Original Article

ANATOMICAL STUDY OF CORNEA IN DEVELOPING CHICK EMBRYOS: A LABORATORY-BASED EXPERIMENTAL STUDY

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Background: The resemblance of the developmental anatomy of the chicken to that of mammals makes it a suitable animal model for understanding the human biological systems. The histological structure of the chick embryo cornea was observed at four developmental stages, with a particular focus on its application to the study of eye development. **Methods:** This laboratory-based experimental study was conducted at the Anatomy Department, Regional Centre, College of Physicians and Surgeons, Islamabad, Pakistan. After obtaining ethical approval, a total of 70 fertilised eggs of *Gallus* domesticus were obtained from the Poultry Research Institute in Punjab, Rawalpindi. The eggs were incubated under standard laboratory conditions. The histological development of the chick cornea was studied at four distinct post-incubation stages: 10th day (n=30), 15th day (n=30) post-incubation, newly hatched chicks (n=5), and adult chickens (n=5). **Results:** at day 10 of incubation, the thickness of the cornea was 171 µm, and the stroma was 160 µm. At day 15 of development, this thickness was 145 µm, with a stroma thickness of 130 µm. The thickness of the cornea of a newly hatched chick was about 170- 200 µ, with a thickness of stroma 135 µ. In adults, the cornea is approximately 250-300 µm thick, with the stroma accounting for about 200 µm. **Conclusion:** This laboratory research presents the differentiation of the chick cornea at various developmental stages, which may contribute to the anatomical understanding of corneal embryology and provide a comparative background for pathological deviations.
Keywords: Chick embryo; cornea; model; histology

INTRODUCTION

Animal models play a crucial role in vision research for ocular diseases and conditions, including corneal injuries. The chick embryo has a long and eminent history as a major model system in developmental biology, with benefits in cost, size, and ease of handling compared to other models. Like most avian species, the chick relies on vision for foraging and escaping predators. The chick eye is relatively large, accounting for 50% of its cranial volume, compared to approximately 5% in humans.[1]

The chick cornea is composed of five distinct layers: An epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and an endothelium on the posterior aspect.[2] histologically, epithelial cells, keratocytes, and endothelial cells form the cellular components, and collagen and glycosaminoglycans constitute the acellular components. The epithelium is stratified squamous, non-keratinised, with 5–7 cell layers. Bowman's membrane lies just anterior to the stroma. The corneal stroma forms the greater part of the cornea and includes approximately 80%–85% of its thickness. It is transparent due to the precise

organisation of stromal fibres and extracellular matrix. The endothelium is a layer of hexagonal cells with an endothelial pump that regulates the water content and maintains corneal transparency.[3] The epithelial cells are derived from epidermal ectoderm. The keratocyte and endothelial cells are derived from the neural crest.

The development of the chick eye is a complex process that starts when a region of the anterior neural plate becomes specified as the 'eye field'. The eye field is then divided into two separate lateral domains. The first sign of eye development is the evagination of the optic vesicles from the lateral domains. Each optic vesicle expands and comes in contact with the surface ectoderm. The ectoderm thickens to form the lens placode, which later invaginates, giving rise to the lens vesicle, whereas the surface ectoderm progresses towards the formation of the cornea.[4]

The epithelial cells start forming an acellular primary corneal stroma by 3rd day of development. This embryonic chick corneal epithelium secretes extracellular matrix that contributes to the formation of the primary corneal stroma. Periocular cells originate

from neural crest cells. These periocular cells migrate to form the corneal endothelium and keratocytes. The primary corneal stroma is invaded by mesenchymal cells on day 6 and begins to produce the collagen that will constitute the adult stroma.[5–7]

The chick cornea has been widely used as a research model due to its histological and anatomical similarities to the human cornea.[4] In some Ocular diseases, it is used for studying corneal wound healing, opacification, transplantation, to understand pathophysiology and pharmacological intervention.[8–10] Chick cornea endothelial cells, with improved techniques for transferring cultured cells to the host, serve as a significant model for Garnier transplant.[11] Moreover, in training procedures like intra-stromal corneal ring segment, the chick cornea is the best model for a beginner surgeon.[12]

Although the literature suggests a variety of applications of the chick cornea as a research model, it is essential to understand its histological development at various stages to validate its effectiveness as a research and training model.[13–15] Therefore, this study aims to investigate the sequential histological development of the chick cornea at different stages, providing a clearer assessment of its suitability and relevance for future experimental and educational purposes, such as a research model or training model.

METHODOLOGY

This laboratory-based experimental study was conducted at the Anatomy Department, Regional Centre, College of Physicians and Surgeons, Pakistan, Islamabad. Ethical approval was obtained from the institutional animal care and use committee. A total of 70 fertilised eggs of *Gallus* domesticus were procured from the Poultry Research Institute, Punjab, Rawalpindi. Eggs with visible cracks or those stored in refrigerators were excluded to ensure viability and optimal development.

Under typical conditions, the chosen eggs were kept incubated at 38±0.5 °C with relative humidity levels of 60% and 70%. At 4 separate post-incubation stages, developmental histology of the chick cornea was considered: at 10th (n=30), 15th (n=30) post-incubation day, newly hatched chicks (n=5), and adult chickens (n=5). Each developmental stage was represented in the same histomorphological studies, with the same sample size, at the same time, to reduce animal use. To ensure reliable, reproducible histological observations, the selected sample sizes were used to minimise loss of potential embryonic tissue during processing.

For every developmental phase, each egg was opened cautiously to dissect the embryos. After that, the dissected embryos were immediately placed in 10% neutral buffered formalin for 48 hours. In the sagittal plane, the embryos' heads were bisected while keeping the right balls solitary. The anterior half of each eyeball was dissected using a sharp blade, further bisected at the meridian plane, and one portion was processed for paraffin embedding. The samples from freshly hatched and adult chicks underwent a similar tissue-processing procedure.

The selected sample tissue was paraffin-embedded, sectioned at 7 µm, and H&E-stained. A light microscope and calibrated ocular micrometre was used for measurement. The total corneal thickness from the apical stroma surface to the basal endothelium was measured. The other measurement was the epithelial thickness, from the epithelial surface to the basement membrane. The number of epithelial cell layers was also measured. Stromal thickness was calculated as the distance from the epithelial basement membrane to the anterior margin of the endothelium. Finally, endothelial thickness was measured from the anterior surface of the endothelial cells to their posterior basement membrane. All were measured in micrometres.

RESULTS

The recorded measurements were summarised as minimum and maximum values for each developmental stage. Given the descriptive nature of this histo-morphological study, no inferential statistical tests were applied. The analysis was based on direct histological comparisons and descriptive measurement ranges, which are presented in Table-1 to illustrate the variations in corneal thickness across developmental stages.

Three distinct layers of cornea can be identified at this stage of development. The epithelium, stroma, and endothelium. The stratified squamous non-keratinised epithelium contained three nuclear layers with a layer of flattened cells at the top that is oriented in the long axis of the cornea. A narrow, eosinophilic acellular band was observed just below the basement membrane of the anterior epithelium, which is the Bowman's layer of the cornea. The stroma exhibited a lamellar appearance, characterised by parallel collagen bundles. The flattened corneal cells called keratocytes are more concentrated in its anterior region. (Figure-1)

The endothelium had a single layer of low cuboidal cells. The thickness of the cornea at this stage was about 171 µm, with the thickness of the stroma 160 µm, and the anterior epithelium and endothelium were about 7- 8 µm and 2 µm, respectively. (Table-1)

Table-1: Shows thickness of different layers of the cornea at different stages of development

Developmental stages	Epithelium µm	Stroma µm	Endothelium µm	Total thickness µm
Day 10 of incubation	7– 8	160	2–3	171
Day 15 of incubation	10–12	130	3–4	145
At hatch	25–30	350	5	170–200
Adults	30–40	200	5–6	250–300

The cornea showed an anterior epithelium with three to four nuclear layers. The stroma had a uniform distribution of corneal corpuscles; however, the thickness of the stroma decreased at this stage of development. The endothelium had the same structure as in day 10 embryos. The corneal thickness at this stage was about 145 μm, with the stroma at 130 μm. The thickness of the anterior epithelium and endothelium was about 10–12 μm and 2 μm, respectively. (Figure-2)

The junction of the cornea with the sclera is the site where the regular arrangement of the corneal lamellae adjoins the irregularly distributed stroma of the sclera. The trabecular meshwork at the sclerocorneal junction, with large open intertrabecular spaces, was present. The intra-scleral vessel is visible as a small vacuole at the posterior limit of the cornea (Figure-3B, arrow). The corneal thickness at this stage was about 350 μm, with the stroma measuring 170–200 μm. The anterior epithelium is approximately 25–30 μm in thickness and consists of 5–6 nuclear layers. The superficial layer had flattened nuclei, while the deep layers had more rounded and oval nuclei. (Figure-3A)

In adult checks, the superficial zone contained about 2 to 3 layers of flat nuclei, and the deep zone contained about 4 layers of round or oval nuclei. The presence of goblet cells in the epithelium, Figure-4 B (arrow), indicates the start of the conjunctival epithelium at the sclerocorneal junction. Furthermore, no Bowman's layer is seen at this junction. The scleral venous sinuses with red blood cells are visible (Figure-5, arrow). The stroma contained relatively fewer nuclei
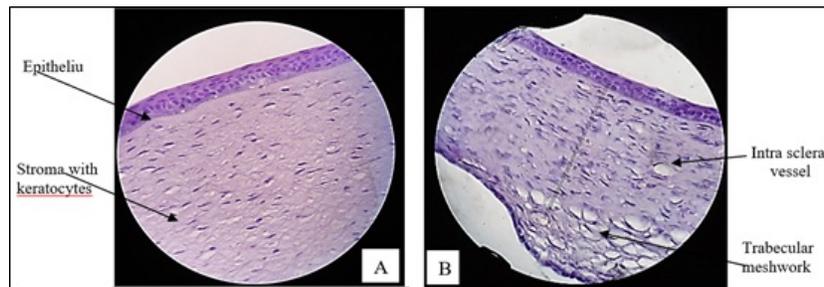
than did the cornea in the newly hatched chick. The thickness of the cornea in adults is approximately 250-300 μm, with the stroma measuring about 200 μm. The anterior epithelium is about 30 μm in thickness and comprises two zones of cells.
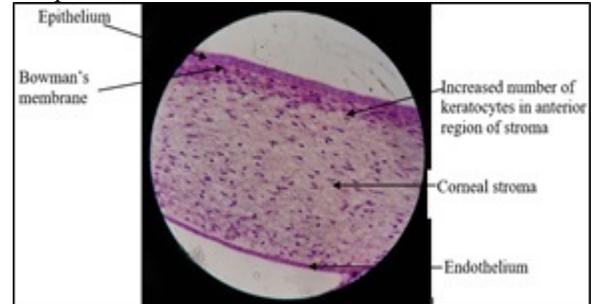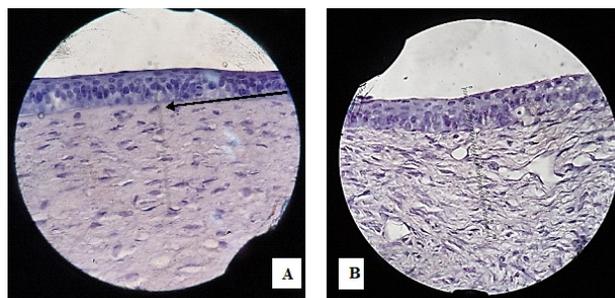

Figure-1: Cornea of chick at day 10 of incubation
Scale bar=200 μm
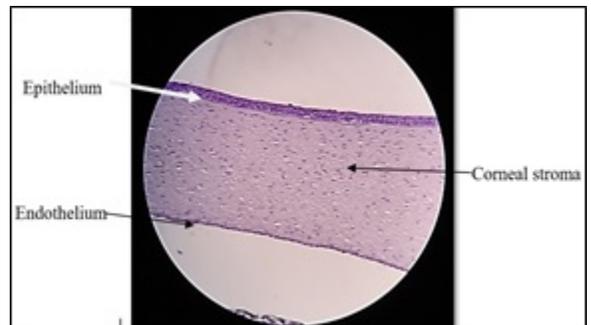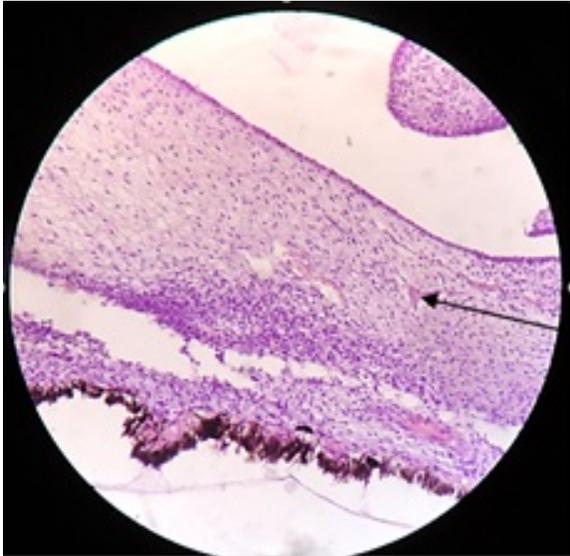

Figure-2: Cornea of chick at day 15 of incubation
Scale bar=20μm

Figure-3: Cornea (A) and sclerocorneal junction (B) of newly hatched chicks
Scale bar=250μm

Figure-4: Cornea (A) and sclerocorneal junction (B) of newly hatched chicks
Scale bar=20μm

**Figure-5: Sclerocorneal junction of adult chicks
with red blood cells in venous sinuses (arrow).**
Scale bar=250µm

DISCUSSION

The present study provides a systematic histopathological analysis of chick corneal development, delineating the structural maturation from the embryonic stage to adulthood. Our findings confirm that the chick cornea is composed of five distinct layers: the epithelium, the Bowman's layer, the stroma, the Descemet's membrane, and the endothelium, like the human cornea. The main goal of the study was to investigate changes in the corneal layers. In our study, we observed two opposing trends: as the epithelium thickens, the stroma becomes thinner and reorganises.

There was a noticeable alteration in the corneal epithelium: it increased in adulthood from ~7–8 µm (three nuclear layers at day 10) to ~30–40 µm with five to seven layers. In the early development of the two-layered epithelium in humans, it appears around 10–20 weeks, and around 22 weeks, it progresses to three to four layers. Finally, it reaches six layers by the end of the term. This maturation, from a simple bilayer to a stratified squamous epithelium, reflects a conserved process of building a resilient ocular surface.[17,18]

Then again, the stroma undergoes a more complex conversion. Before growing to roughly 200 µm in adulthood, its thickness decreased from approximately 160 µm at day 10 to 130 µm by day 15. This is supported by the work of Quantock and Young.[19–20] Rather than tissue loss, this mid-development thinning represents a

crucial stage of matrix compaction. This process, necessary to achieve optical transparency, involves a tighter packing of collagen fibres and a decrease in tissue water content. A clear morphological sign of this condensation is the observed change in keratocytes shape from rounded to flattened. The latter stages of stromal thickening are probably the result of ongoing collagen deposition to satisfy the structural requirements of adulthood.

The anatomical significance of the cheek model is further supported by observations at the sclerocorneal junction. Human ocular anatomy is closely reflected in the distant shift from ordered corneal lamina to irregular scleral collagen, as well as the identification of related vascular and cellular processes.[21] A crucial landmark for study on limbal function, i.e., the corneal conjunctival border, is described by the occurrence of goblet cells at this junction in adults.[21]

The findings further aid the concept that chick as a strong model for corneal research. This further emphasises the histological similarity to humans in terms of epithelial layering and stromal compaction.[22–23] This model is more favourable for studying developmental biology, modelling for human corneal disease, and evaluating new treatments.[24]

This analysis provides a basic histological description, but it's the two-dimensional, illustrative reference that leaves room for improvement. With that said, 3D imaging (e.g., micro-CT or advanced microscopy), molecular profiling to classify the genes underlying these modifications, and functional assays (such as hydration measurements) to link structure to physiology.

CONCLUSION:

This study establishes a corneal development timeline for a chick and identifies histological benchmarks. It demonstrates epithelial stratification to build a protective barrier, indicating maturation, and enabling stromal compaction and transparency. There is a strong similarity between the human cornea and the chick, which supports the chick as a biologically relevant model for ophthalmic research and therapeutic development.

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REFERENCES

1. Mohan RR, Kempuraj D, D'Souza S, Ghosh A. Corneal stromal repair and regeneration. Prog Rretin Eye Res 2022;91:101090. DOI: https://doi.org/http10.1016/j.preteyeres.2022.10109

2. Minhas RS, Shaukat S, Kundi H, Sohail S, Mir A, Qamar A. Differentiation of sclera in chick embryo at various stages of its development–A histological study. Pak J Med Health Sci

2023;17(1):160–2. DOI: https://doi.org/10.53350/pjmhs2023171160

3. Kravchenko SV, Myasnikova VV, Sakhnov SN. The chick embryo and its structures as a model system for experimental ophthalmology. Bull Exp Biol Med 2023;174(4):405–12. DOI: https://doi.org/10.1007/s10517-023-05718-0

4. Bains KK, Young RD, Koudouna E, Lewis PN, Quantock AJ. Cell–cell and cell–matrix interactions at the presumptive stem cell niche of the chick corneal limbus. Cells 2023;12(19):2334. DOI: https://doi.org/10.3390/cells12192334

5. Koudouna E, Young RD, Quantock AJ, Ralphs JR. Developmental changes in patterns of distribution of fibronectin and tenascin-C in the chicken cornea: Evidence for distinct and independent functions during corneal development and morphogenesis. Int J Mol Sci 2023;24(4):3555. DOI: https://doi.org/10.3390/ijms24043555

6. Platzl C, Kaser-Eichberger A, Benavente-Perez A, Schroedl F. The choroid-sclera interface: An ultrastructural study. Heliyon. 2022;8(5):e09408. DOI: https://doi.org/10.1016/j.heliyon.2022.e09408

7. Ma J, Bi L, Spurlin J, Lwigale P. Nephronectin-integrin α8 signaling is required for proper migration of periocular neural crest cells during chick corneal development. Elife 2022;11:e74307. DOI: https://doi.org/10.7554/eLife.74307

8. Murcia-Belmonte V, Liu Y, Shamsi S, Shaw S, Collie-Duguid E, Herrera E, *et al*. Identification of lens-regulated genes driving anterior eye development. Dev Biol 2025;520:91–107. DOI: https://doi.org/10.1016/j.ydbio.2025.01.004

9. Yan F, Wang C, Wilson JA, O'Connell M, Ton S, Davidson N, *et al*. Visually guided chick ocular length and structural thickness variations assessed by swept-source optical coherence tomography. Biomed Opt Express 2021;12(11):6864–81. DOI: https://doi.org/10.1364/BOE.433333

10. Hadvina R, Estes A, Liu Y. Animal models for the study of keratoconus. Cells 2023;12(23):2681. DOI: https://doi.org/10.3390/cells12232681

11. Gao W, Du J, Chi Y, Zhu R, Gao X, Yang L. Minocycline prevents the inflammatory response after retinal detachment, where microglia phenotypes being regulated through A20. Exp Eye Res 2021;203:108403. DOI: https://doi.org/10.1016/j.exer.2020.108403

12. Wilson SE. The cornea: No difference in the wound healing response to injury related to whether, or not, there'sa bowman's layer. Biomolecules. 2023 Apr 29;13(5):771. DOI: https://doi.org/10.3390/biom13050771

13. Almodin FM, Almodin JM, Almodin EM, Fernandes N, Ferrara P, Gonçalves A. Intrastromal implantation of chicken corneal grafts into the cornea of rabbits for corneal thickening: an experimental study. Int Ophthalmol 2021;41:243–55. DOI: https://doi.org/10.1007/s10792-020-01573-4

14. Liu LR, Chen D, Sheng ST, Xu JW, Xu W. Research progress on animal models of corneal epithelial-stromal injury. Int J Ophthalmol 2023;16(11):1890–8. DOI: https://doi.org/10.18240/ijo.2023.11.23

15. Sun M, Zafrullah N, Adams S, Devaux F, Avila MY, Ziebarth N, *et al*. Collagen XIV is an intrinsic regulator of corneal stromal structure and function. Am J Pathology 2021;191(12):2184–94. DOI: https://doi.org/10.1016/j.ajpath.2021.08.016

16. Bradford S, Luo S, Brown D, Juhasz T, Jester J. A review of the epithelial and stromal effects of corneal collagen crosslinking. The Ocular Surface 2023;30:150–9. Doi: https://doi.org/10.1016/j.jtos.2023.09.003

17. Sevel D, Isaacs R. A re-evaluation of corneal development. Trans Am Ophthalmol Soc. 1988;86:178–207.

18. Quantock AJ, Young RD. Development of the corneal stroma, and the collagen-proteoglycan associations that help define its structure and function. Dev Dyn. 2008 Oct;237(10):2607-21. DOI: https://doi.org/10.1002/dvdy.21579

19. Wang K, Hoshino M, Uesugi K, Yagi N, Young RD, Frost BE, *et al*. Cell compaction is not required for the development of gradient refractive index profiles in the embryonic chick lens. Exp Eye Res 2020;197:108112. DOI: https://doi.org/10.1016/j.exer.2020.108112

20. Boote C, Kamma-Lorger CS, Hayes S, Harris J, Burghammer M, Hiller J, *et al*. Quantification of collagen organization in the peripheral human cornea at micron-scale resolution. Biophys J 2011;101(1):33–42. DOI: https://doi.org/10.1016/j.bpj.2011.05.029

21. Fowler WC, Chang DH, Roberts BC, Zarovnaya EL, Proia AD. A new paradigm for corneal wound healing research: the white leghorn chicken (Gallus gallus domesticus). Curr Eye Res. 2004;28(4):241–50. DOI: https://doi.org/10.1076/ceyr.28.4.241.27837

22. Merayo-Lloves J, Yáñez B, Mayo A, Martín R, Pastor JC. Experimental model of corneal haze in chickens. J Refract Surg. 2001 Nov-Dec;17(6):696–9. DOI: https://doi.org/10.3928/1081-597X-20011101-11

23. Mangioris G, Chiodini F, Dosso A. New strategy to study corneal endothelial cell transplantation: the chick cornea model. Cornea. 2011;30(12):1461–4. DOI: https://doi.org/10.1097/ICO.0b013e31821821fe

24. Ibares-Frías L, Gallego P, Cantalapiedra-Rodríguez R, Valsero MC, Mar S, Merayo-Lloves J, *et al*. Tissue reaction after intrastromal corneal ring implantation in an experimental animal model. Graefes Arch Clin Exp Ophthalmol. 2015 Jul;253(7):1071-83. DOI: https://doi.org/10.1007/s00417-015-2959-5