and outer cortex (*Cortex lentis*) portion posteriorly (*Facies posterior lentis*) and then transformed into elongated lens fibres which were similar to the findings of the Barhaiya (2014) in goat, Murphy *et al.* (2012) in dogs, and Hogan *et al.* (1971) in humans.

The newly formed cell fibres became elongated anteriorly and posteriorly and formed U-shaped cells. The cells of successive layer were deposited as secondary lens fibres (*Fibra transitoria*) and lost their nuclei. The outer most cortical zone was having newly formed lens fibres which progressively displaced towards the deeper cortex by newly formed lens fibres as described in various animals (Prince *et al*., 1960; Cogan, 1962; Hogan *et al*., 1971; Murphy *et al.*, 2012). Cortex of the lens showed linearly arranged hexagonal shaped lens fibres oriented in a particular pattern and were closely attached with each other (Figs. 4, 5). Integrity of the lens fibres (*Fibra centralis*) was lost and less recognizable towards the nucleus (*Nucleus lentis*). The nuclear zone was having homogenous structure of densely packed proteins of lens fibres that gradually thickened with advancement of age and it was the reason that difficulty in fixation, embedding and sectioning of the nucleus of adult animals and artifacts like fractured lens fibres and vacuolations were also observed regularly as described in primates and other domestic animals (Gelatt, 2007; Cogan, 1962).

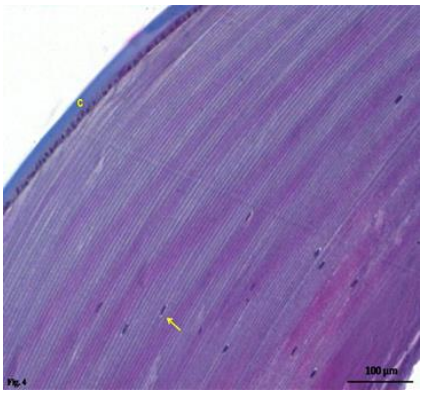

Figure 4: Photomicrograph of the anterior surface of the lens at the equator showing parallel arranged lens fibres and nuclei of the newly formed lens cells at proliferative zone (arrow).Crossman's trichrome X 100 (Bar 100µm)

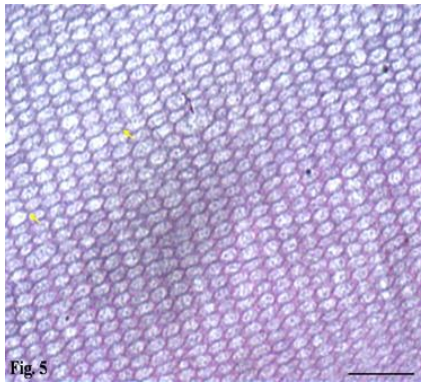

Figure 5: Photomicrograph of the posterior cortex of the lens showing hexagonal shape lens fibres (arrow).Crossman's trichrome X 200 (Bar 50µm)

Scanning electron-microscopy

Lens capsule was closely adhered to the lens vesicle (*Cortex lentis*). The surface of the newly formed cells showed roughness on their outer surface (Fig. 6) and any type of pores were not observed although elevation of the nucleus on the surface was not observed as reported by Hoyer (1982) in pigs. The present study showed button like protrusions on the surface (Fig. 7) which has not been reported earlier. The surfaces of the lens fibres showed variations at different zones of the lens vesicles. The newly formed lens fibres in the cortex were thick and appeared as long elongated fibres and hexagonal shaped in cross sections (Fig. 8) and interlocked together by ball and socket type of joints at each of their hexagonal edge (Fig. 9). Gradually towards the lens nucleus, thickness of the old lens fibres reduced as they were compressed by new, secondary and other younger lens fibres. Ball and socket type of junctions were not prominent towards the nucleus. Their surfaces were not smooth as observed in the outer cortex however; possessed thick micro projections (microplicae) giving wrinkled appearance (Fig. 10). These microplicae formed junctional complexes called as tongue and groove junctions with the opposite surfaces of other lens fibres. These observations were almost similar to the findings in the pig (Hoyer, 1982), humans (Taylor *et al*., 1996;

Versura and Maltarello, 1988), monkey (Willekens and Vrensen, 1982) and rats (Atia *et al*., 2011; Farnsworth *et al*., 1974).

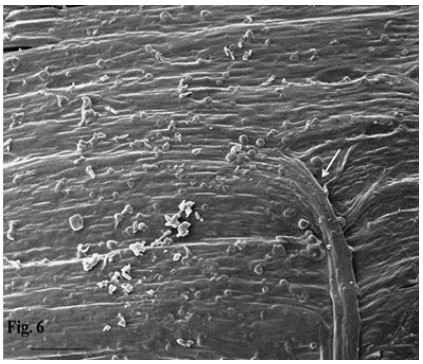

Figure 6: Scanning electron-micrograph of the outer surface of the lens at equator showing lens capsule and attachment for the zonular fibres (arrow). X 300 (Bar 100µm)

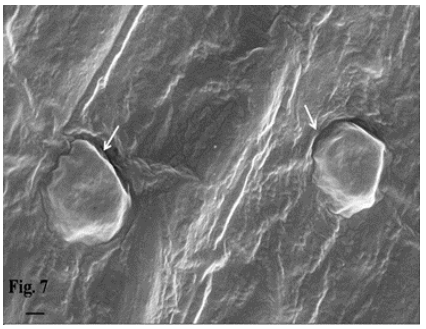

Figure 7: Scanning electron-micrograph of the outer surface of the lens showing button shaped protrusion at high magnification (arrow). X 5000 (Bar 2µm)

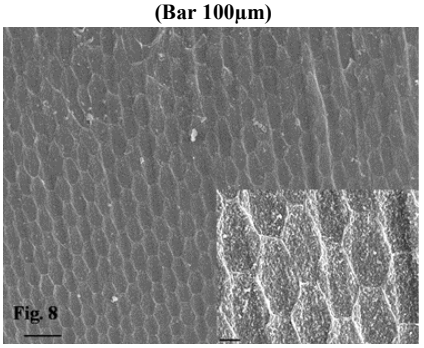

Figure 8: Scanning electron-micrograph of the cut surface of the lens from the cortex showing hexagonal shaped lens fibres (X 1000) (Bar 20µm) and inset photo (X 5000) (Bar 2µm) showed lens fibres closely joint together at their edges.

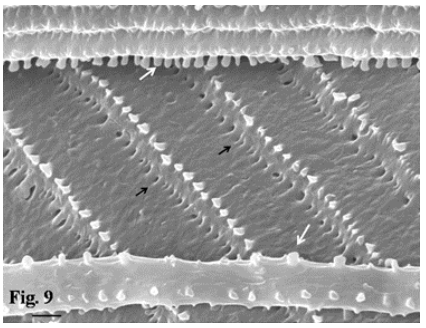

Figure 9: Scanning electron-micrograph of the lens fibres in the cortex showing ball and socket type of interlocking (black arrow) and papillae shaped protrusions (ball) on the surface of the lens fibres(white arrow). X 8000 (Bar 2µm)

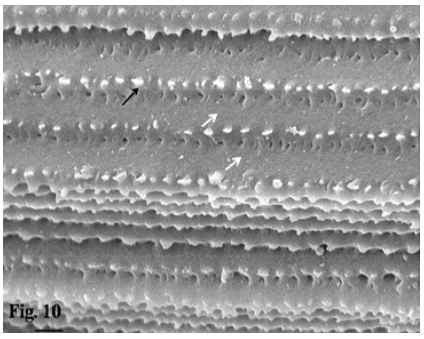

Figure 10: Scanning electron-micrograph of the lens fibres in the outer nucleus showing ball and socket type of interlocking (black arrow) and microplicae that formed tongue and groove type of junctions along the surface (white arrow). X 5000 (Bar 3µm)

Transmission electron-microscopy

Lens cells were hexagonal in shape in cross sections as observed in humans (Hogan *et al*., 1971). Cortical lens fibres showed uniformly distributed granular cytoplasm and usually cell organelles were not observed in the inner cortex and nucleus of the lens (Fig. 11). A very thin intercellular space was observed between the cell membranes of adjacent cells as observed by Hogan *et al.* (1971). They were interdigitated along their long surfaces as well as on the short edges by the interlocking processes (Fig. 11) or ball and socket junctions (Taylor *et al*., 1996; Hogan *et al.*, 1971; Cohen, 1965). The lens fibres from the deep cortex and nucleus of the lens were closely attached without intercellular spaces (Fig. 12) as described in humans (Hogan *et al.*, 1971). The tongue and groove type of interdigitations along the long surfaces was observed but interlocking was usually very less in the outer nucleus and not observed in the inner nucleus as reported by Taylor *et al.* (1996) in humans.

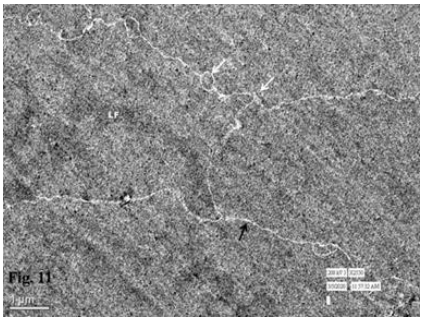

Figure 11: Transmission electron-micrograph of the lens fibres (LF) in the cortex showing intercellular space between adjacent cells (black arrow), interlocking along the short edges as well as on the long surface (white arrow). X 2250 (Bar 1µm)

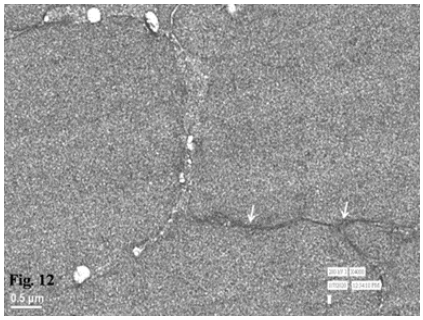

Figure 12: Transmission electron-micrograph of the lens fibres in the nuclear zone showing close apposition of the adjacent cells with no intercellular space (arrow) and interlocking processes were also reduced. X 4000 (Bar 0.5µm)

Conclusion

The present research study was conducted on the lens of adult domestic pig. The lens capsule was thick anteriorly and it showed intense positive reaction for glycogen as well as moderate reaction to the Alcian-blue for acidic mucosubstances. The morphology of lens fibres was different at various zones of lens. Lens fibres of outer cortex were thick, smooth and having large papillae on their surface which fitted into the grooves present on the other adjacent lens fibres. Gradually towards the lens nucleus, thickness of the old lens fibres reduced as they were compressed by new, secondary and other younger lens fibres. Their surfaces were not smooth as observed in the outer cortex and having thick micro projections on their surface and formed tongue and groove type of junctions with the opposite surfaces of other lens fibres. Cytoplasm of the lens fibres of inner cortex and nucleus was devoid of cell organelles as well as intercellular space reduced gradually towards the nucleus and any type of interdigitations and interlocking was not observed in the inner nucleus. The present study of pig lens showed the almost similar structure to the human and may be useful for further various experimental researches in pig.

Disclosure statement

The manuscript was based on bona fide Ph.D. research work. There was no potential conflict of interest by the authors.

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