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Investigation of the eyes histogenesis development in quail embryos at second term in an incubation period

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Abstract:

This study is aimed to identify histological construct development in eye for quail embryo. In this study, we are focused on the tracing appearance of eye from beginning until completion of main structures of the eye at seventh day of incubation. The results shown that there is no structure appear in the optic vesicles at (12 hr) incubator. However, the optic vesicles is began to appear and form the optic cup in the embryo at (24 hr) incubator . After that appeared in fifth day incubation(lens, retina , optic nerve and corona). However, the iris is not formed yet at that age. However, it is appeared in embryo age at seven the days incubation . Whole embryos were separated for ages 12, 24 and 60 hours incubation for whole mount procedure, while the embryos on the fifth and seventh days were Tissue sectioning was performed at the eye region

Keywords: quail embryo, eye development, optic vesicle, incubation

Introduction:

Chick embryos have been a useful laboratory study model for the past 60 years. But, the attention has been drawn to the quail bird as the best model for laboratory studies on developing embryos over the past two decades. That is because of a number of factors, including the bird's small size, ease of care, and quick maturation (Ainsworth *et al.,*2010). Quail eggs are tiny, measuring around 30 mm length and 10 grams in weight. Also, they are typically incubated for 16.5 to 17 days (Ramteke et al.,2013). The eye primordium is formed in the medial anterior neural plate shortly after gastrulation and contains all the neuro-derived. Also, it is known as the eye field (Wilson & Houart 2004). The optic stalk developing in quail embryos after 24 hours of incubation (Ramteke *et al.*, 2013). While Ainsworth *et al.* (2010) mentioned that the optic vesicles appeared in quail embryos at the age of one day from incubation. The

neural ectoderm, surface ectoderm, and periocular mesenchyme are three embryonic tissue sources that contribute to the development of the mammalian eye. (Uemons,*et al.*,2002 ; Heavner & pevny, 2020).

The optic vesicle emerges as a lateral extension of the neural tube in the forebrain area and extends until it makes contact with the surface ectoderm during the early stages of eye formation. So, the surface ectoderm contacted the optic vesicle thickens into a placode, which will eventually form a lens vesicle. While the optic vesicle invaginated to form the double-layered structure of the optic cup (Uemonsa *et al*.,2002) .

Six neuronal and one glial cell type make up the seven main cell types in the vertebrate retina. From multipotent retinal stem/progenitor cells, these cells are derived.(Doh *et al.*,2010) . When the Retina`s chick embryo is broken or removed, it can be fixed or replaced.(Adams *et al.*, 2008) . Stenkamp (2017) Mention , the seven primary cell types found in the vertebrate retina are arranged into three nuclear layers, which are divided by two synaptic or plexiform layers. Photoreceptor cell bodies are located in the outermost layer, as known the outer nuclear layer (ONL) (rods and cones).

The non-neural tissues of the eye, such as the lens and cornea are produced by interactions between the most distal portion of the optic vesicle and the surrounding periocular mesenchyme.

The iris and ciliary body are produced by interactions between the margins of the optic cup and the periocular mesenchyme (stenkamp,2015). The aim of this study is to track the development and histological change of some parts of the quail eye, especially for embryos at stages of the fifth and seventh days

Materials and methods:

Quial Eggs were collected from one local markets for domestic animal . 25 newly laid eggs were taken, their weights ranged from (10-12) grams. The eggs are sterilized with a cloth dipped in potassium permanganate solution. Then, they are placed in an incubator equipped with an external thermometer and a temperature regulator. In addition, to a bowl at the base of the basin was utilized to put water to maintain the required humidity.

The eggs are incubated at a temperature of (37.5) and a humidity of 60% (Uemonsa et al.,2002) for five, and seven days before the eggs hatched . 5 eggs were extracted after (12, 24, 60) hours of incubation, from embryos were isolated and determined the appearance of the ocular primordia at zero age of the eggs remaining in the incubator .

The eggs are placed on a holder horizontally for 10 minutes on stage, Until the embryo settles in the upper region of the egg . The shell is cut to make a window hole through which drops of Bouin's fixated on the embryo. Then, the edges of the area opica cut with sharp scissors for the purpose of lifting the embryo from the egg. (Zareen & Shahid,2011.(

Prepare a Bouin's solution depending on(Suvarna, et al.,2019). The method of whole mount of the embryos was adopted as mentioned by (Ainsworth et al.,2010) and the stain of Borax - carmine is used for this purpose .

Hematoxylin - Eosin stain was used to staining the tissue sections. Also, the routine work was adopted to prepare the tissue sections, as mentioned (Suvarna, et al.,2019. (

The period of placing for the samples in the fixing solutions was ranged from (4-24) hour's, which was depended on the size of the sample. Then, it was washed with distilled water to remove the fixative residue from the sample. The thickness of the tissue sections was 5 µm, The melting point of paraffin was used is (55 – 60) ˚c (Suvarna, et al.,2019).

Results:

In Fig. 1, At the age (12 hr) incubation the formation of the neural tube is started and the appearanced of the neural groove is clearly visible. There is no indication of the appearance of the beginning of the eye**.**

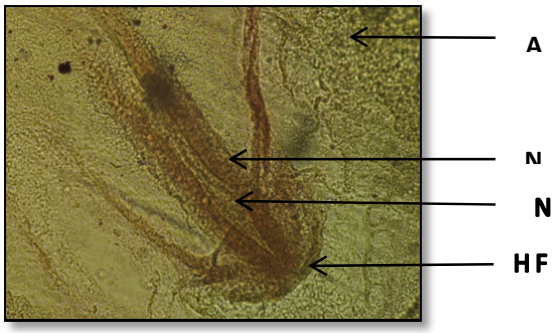

Fig. 1: The embryo of the quail bird at the age of 12 hours incubation shows the beginning of the formation of the neural groove (N G) neural fold (N F) area opeca (A O) head fold (H F) (X 200 stinging by Borax – carmine , whole mount).

The optic vesicle is appeared in the embryo at 24 hours incubation. it is prepared for invagination to form the optic cup . In the embryo 60 hours incubation, the eye is clearly observed in the diencephalon region, but without pigment, as represented in Fig. 2.

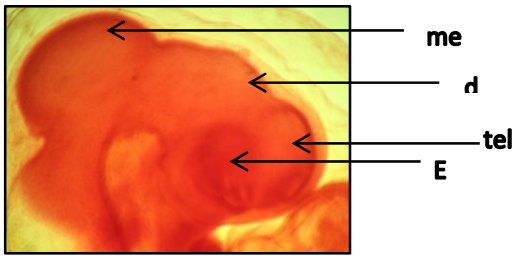

Fig. 2: Embryo at 60 hr incubation E (eye) mesencephalon (mes) diencephalon (di) teloncephalon (tel) (X200 staining by Borax – carmine , whole mount)

In Fig. 3, the lens is appeared and the layers of the retina are formed. In addition to the beginning of the formation of the cornea, the iris has not yet appeared. Then, the optic nerve can be seen clearly , at the age 5 days incubation .

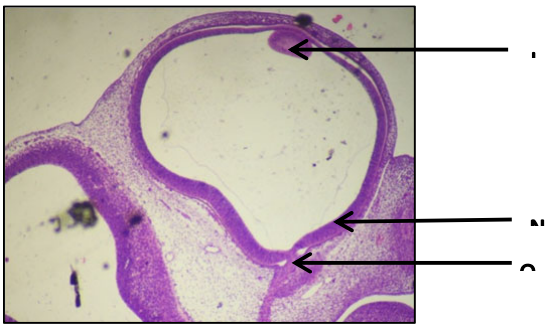

Fig. 3: The embryo of the quail bird at the age of 5 days incubation shows lens (L) , neural retina (N R) , optic nerve (O N). (X 100 staining by Hematoxylin - Eosin , cross section)

Fig. 4 Results shows that it presented the retina undifferentiated layer. The optic nerve is visible in the pictures as well , at (5 days) incubation

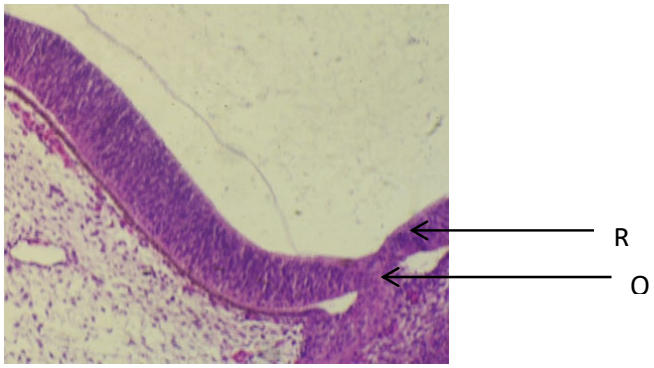

Fig. 4: Embryo at 5 days incubator retina (R) , optic nerve (O N) , cross section X 160**.**

In embryo 7 days incubator showed in the cross section lens more developed , The appearance of the iris in addition to the appearance of the third layers of the retina.

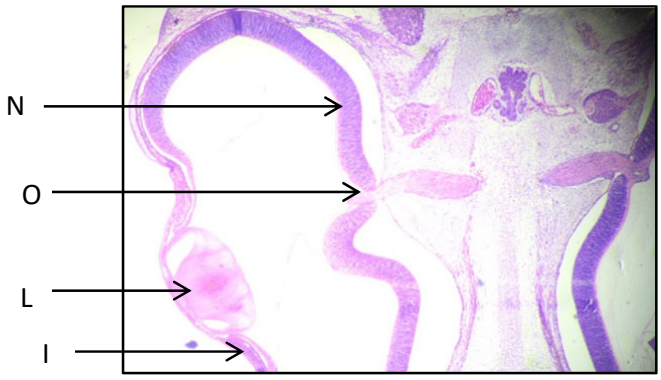

Fig. 5: Embryo at 7 days incubator neural retina (N R) , optic nerve (O N) , lens (L) , iris (I) X 100 cross section staining by Hematoxylin - Eosin

Discussion:

Due to the organism's need on the sense of sight for survival, birds' eyes are better developed than those of the other vertebrates and more effective than those of the other vertebrates. Compared to the size of the skull, the eyeball is enormous (Abd & Almajeed , 2010).

Chicks undergo a series of successive and reproducible changes during development that have been well documented by several embryologists, most notably Malpighi, Lillie, Huxley, and Hamburger and Hamilton . (Adams, *et al* , 2008) . According to the Hamburger-Hamilton approach, it was found that the eye of the quail embryo progresses two stages more growth than the chicken embryo at the same incubation hours (Smith, 2019). This result is agreement with both (Ramteke,2013) . At 12 hours incubation, it was noticed that the optic vesicle was absent from the quail embryo, This matches both (Barratt,*et al.*,2000 and Ramteke,2013). Eyes were clearly visible on both sides of the diencephalon at 60-hours incubation of embryo. While the appearance of this stage of growth in the chicken embryo is on the fourth day of incubation, that is, after 96 hours of incubation(Adams,*el al.,*2008). On the fifth day of growth of the quail fetus, the lens of the eye and the undifferentiated layers of the retina was appeared and the cornea and eye pigment distinct was growed (Ramteke,2013).

On the seventh day of growth, the lens is stained with eosin dye due to the presence of collagen fibers, which are stained with acidic pigments. This result is agreement with (Abd & Almajeed , 2010). The vertebrate retina is contained seven major cell types organized into three layers of cell bodies termed nuclear layers, separated by two synaptic, or plexiform layers.

The outermost layer (outer nuclear layer; ONL) is contained the cell bodies of photoreceptors (rods and cones), which project their inner and outer segments toward the adjacent retinal pigmented epithelium (RPE) (Stenkamp,2015). However, (Abid , 2018) said the retina consist of two layers : nine neural layers are included photoreceptor (rods and cone) and various neurons and pigment layer composed of a single row of cuboidal epithelium.

In this study, the retina of the quail embryo showed that it is consisted of an undifferentiated cell layer at the seventh day of incubation. The retina took a violet color due to the large number of neurons and epithelial cells due to the large number of cell nuclei that took the hematoxylin dye.

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