Pre and Postnatal Histological Changes in Eyelid and Hyaloid Artery in Rabbits (*Oryctolagus cuniculus*)

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ABSTRACT

The purpose of this study was to study the histological changes of eyelids during the eye opining and the regression of hyaloid artery in 20-day old fetus (5,10,15) day old rabbit pups. Paraffin section (5-7) $\mu m.$ of all the eyeball stained with H&E stain and examined with light microscope to follow the histological changes in the eyelids and hyaloid artery in 20-day old fetus and (5,10,15) day old rabbit pups. In 20-day, old fetus the core of the two eyelids was consisted of mesenchymal cells population. The sight of eyelids fusion consisted of mass of irregular pale stained cytoplasm cells with round nucleus. While the junction region covered with one layer of flattened cells of ectoderm from the outer and inner surface. At (5,10) day old pups, there are two depression formed in the outer and inner surfaces of eyelids. The outer surface changed to keratinized stratified epithelia, the conjunctiva surface was thin and non-keratinized, Hair follicles, muscles fibers were noticed at (15) day old pups, 20 days old fetus and at (5-10) day old pups. At the age of 15 days the eyelids were separated completely the previous sight of fusion which covered by keratinized stratified squamous epithelia. The hyaloids artery can be noticed in the intraocular space between the developed retina and the lens, the artery possesses regular lumen full of nucleated red blood cells, the artery was constricted and regressed the endothelial cells showing apoptosis changing, no red blood cells noticed. At the age of 15 days the artery disappeared. The current study revealed that many histological changes occurred within the first three weeks of rabbit's pup's life led to separation of eyelids and the eye reopened. During this period the hyaloids artery regression occurred, at the third week the artery disappeared.

INTRODUCTION

The eyelids formed at the early stage of embryonic development, as two folds from the surface ectoderm and mesoderm core, the upper and lower eyelids continue to grow and fused together (1,2). The time of eyelids reopening is varying in the animal's species, at birth the eyelid is open in human and some animal such as Egyptian water buffalo (3). While in mice the eyelids are open at 12 days after birth, (4) other animal like blind mol lives with closed eyelid along it is life (5). The eyelids cover the eyeball to give mechanical and physiological protection to the delicate internal structure of the eye, the eyelid consist of many layers the outer most the skin, subcutaneous, muscles and conjunctiva. The eyelids are occupied by the, hair follicle, glands, and blood vessels, (6,7). The hyaloid artery is a branch of primitive dorsal ophthalmic artery which enters the optic stalk during the eye development. The hyaloid artery gives blood supply to the lens in the growing fetus. The hyaloid artery is one of the ophthalmic artery branches, the ophthalmic artery is originated from the internal carotid artery. The hyaloid artery passes through the vitreous humour via the optic canal and extends to reach the lens. It is most prominent during fetal live and slowly regresses later. (7,8) The regression or atrophy of the vessels begins in the posterior region (vasa hyloidea propria) and gradually progresses toward the anterior part (tunica vasculosa lentis). (8) The hyaloid vasculature is a complex of transient intraocular vessels (9). The persistent of this artery caused many sight problems (10). The present study aimed to follow the histological events of eyelids opining and the changes in hayloid artery during the evelids opining.

Keywords: Development, rabbit, eyelids, lens, hyaloid

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MATERIALS AND METHODS

This study was carried out on 20 rabbits which divided into four groups (20 days old fetus) and (5-, 10- and 15days old pups) five rabbit for each group. The maternal history with reference to the duration of pregnancy, delivery day of pups and the crown-rump length were used for determined the age of fetuses and pups. The eyeball was fixed with 10% neutral buffered formalin. For histological technique the eyes spacemen were processed with paraffin embedding, the tissues were sectioned at 5-7 μ . and stained with H&E stain (25).

RESULTS AND DISCUSSION

The Eyelids

At 20 days old fetus the core of the two eyelids was consisted of mesenchymal cells population with blood spaces filled with RBC. The sight of eyelids fusion was consisted of a mass of pale stained cytoplasm and round nucleus irregular shaped cells. The area of fusion covered from the outer and inner surface of the eyelid with one layer of flattened cells of surface ectoderm, these two types of cells were originated from basal cell layer which consisted of columnar basal cells. In this age the hair follicles primordial can be noticed into outer surface of the eyelid, glands and muscle fibers were absent (Fig. 1,2). This result was in agreement with the results of (4,11, 12 and 13) in mice and the result of (3) in buffalo embryo and the result of (14) in human in all this animal, with regard to the differences in the period of embryo development, the eyelids had core of mesenchymal cells, the fusing area occupied by polygonal cells the outer and inner surface coated with one layer of flat cells, that represent the future surface epidermal cells. At (5) day old pups, there are two depression appeared on the outer and inner(conjuntival)surface of the eyelids. The outer surface changed to keratinized stratified squamous epithelium, while the conjunctiva surface was thin and non-keratinized stratified squamous epithelium, the keratin invades the fusing sight. Hair follicles, muscles fibers were noticed within the connective tissue (fig.3). At (10) day old pups, the keratin was thick seen in the outer depression, the hair follicles were numerous, it characterized by present of hair shaft. The muscles fiber was increased, Sebaceous gland glands were noticed within the connective tissue of the conjunctiva (Fig.4). At (15) day of pups age the eyelids were separated completely the previous sight of fusion was covered by keratinized stratified squamous epithelium and continues with the outer surface of the eyelid. The conjunctiva surface was coated with non-keratinized stratified squamous epithelia. sebaceous and tarsal gland were developed (Fig.5). The histological changes and the sequence of events on eyelids, that occurred during the two weeks of postnatal life in rabbits' pups, which leads to complete separation of the eyelids, were incompatible with that mention by (4, 13) they noticed that the differentiation and keratinization of cells in the fusion region between eyelids lead to separation. The same observation was recorded by (3) in his study on the eyelids of buffalo. The current study suggests that eyelids closer during the first week of rabbit pups age controlled the effect of light on the eye development and give enough time for the eye internal structures to complete its development and controlled by gene expression. This fact was agreed with what was recorded by (15,16) on their study, they noticed that the development of the eye internal structures in rodents' effect by light exposure. The hyaloid artery : At 20 days old fetus, the hyaloids artery can be noticed in the intraocular space between the developed retina and the lens and passed through the vitreous space, the artery was developed wall with regular lumen full with red blood cells , some cells were nucleated (Fig.6).This finding agree with what was mentioned by (9 and 17) they found that the hyaloids and retinal arteries formed by angiogenesis during early embryonic stage, It was extended through the vitreous space of the eye ball to the caudal border of the lens. At (5) day pups the result showed that, the artery was constricted, the lumen was absent, no red blood cells noticed, and the artery undergone regression, the endothelial cells were apoptotic (Fig.7). The present of hyaloids artery during the first week after birth was reported by (18) in rat. The histological changes during the artery regression were as reported by (17 and 19) They mentioned that the hyaloid artery suffered regression and complete atrophy, the loss of function as result of marked decreased in blood flow and apoptosis in endothelial cells and pericytes. current study suggests that pattern of evolution and involution of hayloid artery is critical developmental process. This suggestion was in parallel with the results of (20) They found that the hyaloids artery had very important role in growth and differentiation of lens and retina during eyelids closer. At the (10-15) day old pups, the artery was not noticed. This result proved the finding of (21 and 22) the found that the involution first affects the proper hyaloids artery branches, and the artery disappear in the postnatal period. The present results were different than the results of (23) They stated that the hyaloid artery was present in embryo and adult gerbil's eye. The latter regression and atrophy of the artery with great effect on light pathway, it controlled by the effect of some genes as mention by (8) and cause by normal cell death(apoptosis) during development as mentioned by (24).



Figure (1) Histological section of fetus eye (20) day showing: A. eyelids, B.Cornea, C.Lens, area of fusion (Arrows)(H&E 4x).



Figure (2) Histological section of fetus eyelids (20) day showing: A. Area of fusion B. Basal columnar cells.C, hair follicle primordial (Yellow arrows) blood spaces. (White arrows) mesenchymal cells. (Red arrows) Outer flattened cells. (H&E stain40 x).



Figure (4) Histological section of eyelids showing: In-internal depression, o- outer depression, Mmuscle, h-hair follicles,G-glans(H&E stain x40)



Figure (4) Histological section of eyelids showing: In-internal depression, o- outer depression, Mmuscle, h-hair follicles,G-glans(H&E stain 40x)



Histological section of eyelids showing: epi-epidermes, co- conjunctive surface,s-sight of sepration M- muscle, h-hair follicles(H&E stain x 40)



Figure (6) Histological section of eye ball, (20) day fetus showing; R-retina,L-lens,h- hyaloids artery,R-RBC(H&E stain x 400)



Figure (7) Histological section of eyeball, (5) day pup showing; raregressed artery, ac- apoptotic cells (H&E stain x 400)

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