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EMBRYONIC AND FETAL HISTOMORPHOLOGICAL DIFFERENTIATION OF THE OCULAR STRUCTURES IN SPRAGUE DAWLEY RAT (*Rattus norvegicus*)

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ABSTRACT

Histological sections of the embryos and fetuses of the Sprague Dawley rats were used to study the ocular developmental stages. Microscopic examination indicated that the primordial tissue related to the eye is found in the head fold region as an optic pit, then form the optic vesicle. The latter is invaginated upon itself to form the optic cup. The lens vesicle, which had separated from the ectoderm, was distinctly visible. Hence, lens capsule and fibers were evident. The front lens of the eye is derived from the superficial ectoderm and from the cornea. The optic vesicle is destined to form the retina. The mesenchymal cells found between the margins of the cup and the lens is involved in the formation of the vitreous body. In conclusion, the organogenesis of the ocular tissues in studied rats becomes evident when the optic cup and invaginated lens placode were begun to be formed which can be morphologically identified on the 12th embryonic day. The current information about the embryonic and fetal development of the rat's eye gives more concepts for subsequent morphological and physiological works or experiments.

INTRODUCTION

The eye is a complex structure and it was the one of the organs that was experimentally studied by the earliest embryologists. The eye of vertebrate is developed through a complicated series of morphogenetic events concerning the differentiation of structures with different embryonic origin (1). In fact, the visual system has been considered as among

important sensory system in animals' life span. The complicated structure of the eye is originates from the primordial tissues derived from a number of sources, mainly the neural ectoderm, the surface ectoderm and the periocular mesenchyme (2). The structural details of the early development of the eye have been described in the hamster (3), kittens (4), dog (5), mouse (6), chicken (7) and human (8). In developed countries, malformations of the eye are among the most common causes of serious visual impairment in newborns (9).

The optic vesicle is initiated from the eye primordium which is basically formed as a simple outgrowth from the neural tube, early forebrain and surrounded by the cephalic mesenchyme and ectoderm. The optic vesicle invaginates and becomes patterned into the optic stalk, neural and pigmented epithelium of the retina. The optic vesicle adopts a more complex configuration to form the optic cup. Subsequently, the lens, external corneal epithelium, stroma of conjunctiva, choroid and sclera originates from the surface ectoderm. Surface ectoderm of the embryo results in formation of the lens pit. The invaginated epithelium of this pit pinches off to form the lens vesicle, which then develops into the lens proper. It was established that lens induction from the surface ectoderm is dependent on the underlying optic cup. Iris and ciliary body are consequentially formed from the wall of the diencephalon via optic vesicle and optic cup (10).

Both neural retina and retinal pigmented epithelium are raised from the dorso-ventral expanded optic vesicle and that the optic stalks and optic nerve are developed in a position directed by the proximal narrowed end of the optic vesicle. The choroid or optic fissure forms from along the proximo-distal axis at the eye at a predictable position. Both layers of the neuroectoderm begin to fuse in the middle of the long axis and this process continues proximally towards the optic stalk and distally towards the future iris (9).

The goal of this study was to exposed information about the embryonic and fetal development of the rat's eye in order to give more concepts for morphological and physiological works or experiments.

MATERIALS AND METHODS

Thirty six samples of embryos and fetuses were provided from the Animal House Unit at Faculty of Veterinary Medicine/ Baghdad University. Three pregnant Sprague Dawley rats were euthanized on the afternoon of days 1, 5, 7, 9, 12, 15, 17, 20 and 23 of pregnancy. The whole uteruses were removed and placed immediately in 10% neutral buffered formalin solution. Directly, deciduas swellings were freed from the uterine wall and embryos dissected from them. Embryos in good conditions were selected for subsequent histological procedure.

Embryos were left for 24 hours in the above fixative then dehydrated in a descending series of ethyl alcohols, cleared in 100% xylene and then embedded and blocked in paraffin wax. Serial sections of 6 μm thickness were prepared from the blocked samples and stained with hematoxylin and eosin stains. Sections were examined with Olympus (BX 51, Japan) light microscope.

RESULTS

Cross section through the head region of the embryo revealed many changes such as formation of the optic vesicle which was extended from the diencephalon. The latter was thickened and folded as an optic cup. The optic cup induces the lens formation as the lens placode was formed from the epithelial ectoderm, subsequently infolds as future lens. Subsequently, lens with or without optic cup induces the formation of the cornea.

In the 1st and 5th old embryos, light examination did not detect any primordial structures related to the ocular development. **Embryos of 7th days** revealed the presence of primordial placode that were appeared as thickened areas on either side of the primitive forebrain(.Fig.1) **In the 9th day's embryo**, two lateral diverticula were initiated on both sides of the diencephalon of the embryo. It was started by the appearance of an evagination on both sides of diencephalon. This evagination leads to the development of the optic vesicle. The optic vesicle was maturated by cellular and vesicular shape differentiation. The early optic vesicle was distinguished by the presence of a flat plate of cells as an optic primordial structure. As the optic vesicle approaches the surface ectoderm, it involutes to form the optic pit which subsequently continued in invagination to form the optic cup. This stage of embryo showed optic vesicles originally have a broad attachment to the related diencephalon.(Fig.2).

In the 12th old embryos, the cells underwent dramatic changes in growth and morphology during eye development. A true optic stalk appeared to connect the optic vesicle to the brain. Once the vesicle reached the surface ectoderm, it involutes to form the optic cup which appeared as C-shape with the appearance of the optic stalk. The formation of optic cup was associated with the initiation of the lens placode. The latter was started to form by an invagination of the corresponding surface ectoderm. The optic cup was composed of two layers of neural ectoderm, the inner layer being thicker than the outer layer. The optic stalk was also formed which was hollow structure at this stage of development. The invagination of the lens placode was evident. The lens placode was lined by a single layer of epithelial cells continuous with the surface ectoderm of the formed lens pit. The lens vesicle was formed which subsequently was separated from the surface ectoderm and typically remains in the optic cup when the surface ectoderm is removed.At such stage, a goblet-shaped optic cup was appeared with the lens vesicle floating in its open end

The optic stalk lengthens considerably so that the optic vesicle comes to lie farther from the brain and closer to the surface ectoderm. Synchronously, the optic vesicle begins its characteristic involution to create the optic cup. A shallow furrow forms on the ventral surface of the optic cup. The furrow, the presumptive optic fissure, is bounded on either side by folds of neuroectoderm and filled by mesectodermal tissue.(Fig.3).

In embryo of 15th days, the cup was underwent some changes. It deepens and creates a prominent rim on all but its ventral side where it left a cleft in the ventral portion of the optic cup called the optic fissure. The optic fissure was extended to permits the passage of the intraocular vessels which may be called hyaloid vessels. In another aspect, the optic cup was occupied by the forming lens and mesectoderm. The newly lens vesicle which developed from the surface ectoderm was detached at this period. The lens vesicle was nearly spherical in shape characterized with a large central cavity. The epithelial cells of lens were formed form simple layer of epithelial cells. The mesenchymal cells present in this area were differentiated to fibroblasts. The fibroblasts then migrate between the lens and the epithelium and start to lay down a dense stroma to constructs bundles of parallel collagen fibers.

The optic fissure closes completely. All that remains of the fissure at this time is a shallow furrow on the ventral aspect of the eyeball. Intraocular vessels enter the optic cup through a small persistent portion the optic fissure.

As soon as the lens vesicle is formed, the cells at its posterior hemisphere (closer to the optic cup) begin to elongate to form the lens fibres, and the lumen of the lens vesicle is completely filled in. The lens continues to increase in size by the formation of new fibres from the epithelial cells located at the equatorial region of the lens. The inner fibres start to undergo terminal differentiation, which results in denucleation and loss of other organelles. Because the fibre elongation is synchronized, the nuclei of the fibre cells become arranged in very neat and regular pattern.

Major inductive events were takes place to establish the cornea in which processes of transformation were takes place to change the typical surface ectoderm to a transparent, multilayered structure with a complex extracellular matrix and cellular contributions from several sources. At first, ectodermal cells were stimulated by the lens vesicle to form a multilayered structure. In the next step, these cells begun to secrete various collagen types to form the primary stroma**.** Later, two waves of neural crest cells were reached the optic cup. Later stages showed that the central lens fibres began to differentiate; the bow region of the lens becomes distinguishable. The bow region was clearly located closer to the posterior pole of the lens. The lens nucleus, composed of the primary lens fibres, is flattened, and the secondary lens fibres are irregular and misshapen. These cells were considered as primary lens fibers. The cells at the anterior pole of the lens vesicle remain as epithelial cells. At this prenatal stage; a germinative ring of mitotic active cells was formed around the central region. Many daughter cells from this germinative region were moved into the equatorial region or bow of the lens, where they soon elongated tremendously and differentiated into secondary lens fibers. They form concentric layers around the primary fibers of the lens nucleus. Both, the primary and secondary fiber cells lose their mitochondria and cell nuclei during the final processes of the differentiation. The two layers of the optic cup begin to differentiate in distinct directions. The cells of the outer layer produce pigment and eventually form the **pigmented layer of the retina.** At the same time, the outer lips of the optic

cup, where the developing neural and pigmented retinas meet, undergo a different transformation into the **iris** and **ciliary body.**(Fig 4)

Eighteen day old embryos: The margins of the optic cup overlap the lens edges. The overlapping portions of the optic cup form the epithelial part of the iris and the reduced opening in front of the lens represent the pupil. The retina develops normal histogenesis with cells being added peripherally during embryonic development. The space between the lens and part of the optic vesicle filled with a gelatinous material and constitute the vitreous body. At this stage of development the rat retina, in hematoxylin and eosin section, is composed of undifferentiated epithelium with an indication of formation of the optic nerve layer. The lens has separated from the covering ectoderm and is still developing. The ciliary body has not yet formed The cells of the inner layer of the optic cup proliferate rapidly and generate a variety of glia, ganglion neurons, interneuron, and light-sensitive photoreceptor neurons. All these cells together constitute the **neural retina.** The axons from the ganglion cells of the inner layer of the retina meet at the base of the eye and travel down the optic stalk. Initially, the optic stalk represents a narrow neck that connects the optic cup to the diencephalon. Once the axons reach the optic stalk, they grow into it and relay the eye with the visual centers of the brain. The optic stalk is now referred to as the optic nerve One of the most prominent features of the eye of rat was its very large and highly pigmented ciliary body. The iris primordium can be distinguished from the prospective ciliary body, and slight folding of the ciliary processes is evident. The morphogenesis of the ciliary body and the outgrowth of the iris continue postnatally. Interestingly, the iris primordium is not identifiable in the eye of the rat until the ciliary fold morphogenesis is well under way. The ciliary body and iris morphogenesis is completed in this animal by the time of birth.(Fig 5).

In the twenty one day old embryos, the lens were almost fully formed. The developing cornea and sclera can be identified. In the retina, the nerve fiber layer and the beginning differentiation of the ganglion cell layer can be recognized but the ciliary body was still incompletely developed.(Fig.6).

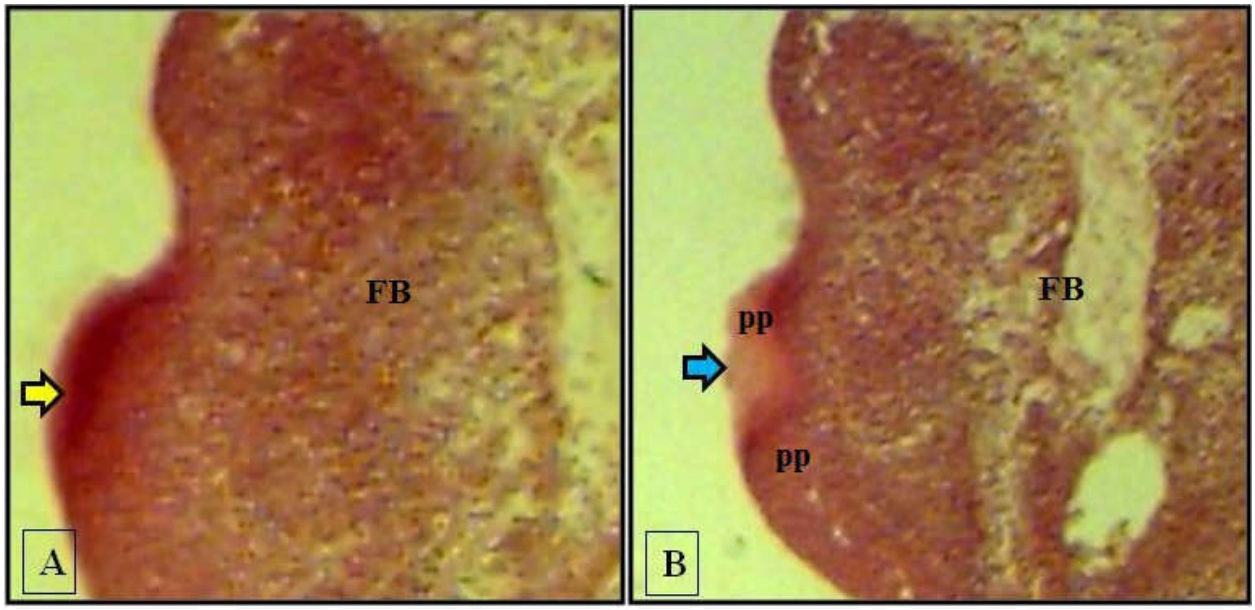

Figure 1: Micrograph Embryos of seven days (A) shows lateral evagination (Arrow) of diencephalon represents eye primodiaum. Micrograph (B) shows creation of optic cup (Arrow), primodial placode (pp) ,fore brain(diencephalon) 400x (H&E stain**. Embryo of 7 days**.

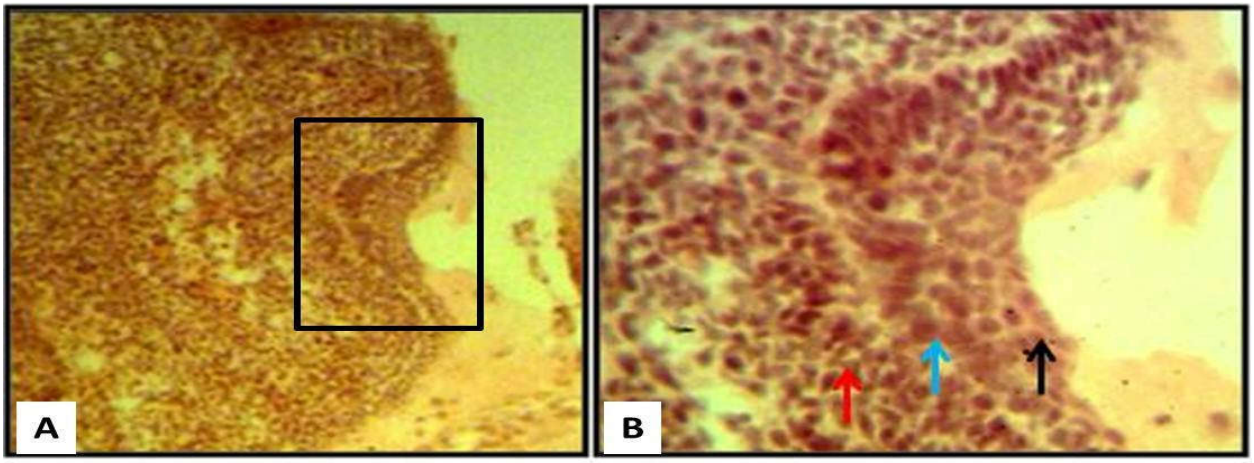

Figure 2: Micrograph In the **9th day's embryo** (A) shows invagination of double layer structure (rectangle area) of diencephalon represents eye cup (100x) . Micrograph (B) shows sensitive layer (blue arrow) , pigmented layer (Red arrow) & permordium of lens from superficial ectoderm optic cup (Black arrow).400x (H&E) stain**.**

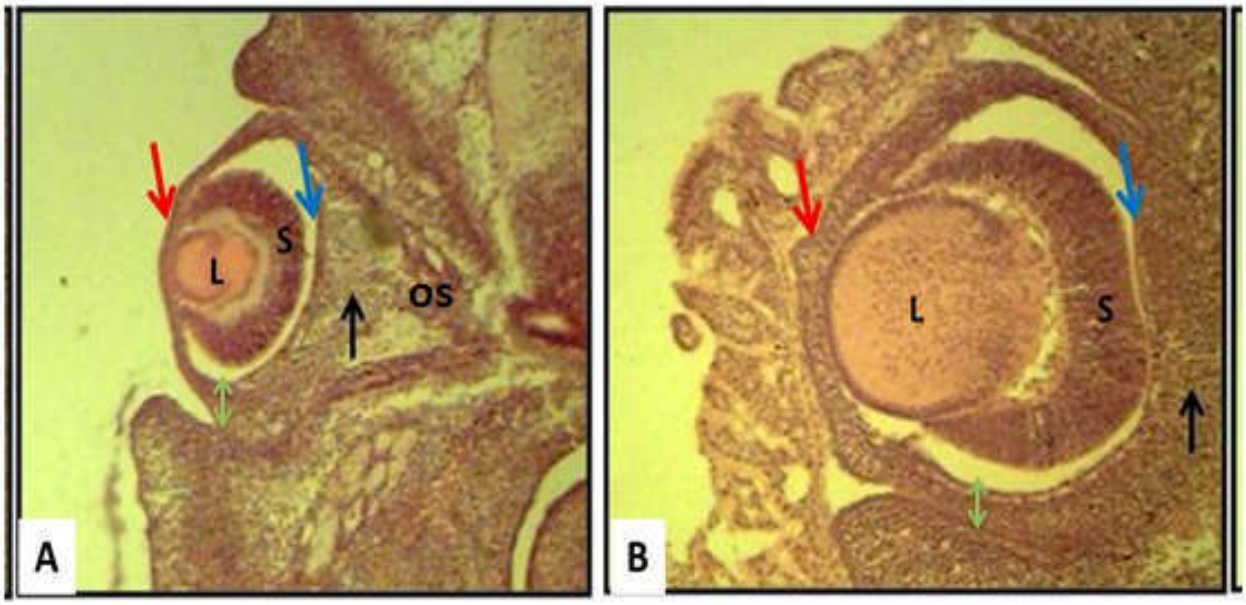

Figure 3: Micrograph In the **12th old embryos**(A & B) shows eye ball had lens (L), corneal (Red arrow), sensitive layer (S), pigmented layer (Blue arrow), and condense mesenchymal tissue of sclera (Black arrow) , optic cup(green arrow) , optic stalk (os).40x & 100x (H&E) stain.

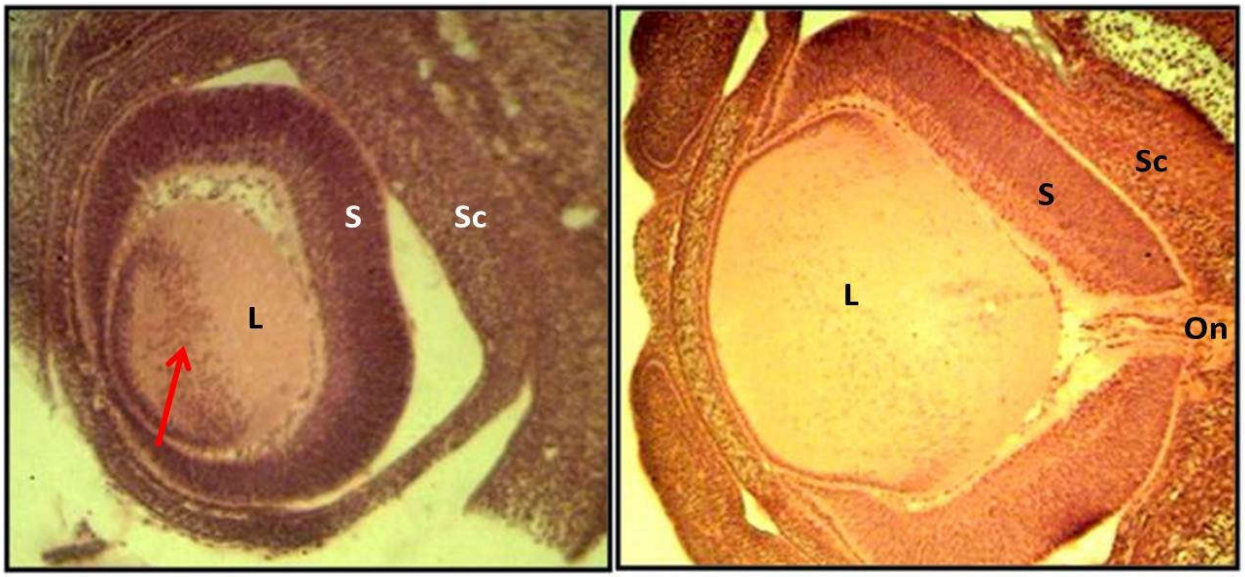

Figure 4: Micrograph of **embryo of 15th days,** eye ball shows: lens (L), nuclei of lenticular fibers (Red arrow), sensitive layer (S), primordial tissue of sclera (Sc) & optic nerve (On) 40 &100x (H&E) stain.

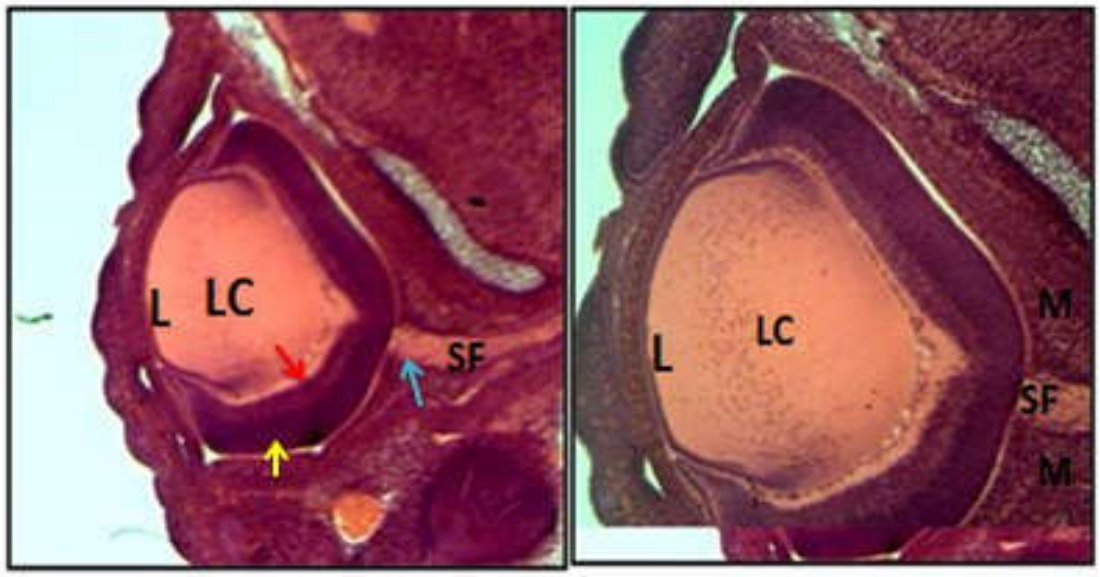

Figure 5: section of **embryo of 18th days,** eye ball shows: lens (L), nuclei of lenticular centre (LC), sensitive layer (Red arrow),Pigmented layer of retina(yellow arrow)Shallow furrow(SF), Mesenchyme(M)40&100x (H&E) stain.

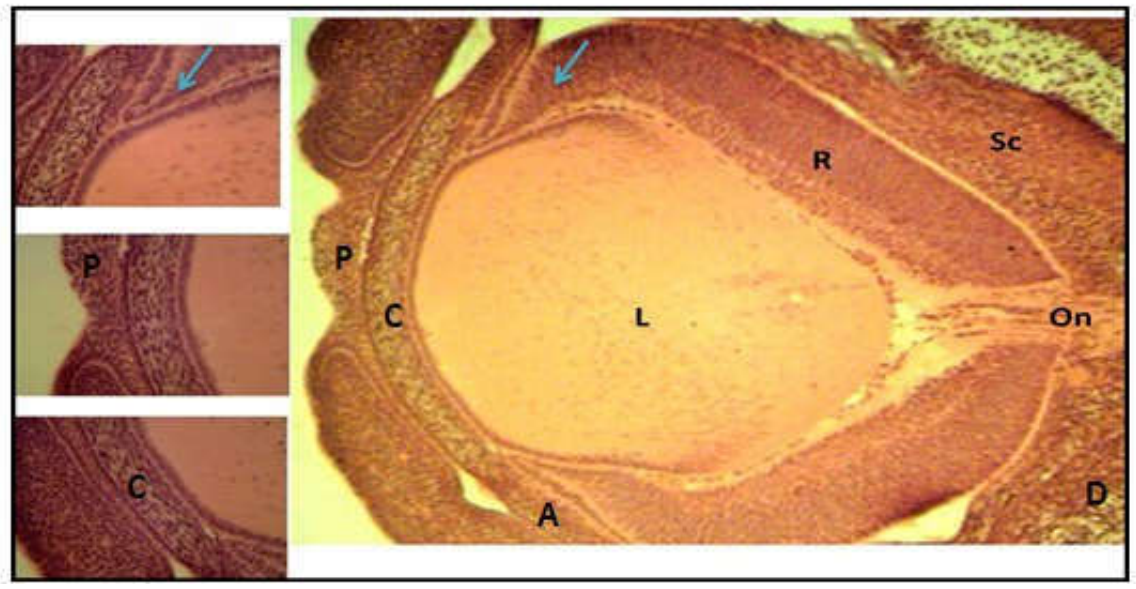

Figure6 : Micrograph of the **21 day old embryos** (A & B) shows eye ball had lens (L), cornea (C) ,Retina(R) ,Attachment of ciliary body and iris (Blue arrow),Pupil(P),Diencephalone(D) ,optic nerve (on)and condense mesenchymal tissue of sclera (SC).40x & 100x (H&E)

DISCUSSION

The initiation steps of the ocular development in the studied rat's embryos were generally similar to those events recorded in mammalian species specially rodents such as mice, hamster ….etc.

The developmental morphogenesis in the 15th days aged rat embryos were critical in this period of differentiation which was approximately parallel to those of the mouse embryo but with slight difference where the critical period was recorded at 13 days.

The formation of optic cup may be stimulated by the initiation of the lens placode. In fact, (7) previously hypothesized the presence of a signal coming from the pre-lens ectoderm that induces the optic vesicle to form an optic cup.

Numerous experimental studies have demonstrated that **the lens serves an essential** role in the morphogenesis of the anterior eye structures (cornea, ciliary body and iris) (**11,12).**
In the mouse, eye development starts with the lateral outgrowth of the optic vesicles from the diencephalon, which reach the overlying surface ectoderm, flatten and then invaginate, forming the optic vesicle by E10.5 (13). By E11.0, the lens vesicle is formed by invagination and "pinching-off" from the overlying ectoderm. These initial stages of eye morphogenesis appear to occur similarly in the Cape dune mole rat eye.
The findings of this study, combined with data obtained from the literature, suggest that the period during which ocular development takes place is earlier in larger mammalian species than in the smaller ones.

Previous descriptions of ocular development in other mammalian species, and one in the dog, reported that the development of the lens capsule starts posterior and proceeds towards the anterior part of the lens vesicle.(10)
Eye formation in the human embryo begins at approximately three weeks into embryonic development and continues through the tenth week
The major development of the eye takes place between week 3 and week 10 and involves ectoderm, neural crest cells, and mesenchyme.
When the optic vesicle contacts the overlying surface ectoderm, an exchange of inductive signals between these tissues is thought to take place, which results in their

coordinated invagination to form the lens vesicle and the optic cup. A signal from the distal portion of the optic vesicle induces the part of the overlying epithelium in contact with it to thicken and form the lens placode, which in turn, promotes the invagination of the optic vesicle to form the optic cup.(14)

Numerous experimental studies have demonstrated that the lens serves an essential role in the morphogenesis of the anterior eye structures (cornea, ciliary body and iris) (15).

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