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Morphological and histological study of eye development in embryos and larvae of common carp fish *Cyprinus carpio*(L.1758).

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Abstract

Growth and development of the eye in common carp fish were studied by using a several samples of the eggs, larvae and juveniles during artificial fertilization in the marine science center hatchery of fishes. Temperature of the Water in the incubators was 26C and the incubation of eggs was 38 hr. The study included the morphological and histological of the formation of the eye, optic primordial appeared after 12 of fertilization in a form evagination from both sides of the forebrain and slightly oval elongated and then characterized optic vesicle, then lens was appeared , it was spherical shape, histological the optic cup characterized at 24 and 28 hours of incubation is spherical and regular with the advent of the cornea lens and iris in addition to the room of the organization of the eye and the vitreous chamber. the retina was formed at the age of 24 hours of incubation from ganglion cell layer (Gcl)inner nuclear layer(Inl).And then followed by the other layers that fit their appearance with the age of the embryo, completed growth and the emergence of layers of the retina at the age of almost 30 days after hatching.

Introduction

Common carp fish (Cyprinus carpio) was returned to the family Cyprinidae, which have spread over the wide extends from the shallow lakes, slow runoff rivers and brackish water and because of higher adaptation to different environments that has able of this species from living in a deep lakes and rapid run off rivers which characterized by the presence of gravel and sands in layers (Mohamed *et al.*,2006),And it is one of the main types of freshwater fishes in Turkey, as there is in East Asia, in addition to Iraq(Kohlmann *et al*.,2003; Coad, 2010).it is a bear the high temperatures to more than 35ºC and a low level of dissolved oxygen up to 3 mg / L (Coad, 2010).

The eye is a visually member that has complex composition different degree of development in others vertebrates and because the importance of this member which has taken a large part of the study (Muller, 1975; Lamb *et al*.,2007 and Neuhauss, 2010). It was that a great interest in the study of embryonic configuration of the eye in the last decade,Bejarano-Escobar *et al*.,2010 has described the evolution of the eye and the retina in Senegalese sole fish (*Soleasenegalensis)* .It was incubated at 19.5ºC , as pointed to the most of a major events that contribute to the formation of the eye and the retina that occurs early and after hatching leading to the formation of layers of optic cup, while the Al-Mosawai (2008) has pointed that the eye was appeared in embryos Barbus sharpeyi fish after 22 hours of fertilization as a form ofevagination from the sides of the beginning of the forebrain and of which later grow to the eye. EyesConsists in vertebrates as a resultsof the interaction of tissue between the surface nervous ectoderm neuroectoderm which includes a neural crest with the mesoderm, and the cells of neural crest as clusters of specialized cells from a mesenchyma, which migrate from the edge of dorsal nerve folds which coincides with the lock of the neural tube (Ozeki&Shriai, 1998).Neural crest cells divide into two groups, a nervous cranial neural crest, which extends from the center of the diencephalon to the end of the fifthsomite, while trunk neural crest which extends from the fifthsomite to the caudal end of the embryo (Sinning, 1998). The primordium of the eyes appeares as a form of evagination from both sides of the forebrain and appearance period varies depending on the fish species (ALMosawai, 2008).Then take this evagination in the form of a hollow optic vesicle in the beginning but later gaining bores as in some final ostiechthyes (Balinsky, 1981).Optic vesicle grows on both sides of the forebrain until each and every one of them close to the outside endoderm, accompanied by elongation in the contact area of the brain after narrowing made up the so-called optical stalk, then lock invagination occurs in the wall near the vesicle to the ectoderm leading to the formation of a dilayer configuration know as the optic cup is associated with a thickening in the ectoderm to formation of the lens in the end, as it grows in parallel with the lens encapsulate the retina and form the optic cup (Lamb *et al.*, 2008 and Al-Mosawai, 2008). This study describes the embryonic development of the eye for common carp fish in artificial propagation process and its goal is to see the emergence of members mechanism during embryonic development and the statement of the effect of temperature and the amount of oxygen in the water.

Materials and methods

Samples of embryos, larvae and young were obtained from the process of Artificial fertilization and carried out in the marine science center hatchery of fishes. Embryos was collected after 11 hours of incubation in

the blastopore closure stage and at a rate of 6-8 samples per hr then increased period every two hours until hatching, then the samples of the larvae is take of the first, second and third days, respectively, to the tenth day and at the same rate then increased the interval gradually after the tenth day. Samples fixed by using 10% formalin and recorded according to the method of Bancrottand& Stevens (1982), for a period ranging from 8-48 hours, and worked histological section of larvae ,young and eggs. Samples were washed by using tape water for several hours to remove the excess of the fixative, then its passed after washing with series progressive ethanol for two hours for each concentration, sample is Clearing by using 1:1 (xylene - alcohol) for 20 minutes and pure xylene for half an hour, it infiltration with using Paraffin Wax with melting 56.6 ^{o}C, as put in the oven at a temperature 60^{o}C for two hours and replace the melted wax twice, for an hour each then left the samples overnight in the oven. the sample was embedded at the same type of wax that used in the infiltration , samples is cutting by use the Rotary Microtome with thickness of 5-7 microns , Then transferred to a water bath at 45 C for the purpose of brushes a sections, it was picked up by using a slide coated with Myers albumin and transferred to a hot plate type Fisher slide warmer with a temperature 35C, stained with Cole's Haematoxlen –Eosin stain , mounted with use a D.P.X, Examined the microscopic sections by using an optical compound microscope type (Olympus, japan) and picked up images of eggs samples and tissue sections by optical imaging microscope type Kruss (German)and provide with a digital camera type HDCE-50B .

Results

Table 1 shows the results of the process of artificial fertilization for common carp fish that used in the experiment, the water temperature of incubators was 26 ^{o}C, oxygen 8.5 mg / L , the rate of eggs weight that laying by the Fish 1 kg, the period of incubation 38-hour and the proportion of hatching was 85% .

Table (1) result from the process of artificial fertilization for common carp fish.

Type of fish	No. of Female	No. of male	Egg Weight	proportion of hatching	Temp. ^{o}C	PH	O_2 mg/L
Common carp	` 10	6	1 k	85%	26	8	8

The results of the current study was Included the morphological and histology of the evolution of eye in the embryos and larvae of common carp fishes, the first sign of the emergence of visual optic primordium stage that appeared after blastopore closure and appearance the somite after passage of 11 hrs from incubation plate 1(a). After 12 hours of incubation the visual optic primordium stage was came and characterized by the appearance of optical primordium that were slightly elongated oval-shaped with a high incision in the lower edge of the prefixes and the embyo

occupies about one-fifth to one-sixth of yolk plate1(b). while in optic vesicle stage plate 1(c-d), which Characterized by an increase in size visual primordium with prolonged larger than the previous stage and the embryo became surrounded by most of the yolk with the emergence of notochord. .Then, observed the appearance of olfactory placode and caudal bud stage, As characterized by the emergence of olfactory highest optic visual but it is difficult to clearly distinguish with the continued growth of optic vesicle and increase the number of somite with the appearance tail clearly plate1(e). in otic capsule stage was after 18 hours of incubation characterized continually the growth optic vesicle and converts them into Oval shape and differentiation of the brain larger with the clarity of tail that containing on the caudal vesicle and an increase in the number of somite plate1(f). After 20 hours of incubation was observed the appearance caudal fin stage with elongated the embryo and the eye becomes a circular motion and the yolk sac takes the form of kidney shape plate2(a). Followed by the stage of lens formation, as characterized by the lens as having spherical or oval shape and appearance of muscle effects on the embryo at this stage and the later stages since the start of embryo movement and gradually with the disappearance of caudal vesicle and clarity of the yolk sac plate 2(b-c). After that noted the embryo and increase the size of the head and eye became clarity and increased in movement of embryo with the appearance of heart and the caudal fin become more in length and embryo movement automatically plate2(d-f). After this stage, the embryo becomes a straight up while the head is still curved around the front yolk sac with movement of embryo, heart starts pumping and contraction and embryo circulation continuously then it becomes a thin membrane of the egg and rupture the wall of the it to come out and be free swimming larva plate3(a-f). eye tissue at age 24 and 26 hours of incubation was appeared it consists of the optical cup which have a spherical shape and regularly either cornea is a connective tissue is a continuation of a layer a Sclera that surround eye ball and be transparent covering the front of the eye in front of lens and the iris, the lens is small oval or spherical and cells long thickened that surrounded from outside by a simple cubic cells, , iris is Consisting of two parts that touches the lens from outside and there is a water chamber called the opening organizer of eye, which is located in front of the lens and the iris, which are small at this age, as well as vitreous mixing Chamber, which is located behind the glass lens. Retina at this age consists of two layers arranged from inside to outside, the ganglion cell layer, which are narrow and inner nuclear layer in addition to retinal pigment epithelium layer of the retina plate 4(a-d). Increase the growth of the optic cup at the age of one day after hatching with differentiation anew layers of the retina, Noting differentiation a ganglion cell layer, inner plexiform layer ,inner nuclear layer which becomes more systematic and retinal pigment epithelium plate 5 (a-b). at the age of 4 days after hatching noted increase in eyes size with differentiation the layers of retina, plate 5 (c-d). at the age of 5 days after hatching note expansion of a ganglion cell layer and axes, inner nuclear layer which is a regular thick layer of cells with a few differentiation inner plexiform layer with appear the outer nuclear layer plate 6(a-b). While at the age of 16,22 days after hatching observed increase in size of the eye, which are spherical shape with a differentiation a most layers that consisting of the optic cup 6(c-d).at the age of 30 days after hatching which Observed complete differentiation of optic cup of retina plate 7(a-c).

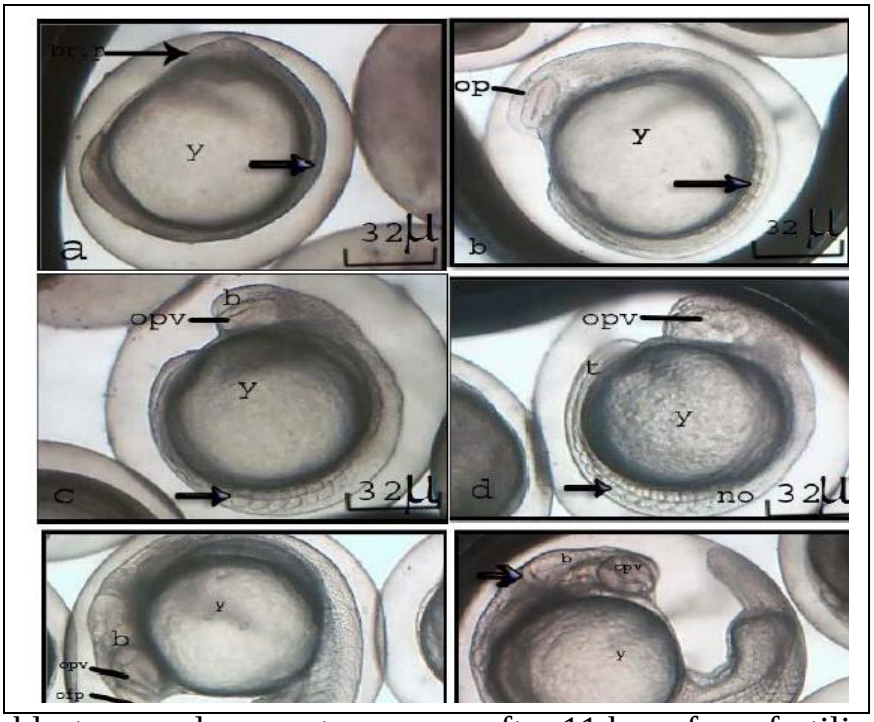

Plate 1: (a) blastopore closure stage occur after 11 hour from fertilization, (b) optic primordium stage after 12 hrs from fertilization,(c-d) optic vesicle stage . Optic primordium (op), yolk(y), (⟶) somite, optic vesicle(opv), tail(t) and brain (b). (e). Olfactory placode and tail bud stage , (f) otic capsule stage after 18 hrs from fertilization , (opv) optic vesicle, (ofp) Olfactory placode , (b) brain , (y) yolk, (t) tail, (⟶) somite, (no) notocord. x4

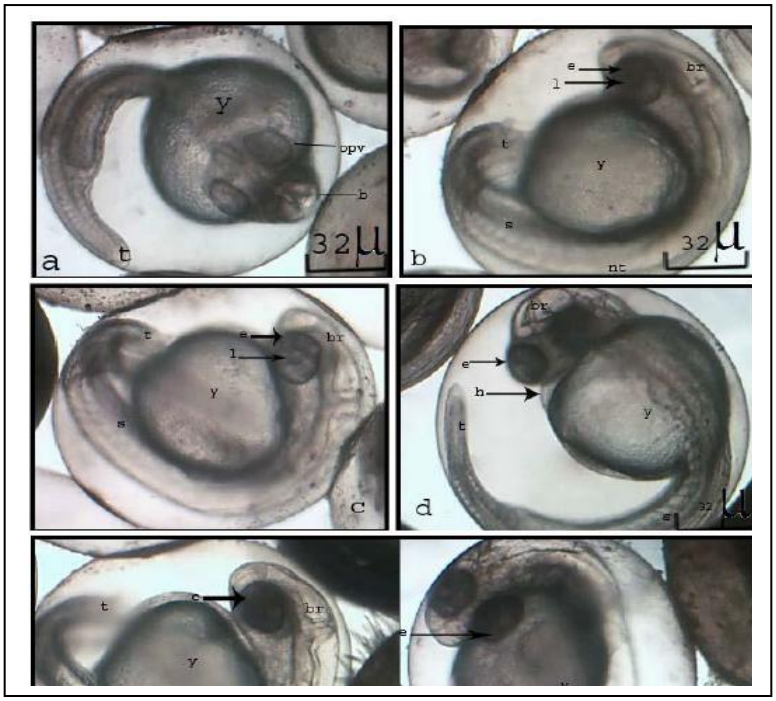

Plate2: (a-f) caudal fin stage & lens formation , (e) eyes , (I) lens , (br) brain, (t) tail . (s) somite, (y) yolk , (nt) notocord , (opv) optic vesicle , (h) heart . x4

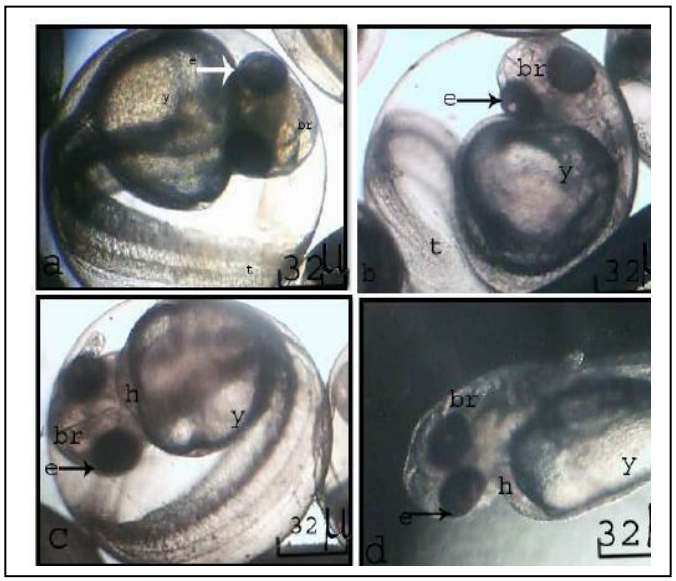

Plate 3:(a-b) heart formation stage , (c-d) hatching stage, (e) eye, (br) brain, (y) yolk , (t) tail , (h), heart , x4

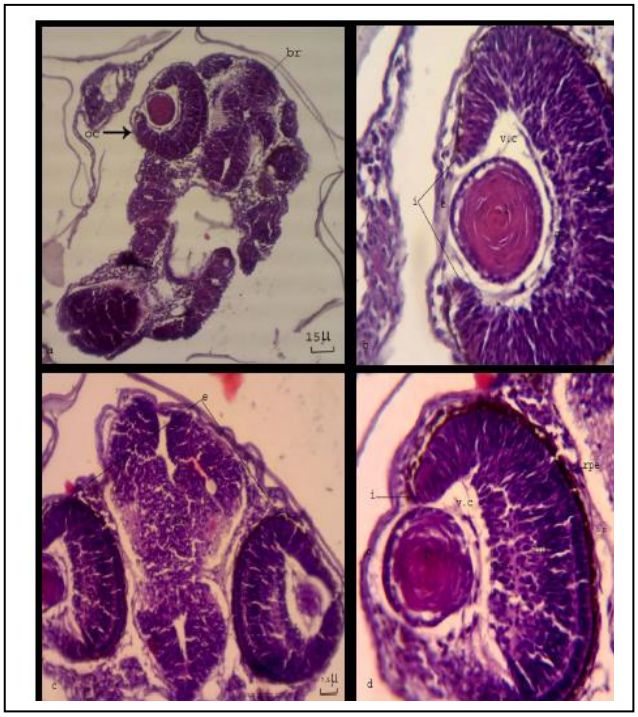

Plate 4: longitudinal section of eye in egg embryo (a-b) embryo at 24 hour after fertilization shows (oc) optic cup, (br) brain, (i) iris, (l) lens , (v.c) vitreous chamber , (gcl) ganglion cell layer , (inl) inner nuclear layer , (rpe) retinal pigmented epithelium ,(c.m) choroidal melanocyte , (c-d) embryo at 26 hour after fertilization.

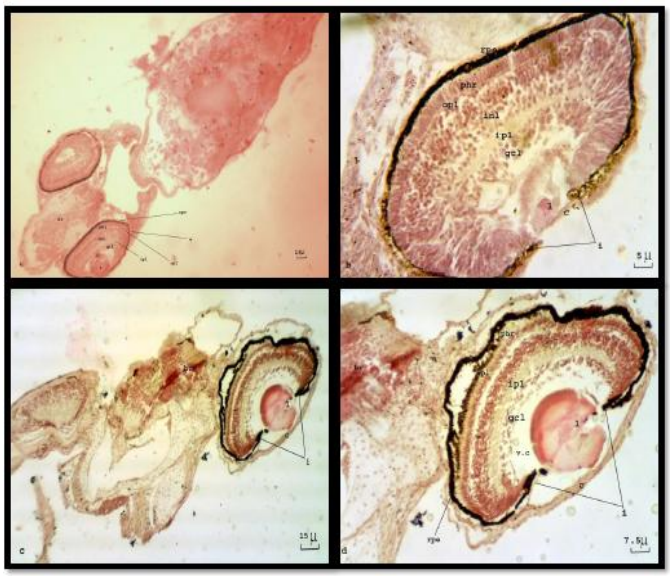

Plate 5: saggital section of carp larvae , (a-b) after 1 day from hatching and (c-d) larvae after four day from hatching shows increase in the layers size and appear new layers, (oc) optic cup, (br) brain, (i) iris, (l) lens , (v.c) vitreous chamber , (gcl) ganglion cell layer , (inl) inner nuclear layer , (rpe) retinal pigmented epithelium.

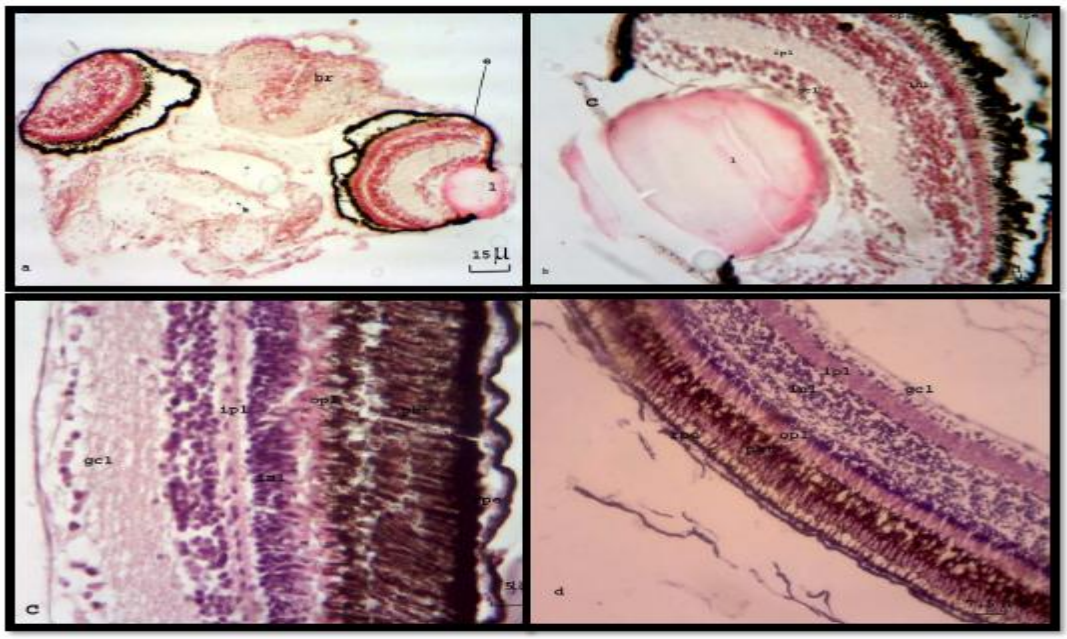

Table 6:cross section in head of carp larvae, (a-b) after 5days from hatching, (c) larvae after 16 days from hatching of retina and(d) larvae after 22dayes of retina . shows (br) brain, (i) iris, (l) lens , (gcl) ganglion cell layer , (ipl) inner plexiform layer , (inl) inner nuclear layer , (ool) outer plexiform layer,(phl) photoreceptor layer , (rpe) retinal pigmented epithelium.

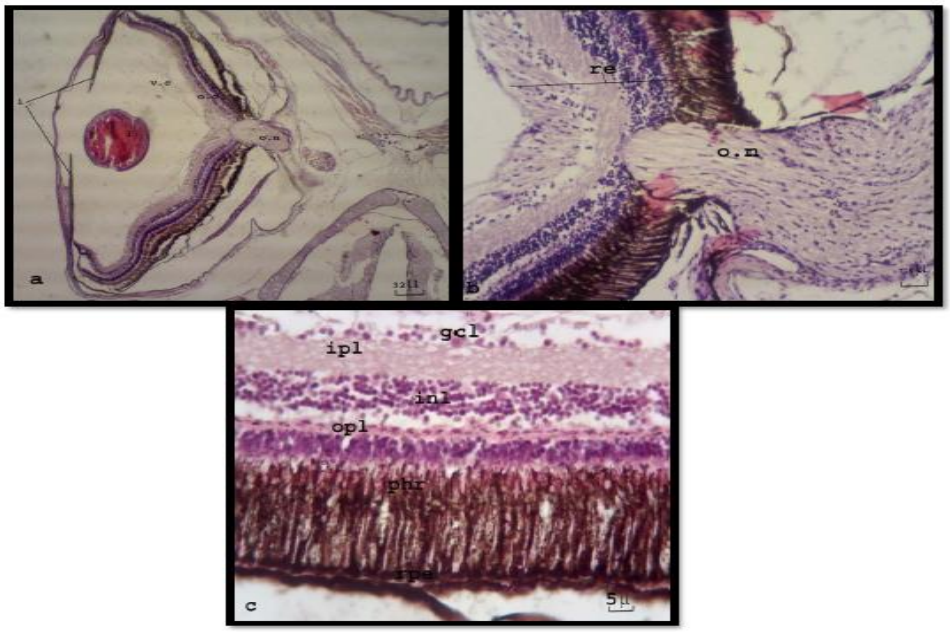

Plate7:cross section in eye of carp larvae (a-c) after 30 days from hatching shows (oc) optic cup, (br) brain, (i) iris, (l) lens , (v.c) vitreous chamber,(o.n) optic nerve, (gcl) ganglion cell layer , (ipl) inner plexiform layer , (inl) inner nuclear layer , (ool) outer plexiform layer,(phl) photoreceptor layer , (rpe) retinal pigmented epithelium..

Discussion

The results of the current study appear the formation of the eye in the common carp fish that incubated at 26º C, the first sign of the emergence of optic primordium was after blast pore closure with the appear the somites after passsage 12 hours of incubation, while Chapman (2006) noted that the period of the emergence of optic primordium was at a different times when he studied initial fetal growth of four types of carp in China. As it appeared those prefixes in grass carp fish after 14 hours of fertilization, while it was in black carp and silver carp fishes after 16 hours , while in the big head carp fish after 15 hours of fertilization and all were incubated at a temperature ranging between 18-24º C. Jafari *et al*., (2010) pointed when he studied the embryonic development of Caspian kutum fish and which incubated at a temperature between 14 – 16 ºC , the eye primordium was appeared after 60 hours from incubation. Al-Mosawai (2008) said that the time for the emergence of eye primordiun was vary depending on the species of fishes that appears in embryo *Barbus sharpeyi* fish at age of 22 hours of incubation ,while showing in *Barbus grypus* embryo after 22 hours from incubation (Salman, 1999). While saleh *et al*.,(2011) that the optical prefixes appear in common carp fish which were incubated at 23º C after 30 hours of incubation at a two ball form in front of the head. The reason of difference is attributed to the multiplicity of fish species in addition to the effect of water incubators temperature, as the temperature and oxygen is one of the affecting valuable environmental factors in fetal growth of fishes, as the rapid development of the fish embryos caused by appropriate availability of environmental conditions as well as the qualities of good life to adults that bearing the eggs (Kange & Kishimoto, 2002; Nakagawa *et al*., 2002). And low temperature of water incubators delay the development of embryos and has effects on

the percentage of survival, more ever lead to the numerous distortions resulting in embryos (Rechulicz, 2001; Arenzon *et al*., 2002 and Ojanguren & Brana, 2003). Eyes primordial appear in the form of evagination then take this to form optic vesicle and this is consistent with what was said by Al-Mosawai, (2008) and Bejarano-Escobar *et al*., (2010), they indicated the appearance of visual primordium as a form of evagination which quickly increases in growth and be form optic vesicle which is getting growth on both sides of the forebrain and become close to the surface epithelium is associated with elongation in the contact area of brain composed optic stalk then the cup was formed. The current study showed the differentiation of layers of the retina of eye in embryos of common carp fish which aged 24 hours after fertilization and continued to differentiate until almost complete in age 30 days after hatching, and many researchers have found that there is a wide variation in times of appearance of layers of the eye retina and differentiation in different fish. Gruan (1975) observed that the cells of the inner wall of optic cup in tilapia fish (*Tilapia leucostica*) that appear on the third day to the sixth day after hatching, while Lagler (1956) notied that the layers of retina in the salmon fish ,which lives in cold water appeared after 80 days of fertilization. While the layers of retina in *Barbus sharpeyi* fish appear at aged 72-94 h (Salmen, 1999). that is explained because of different types of fishes as well as the impact of environmental conditions on embryonic growth. stated Fishelson (1968) explained the difference in rate temperature from 2 – 3 ºC leads to a difference of 25% -35% in the rate of formation members during embryonic development. Mallya (2007) observed that the ratio of saturated oxygen in the water gives a good effects on the growth and development of embryos and this corresponds to the current study, the ratio of dissolved oxygen rate was 8 mg / L, while the pH was 8. Oyen *et al*., (1991) has pointed that the pH has a grainy effects on embryonic growth of common carp fish. This study results showed the rate of pH between 4.5-5.5 effects is not good on embryonic growth coincided with the emergence of several distortions in developing embryos, While the rate was between 5.5 to 5.7 the effects was less. Results of the current study showed that the rate of hatching was 85% and the incubation of eggs was 38 hr.

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دراسة نسيجية ومظهرية لتطور العين في أجنة ويرقات اسماك الكارب الشائع

Cyprinus carpio(L.1758)

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المستخلص

درس نمو وتطور العين في اسماك الكارب الشائع باستخدام عدة نماذج من البيض واليرقات واليافعات أثناء التلقيح الاصطناعي في مفقس اسماك مركز علوم البحار وبدرجة حضن بلغت 26 م°، استغرقت فترة الحضن 38 ساعة. شملت الدراسة الجانب المظهري والنسيجي لتطور العين، ظهرت البادئات البصرية بعد مرور12 من الحضن على شكل اندلاق evagination من جانبي الدماغ الأمامي وبشكل بيضاوي متطاولة قليلاً ثم تميزت الحوصلة البصرية بعدها ظهرت العدسة التي كانت كروية الشكل، نسيجياً تميز الكأس البصري عند عمري 24 و28 ساعة من الحضن بشكل كروي ومنتظم مع ظهور القرنية والعدسة والقزحية إضافة إلى الغرفة المنظمة للعين وغرفة الخلط الزجاجي، تكونت شبكية العين عند عمر 24 ساعة من الحضن من الطبقة العقدية (Gcl) والطبقة النووية الداخلية(Inl) ثم تلتها الطبقات الأخرى والتي تناسب ظهورها مع عمر الجنين، اكتمل نمو وظهور طبقات شبكية العين تقريباً عند عمر 30 يوم بعد الفقس