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Histogenesis of the cornea in sheep fetuses

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Abstract

In this study, 36 samples of sheep fetuses at different ages were used. After preparation and determination of age, the samples were immediately fixed and subjected to gross anatomy and histology examination. In addition to routine H&E staining, two specific staining methods, Verhoeff and Masson's trichrome, were used in the histological study. The results showed that the corneal forming layer completely loses its contact with the lens and posterior layers and the eyelid ridges start to emerge at around 30 days of gestation. However, the cornea becomes fully differentiated in the second month of gestation and only undergoes growth stages in subsequent ages. From the beginning, the cornea in sheep fetuses is formed as a horizontal ellipse, with its inner curvature larger than its outer curvature. The growth process in terms of dimensions, including longitudinal diameter, transverse diameter, and corneal height, shows a regular ascending trend with increasing age. The upper and lower eyelids fuse in early second month and separate again in late third month. The third eyelid appears at approximately 120 days of gestation in the inner angle of the eye. The outer epithelial covering of the cornea changes from simple cuboidal to stratified squamous epithelium. With increasing age, the matrix is strengthened both in terms of cellular components (fibroblasts) and fibers (collagen). Additionally, an increase in elastic fibers is noticeable in the late fetal stages. The Descemet's membrane appears as an inner limiting membrane beneath the inner epithelial covering in early second month and also undergoes growth with increasing age. Descemet's membrane lacks elastic fibers. Bowman's layer or the outer limiting membrane was not observed in this animal. Masson's trichrome staining confirms the growth of collagen fibers, while Verhoeff staining confirms the growth of elastic fibers with increasing age.

Keywords: Cornea; Histogenesis; Sheep; Fetuses; Eye

1. Introduction

After the closure of the neural tube, the lateral expansion of the prosencephalon forms visual vesicles, which remain connected to the prosencephalon by optic stalks. Each visual vesicle grows towards the periphery and connects to the adjacent surface ectoderm. As a result of this contact, the surface ectoderm thickens and forms the lens placode, giving rise to the lens vesicle. The corneal page subsequently undergoes invagination and forms the lens vesicle, which separates from the surface ectoderm and also forms the anterior layer (surface) of the cornea (Cornea). Deeper layers of the cornea are formed by mesenchymal cells. The differentiation of these mesenchymal layers forms the anterior parts of the eye. The formation of a spatial tissue cavity called the anterior chamber occurs, which divides the mesenchyme into two layers. The inner layer is located opposite the lens and iris, and the outer layer is continuous with the sclera and is called the corneal stroma. Thus, the corneal epithelial layer is derived from the surface ectoderm. The corneal stroma, which is continuous with the sclera, forms the main substance of the cornea. Finally, a posterior epithelial layer is located at the border of the anterior chamber [1-24].

The eye is one of the most important and sensory organs of the body, playing a significant role in establishing communication between living organisms and the environment. Due to its importance and sensitivity, researchers have

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long been studying it. The eye consists of three important layers: neural, vascular, and connective tissue layers. The cornea is part of the connective tissue layers of the eye and is one of the transparent layers of the eye. As the most anterior and outermost layer of the eye, it is exposed to various injuries and is also examined and evaluated in many diseases. Before examining any abnormalities of this organ, it is essential to have sufficient knowledge about its normal state, its constituent parts, and how this structure is formed. This study was conducted in this regard.

2. Material and methods

This study was conducted on 36 sheep embryos collected from a slaughterhouse. After determining the age using the formula, the collected embryos were divided into 9 groups of 4 embryos each. The groups included embryos of 80, 70, 60, 50, 40, 30, 120, 90, and 150 days. Embryos smaller than 7 cm were completely fixed in 10% formalin, and embryos larger than 7 cm, which could be removed from the eye, were fixed by placing the eye in 10% formalin for 48 hours. To ensure complete fixation of the internal parts of the eye, 10% formalin was injected into the anterior chamber using a syringe. The dimensions were measured using a ruler and calipers.

Tissue samples were prepared using the conventional histological method. Histological slides were stained with three types of staining: hematoxylin-eosin (for general tissue examination), Verhoeff (for elastic fibers examination), and Masson's trichrome (for collagen fibers examination). The slides were then examined under a light microscope.

3. Results

3.1. The results of the study are as follows

Results of the 30-day embryo group: At this time, the eyelids were closed, and the eye globe was small and round. The mean longitudinal diameter of the eye globe was measured to be 0.20 ± 0.0081 cm, and the mean transverse diameter was measured to be 0.10 ± 0.0081 cm.

Histological findings indicate that the corneal tissue structure is forming at this stage, with a row of cuboidal cells with round nuclei, and occasionally, cells of the second row are also observed scatteredly. The structure of the basement membrane under this epithelial covering is not very clear. The stroma is completely visible, and the initial and primary collagen fibers are discernible in this area with low amounts and thin thickness. Numerous connective tissue cells are seen in the stroma area, with a high percentage of them being fibrocytes with clear and euchromatic nuclei. Some round and dark-nucleated cells that appear to be lymphocytes are distinguishable in the connective tissue cells layer. The inner endothelial layer and its underlying membrane are not identifiable. In the first month's staining, no evidence of elastic fibers in the matrix of this stage was observed, and fine strands seen in the matrix were identified as collagen, which appeared golden yellow.

3.2. Results in 40-day-old fetuses

The eyelids are closed and in the early second month, the eye globe appears as a unified, small, and round structure. The average longitudinal diameter of the eye globe is 0.25 ± 0.0081 cm, and the average transverse diameter is 0.15 ± 0.0081 cm.

In the early second month, the anterior epithelium tissue is clearly distinguishable, and the second row of cells is forming on top of the first row. Some of these cells are cubic and a few are elongated. Under the epithelial covering, a thin and distinct layer equivalent to the basement membrane is visible. The number and thickness of collagen fibers have increased compared to the first month but are still considered fine strands. Connective tissue cells in the matrix are abundant, and in addition to fibroblasts and lymphocytes, some fibrocytes are also recognizable. The underlying endothelial membrane or the posterior limiting membrane is not very clear, and the endothelial covering cells are appearing as cubic cells in the process of emergence and becoming evident. In the staining, some partial traces of elastic fibers gradually appear in the stroma.

3.3. Results in 50-day-old fetuses

In these samples, the eyelids are still closed, and the eye globe remains small, round, and unified. Further histological examination is required to assess the corneal tissue, and anatomically, nothing is distinguishable. The average longitudinal diameter of the eye globe is 0.30 ± 0.0081 cm, and the average transverse diameter is 0. 20 ± 0.0081 cm.

The corneal epithelial tissue is clearly distinguishable in these days, consisting of one to two rows of covering cells. The first row contains round-nucleated cells, and the second row contains round to elongated nuclei. A thin membrane, which is the equivalent of the basement membrane, is visible. The density of connective tissue cells, especially fibroblasts, is high in the matrix, and collagen fibers have increased in thickness and density. The endothelial layer or the inner layer is clearly distinguishable, composed of cells ranging from cubic to squamous. The posterior limiting membrane between the matrix and the epithelial cells is not very clear. In the staining, the presence of some black elastic fiber strands in the matrix is confirmed, with their number increasing compared to previous samples.

3.4. Results in 60-day-old fetuses

The eyelids in these samples are still closed, and after opening them, the cornea appears as an oval shape. The average longitudinal diameter of the cornea is 0. 56 ± 0.0081 cm, the average transverse diameter is 0. 40 ± 0.0081 cm, and the average height of the cornea from the limbus is 0.12 ± 0.0081 cm.

In the sections prepared from the cornea in the last decade of the second month, it was evident that the corneal epithelial tissue consists of one to two rows of covering cells, with the first row containing round-nucleated cells and the second row containing round to elongated nuclei. A thin membrane, equivalent to the basement membrane, is visible. The density of connective tissue cells, especially fibroblasts, is high in the matrix, and collagen fibers have increased in thickness and density. The endothelial layer or the inner layer is clearly distinguishable, composed of cells ranging from cubic to squamous. The posterior limiting membrane between the matrix and the endothelial cells is not very clear. In the staining, the presence of some black elastic fiber strands in the matrix is confirmed, with their number increasing compared to previous samples.

3.5. Results in 70, 80, and 90-day-old fetuses (third month)

In the 70-day-old fetuses, the eyelids are still closed, and after opening them, the cornea appears as an oval shape inclined towards a triangular shape. The internal curvature of the cornea is larger than the external curvature. The average longitudinal diameter of the cornea is 0.90 ± 0.0081 cm, the average transverse diameter is 0. 70 ± 0.0081 cm, and the average height of the cornea from the limbus is 0. 20 ± 0.0081 cm.

In the 80-day-old fetuses, the eyelids are still closed, and after opening them, the cornea appears as an oval shape inclined towards a triangular shape. The internal curvature of the cornea is larger than the external curvature. The average longitudinal diameter of the cornea is 1.10 ± 0.0081 cm, the average transverse diameter is 0. 80 ± 0.0081 cm, and the average height of the cornea from the limbus is 0. 20 ± 0.0081 cm.

In the 90-day-old fetuses, the eyelids begin to open. The cornea appears as an oval shape inclined towards a triangular shape. The internal curvature of the cornea is still larger than the external curvature. The average longitudinal diameter of the cornea is 1.23 ± 0.0081 cm, the average transverse diameter is 0. 90 ± 0.0081 cm, and the average height of the cornea from the limbus is 0. 20 ± 0.0081 cm. In 150-day-old fetuses, the eyelids are observed in a completely open and separate state. The cornea is observed in an oval shape, asymmetrical, and non-symmetrically. It is positioned in the inner curve of the base of the triangle. The inner curve of the cornea is larger than the outer curve. The third eyelid is fully formed and visible in the inner curve. The average longitudinal diameter of the cornea is measured to be 1.60 ± 0.0081 centimeters, the average transverse diameter is 1.10 ± 0.0081 centimeters, and the average height of the cornea from the scleral edge is 0.20 ± 0.0081 centimeters. In the fifth month, the number of cell layers in the epithelial tissue increased, and in some parts, it reaches up to six rows. The underlying cells are basal cells with bright, round to oval nuclei. The second and third rows of cells are cuboidal with round nuclei, and the upper rows of cells have a pavement-like appearance. The basement membrane in this layer is very distinct and prominent, located at the boundary between the epithelial tissue and the matrix, which is clearly visible in trichrome staining. The matrix is very thick and composed of collagen and elastic fibers, with the collagen fibers being thicker. The cell density is lower, and fibroblasts and fibrocytes are observed in the matrix. The Descemet's membrane is clearly visible, and endothelial cells are located on this membrane. The presence of elastic fibers in the corneal matrix is indicated by Verhoeff's staining.

4. Discussion

As mentioned in the results section, in the one-month samples, due to the small sample size and the inability to perform detailed anatomical examinations, only histological examinations were conducted. At the end of this period, visual cups are formed and can be seen as two distinct prominences under the microscope. These visual cups protrude from the sides of the optic region towards the outer sides. However, in the sheep embryo at this time, no signs of differentiation of the visual cup and the surrounding mesenchymal tissue into the choroid and sclera layers were observed. However, the continuation of the mesenchymal tissue around the visual cup towards the anterior side revealed initial signs of the

cornea. In humans, the first signs of eye formation appear as a pair of shallow grooves on both sides of the anterior brain at 22 days of gestation. It has also been reported in humans that with the closure of the neural tube, protrusions from the anterior brain called optic vesicles emerge. Furthermore, in humans, the optic vesicles move towards the sides and the visual cup is fully formed in the late first month. In dogs, the optic vesicle forms at 17 days of gestation, while in cows, it forms at 25 to 30 days. According to the same report, the formation of the visual cup occurs at 19 days in dogs and at 30 days in cows. The overall reports indicate that in most animals, the formation of the visual cup occurs approximately in the first month of pregnancy or the one-month-old embryo. Additionally, in the first month, the movement of the ectodermal layer begins as invaginations in front of the cornea, but the eyelids have not yet reached each other and are not fully formed. The appearance of the third eyelid in other animals has not been observed. From a histological perspective, the growth process continues in such a way that the outer epithelial cells are arranged in more than two rows and the basement membrane becomes completely evident. Additionally, a glycocalyx membrane appears on this epithelial tissue. The corneal lamellae and cells are strengthened in the corneal matrix, and the Descemet's membrane is clearly visible without elastic fibers. In the fifth month, the anatomical growth process is completed, and the oval-shaped cornea shows gradual and progressive growth in terms of longitudinal and transverse diameter, as well as height from the limbus. Approximately at birth, the longitudinal diameter of the cornea will be 6.1 centimeters and the transverse diameter will be 1.1 centimeters. Additionally, the height of the cornea from the limbus reaches 2.0 centimeters. From a histological perspective, the number of layers of the outer epithelial tissue becomes extremely high, even reaching 6 layers. The basement membrane is completely evident. In the matrix, there is a high number of collagen fibers, but elastic fibers are rarely seen. Overall, the number of fibers exceeds the number of cells. The Descemet's membrane is clearly thick, and the squamous cells are seen as posterior epithelial tissue. Based on the overall anatomical studies, it can be concluded that the main differentiation of the cornea occurs in the second month, and its growth continues in the following months. The overall shape of the cornea is oval and positioned horizontally, with the inner angle being larger than the outer angle. This process continues until birth, and its gradual growth occurs in terms of longitudinal and transverse diameter, as well as height. The adhesion of the eyelids occurs in the early second month and their separation takes place at the end of the third month. Histological investigations have also revealed that the main differentiation of the lamellae and cellular layers occurs in the second month, and as we approach the end of the embryonic period, growth occurs in terms of the epithelial tissues, cells, matrix fibers, and Descemet's membrane. It should be noted that in the sheep embryo, the Bowman's layer, which is reported to exist in humans, is absent [25-43].

Figure 1 Fetal sheep eye cornea at 4 months

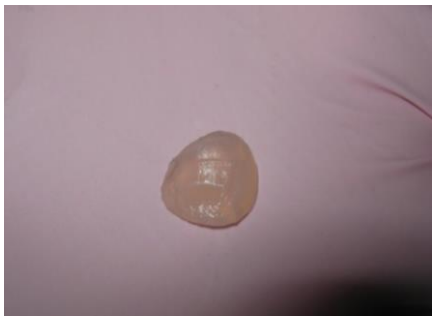

Figure 2 Cornea separated from a 3-month-old sheep fetus

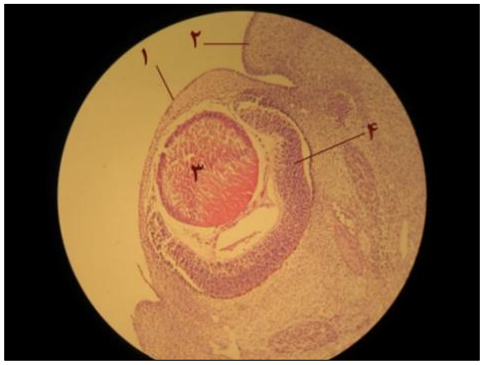

Figure 3 Fetal eye segment at one month. X40

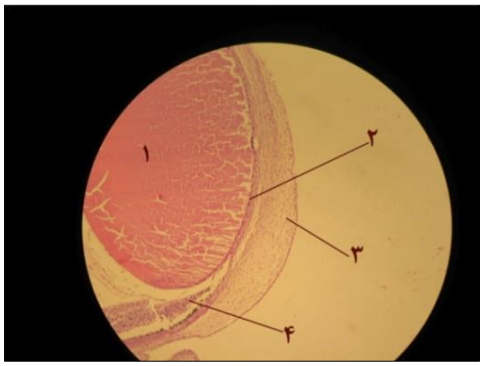

Figure 4 Embryonic eye segment at two months, X100

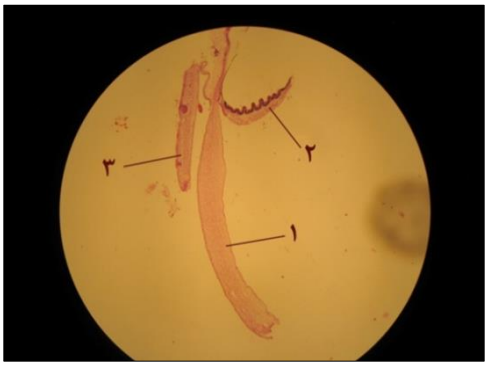

Figure 5 Embryonic eye segment at two months, X40

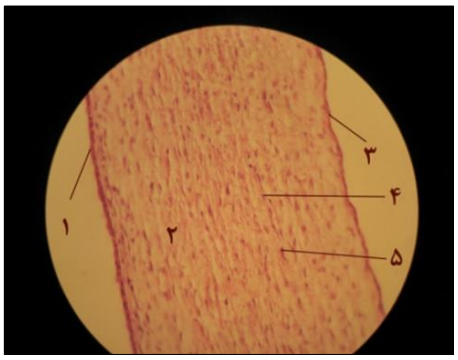

Figure 6 Corneal segment in a two-month-old fetus, X40

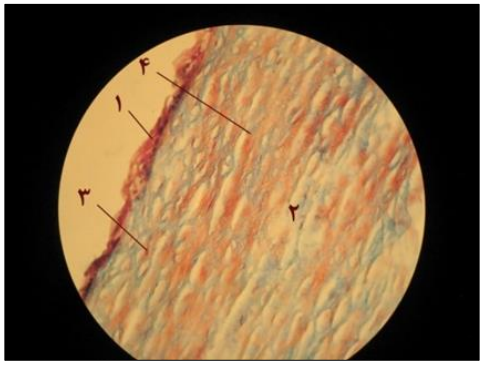

Figure 7 Corneal section in a 3-month-old fetus, trichrome Mason, X1000

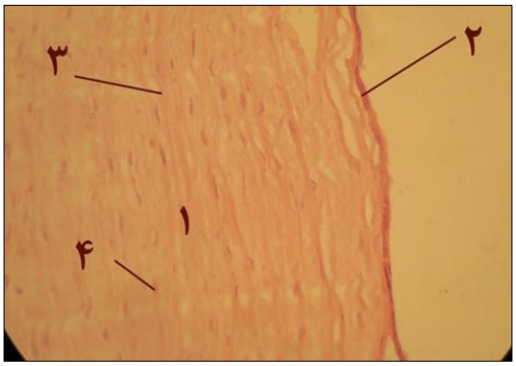

Figure 8 Corneal section in a 4-month-old fetus, X100

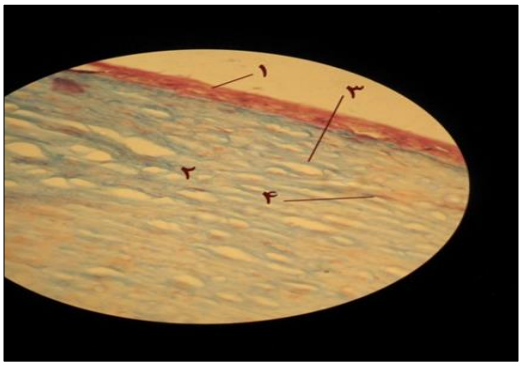

Figure 9 Corneal section in a 4-month-old fetus, trichrome Mason, X1000

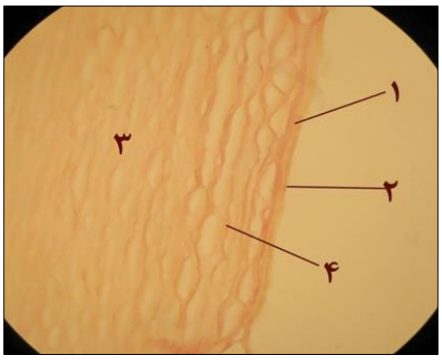

Figure 10 Corneal section in a 5-month-old fetus, Verhoeff, X1000

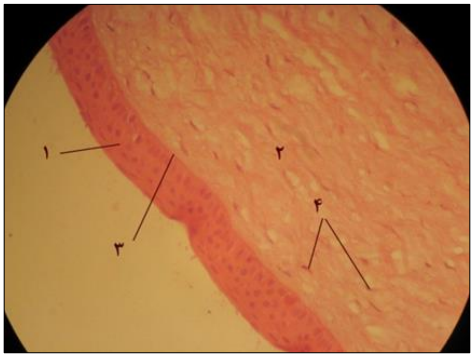

Figure 11 Corneal section in a 5-month-old fetus, X1000

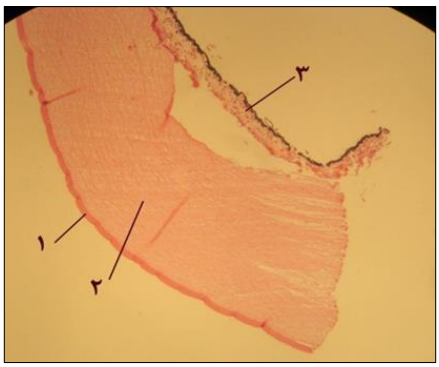

Figure 12 Fetal eye section in a 5-month-old fetus, X1000

5. Conclusion

In conclusion, sheep eye development follows a similar timeline to other mammals, with the visual cup forming around the first month and the cornea undergoing its main differentiation in the second month. While the overall shape and histological features resemble those of adult sheep eyes, some key differences exist, such as the absence of Bowman's layer. Further research could explore the functional implications of these variations and their potential links to vision capabilities in sheep compared to other species.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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