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# The Embryonic Development Of The Heart In Sailfin Molly Fish (Poecelia Latipinn)

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***Abstract***
*This included the study of embryonic development of the heart in different lengths of embryos of Sailfin molly (Poecelia latipinna) fish, which is one of the invasive fish species in Iraq and is spread acrosswater bodies, especially in the marshes that are located in the south of Iraq. The fish samples were collected from the AGhazl market in Baghdad province, and dissected to remove the ovary that containing embryos at different lengths using fine forceps. All the samples were fixed using formalin after making a hole in the gas sac, especially in advanced embryos stage. In a 3 mm embryo, the heart was completely formed and the blood vessels and optic cup were clear, while the lens of the eye was beginning to form. In 4-5 mm embryo, it was found that the formation of the atrium and ventricle was developed clearly, in addition to that the appearance of the bulbus arteriosus were detected. Furthermore, In a 7-6 mm embryo, several changes were revealed including that the atrium, ventricle, blood clots containing hematopoietic cells and blood cells are more clearer than in the previous stage. In a 9 mm embryo in length (birth stage), the heart and its parts were clarified than in the previous stage. In a 10-11 mm embryo (larval stage), it was noticed the completion of the heart development, clarity of the atrium, ventricle, and bulbus arteriosus, as well as development of the epicardium formation, while the myocardium muscles were very clear.*
***Keywords***:*molly fish , Sailfin, embryonic development, Atrium, Ventricle.*

## INTRODUCTION

The circulatory system in fish is a simple system compared to a number of living organisms, and consists of the heart muscle, blood vessels, and blood (Molnar and Gair, 2022). The heart is an important organ in fish; it consists of two halls or chambers (a thick muscular ventricle and a membranous atrium). In addition, the heart is surrounded by a thin membrane and consists of venous sinuses and a tube called the bulbus arteriosus. Fishes have an arterial and venous circulatory system, and the blood contains white and red blood cells with a nuclei and hemoglobin, which gives them their red color. Moreover, the blood circulation is usually a single circulation, meaning that the blood passes through the heart once in each circulation (Monahan-Earley et al., 2013; Barresi and Gilbert, 2017).

The study of embryonic heart development in teleost fishes offers valuable insights into vertebrate organogenesis, particularly the early formation and function of the cardiovascular system (Bakkers, 2011). Poecilia latipinna, commonly known as the sailfin molly, is a live-bearing freshwater fish frequently used in developmental biology due to its relatively short gestation period and transparent embryos (Tiedemann et al., 2024). This fish lives in fresh and salt water in different parts of the world including North Carolina, Texas, and the Yucatan Peninsula of Mexico (De Magalhaes and Jacobi, 2010). Furthermore, sailfin molly fish prefers to live in swamps, low river waterways and estuaries, whereas it is common in the Florida peninsula, New Zealand, the west of United States and Hawaii (Rypel, 2005).

As one of the first organs to differentiate and function, the heart plays a crucial role in supplying nutrients and oxygen necessary for the proper growth of embryonic tissues (Tan and Lewandowski, 2020). Investigating heart development in P.latipinna not only enhances our understanding of normal morphogenesis but also provides a comparative model for studying congenital heart defects, environmental toxicity, and evolutionary biology (Ullah et al., 2024).

The development of the heart, like that of other organs, requires the identification of progenitor cell populations that will eventually form the differentiated cell types for the functional organ (Scott, 2012). Among the recent and exciting developments in cardiac research is the identification of cells that have the ability to form the main types of heart cells (cardiovascular progenitor cells, cardiomyocytes, smooth muscle, endothelium, and pericardium) (Barreto et al., 2019). The fate of cardiovascular progenitor cells is determined during the formation of the heart, and it was observes that the final shape of the heart, especially the ventricle chamber, varies significantly among species based on general physiological traits related to swimming ability (Li et al., 2024). Species that do not swim continuously, such as flatfish and small fish, have hearts with bulbous ventricles without coronary circulation, while fast and continuously swimming species, like salmon, have hearts with muscular conical ventricles and coronary vessels (Anokye-Danso et al., 2011).

The stages of heart development include the following: Formation of the heart tube: The heart tube is formed from mesoderm cells and begins to contract irregularly. It forms from precursor cells located in the lateral anterior layer of the embryo, where these cells gather to form a heart disc, which then transforms into a linear heart tube. Cardiac bending; bending occurs in the heart tube, causing it to take on an S-shape, which defines the locations of the atria and ventricles. Over time, the heart tube begins to twist and shape itself to become a two-chambered heart, with the atria at the front and the ventricles at the back, and this process continues after fertilization. Formation of heart chambers; the heart tube differentiates into specific chambers including the atrium and the bulbus arteriosus. Finally, beginning of cardiac pulsation; the heart begins to beat regularly, contributing to the distribution of oxygenated blood throughout the fish (Stainier et al., 1993; Incardona and Scholz, 2016).

The hearts of P.latipinna fish are characterized by their high regenerative ability, as the heart cells remain capable of division and reproduction throughout the fish's life. Studies show that heart cells in guppies express proteins such as SOX9, Myostatin, NF-kB, and S100, indicating their capacity for regeneration and response to injuries. They also contain immune cells known as Rodlet cells that express proteins like S100 and NF-kB, suggesting their role in the immune response within the heart (Zhang et al., 2001). The importance of P.latipinna fish lies in studying heart development in vertebrates due to their transparent bodies, which facilitate monitoring heart development, and also because the studies contribute to understanding the mechanisms of heart development and cardiac regeneration, benefiting the evolution of treatments for heart diseases in humans (Farrell, 2023).

This aim of the present study is to identify the morphological and histological stages of heart at the embryonic development stages in sailfin molly fish (Poecilia latipinus) different stages and in different lengths of embryo, as the studied fish is an invasive fish in Iraqi rivers.

## MATERIALS AND METHODS

The sailfin molly fish is an invasive fish in Iraq, The fish is characterized by being hermaphrodite, and the males having a longer dorsal fin than females, resembling a sail, while females have a smaller fin but a larger body than males.

**Scientific classification**

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cyprinodotifrmes
Family: Poecilidae
Genus: Poecilia
Species: Latipinn
Binominal name: Poecilia latipinna (Brands, 2018).

After collecting samples (n=12), the fish placed in the tanks and their behavior was monitored, then the antenatal fish was isolated and dissected. The abdomen of the pregnant fish was opened using forceps and saline solution was added. The embryos at different stages 2-13 mm were isolated and placed for several hours in a 10% diluted formalin solution while the organs in 70%( Monaco 1983); then each stage of the embryo was photographed by measuring their different lengths using a ruler under the petri dish in which the embryo was placed.

After dissecting the fish using the Billet and Wild (1975) method and removing the yolk from the embryo, all images are taken using a compound microscope adapted with a camera. After that, the stages of preparing the sections for histological study were carried out to prepare a ribbon of serial sections of samples, and after preparing of mounted slides in the laboratory, the stages of heart formation were photographed using a compound microscope adapted with a camera.

## RESULTS AND DISCUSSION

This study was relied on the lengths of the embryos in addition to the morphological and histological characteristics of the fish.

1- **Embryo at 3 mm in length (Heart formation stage)**

As shown in Figure (1), it was noticed the completion of the heart formation and the blood vessels were developed. Furthermore, it was detected the formation and clarity of the optic cup of embryo; the blood is distributed to all parts of the embryo's body, especially pronephric kidney, which continues to contain blood coming from the dorsal aorta. In contrast, this study revealed the increasing the optic cup growth, beginning of the lens growth, the clarification of eyeball, the pigment cells was with small quantities in the head area, and the beginning of the appearance of the two pectoral fins.
In this study, the formation of the heart appears in a 3 mm embryo in length (Fig. 2). This is not consistent with Drummond and Davidson (2010) study, as it was proven that hematopoietic stem cells appear in the embryo with 4 mm in length. However, this result is in agreement with study of Al-Rawi et al. (2004) on the mosquito fish, which is an oviparous fish, as the heart appeared in a 3 mm embryo in length.

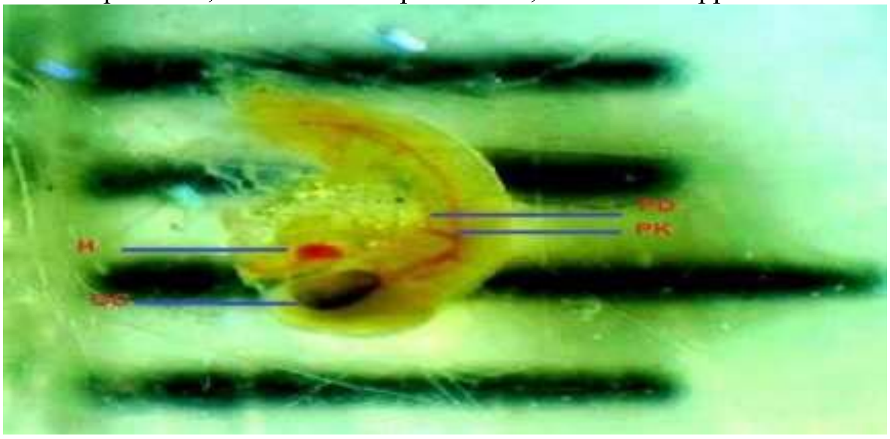


**Figure (1):** The embryo with 3 mm in length. It is notice the formation of the heart (H), Pronephric Kidney (PK), optic cup (OC), and the primary renal duct (PD) (14).

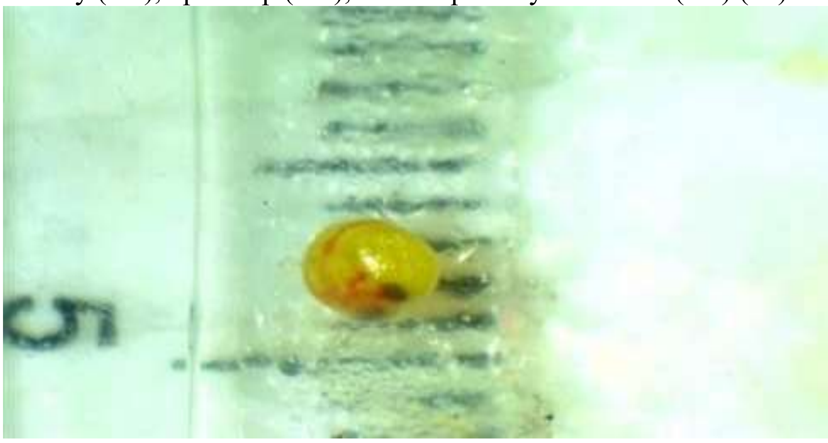

**Figure (2):** The embryo attached to the yolk.

**2- Embryo at 4-5 mm in length**

In this stage of embryo, it was observed from the external morphological features that the formation of heart and optic cup were much clearer than in the previous stage, as well as the growth of pronephric kidney and renal duct was obvious, as it is the working kidney in the embryonic stage in fish (Fig. 3). In addition, the formation and growth of the dorsal and pectoral fins was clear; it was also showed that the embryo body is curved due to the attachment to the yolk, as it is feeds on the yolk vessels, which are completely formed (Fig. 4). Furthermore, it was found the clarity of blood cells, hematopoietic stem cells, and the dorsal aorta (Rada, 2022). The current study also noticed from histological characteristics the clarification of the atrium and ventricle formation, as well as the appearance of the bulbus arteriosis (Fig. 4). The study of Gering et al. (1998) showed from the mechanism of gene expression that there are some genes found in the hematopoietic stem cells that originate in the lateral mesoderm in zebrafish. Additionally, previous study revealed that the embryonic blood formation in bony fishes (Osteichthyes) develops early for the first time within the lateral mesoderm, and then the cells migrate to the middle to form the intermediate cell mass (ICM), which is connected to the dorsal aorta (Liao et al., 1998).

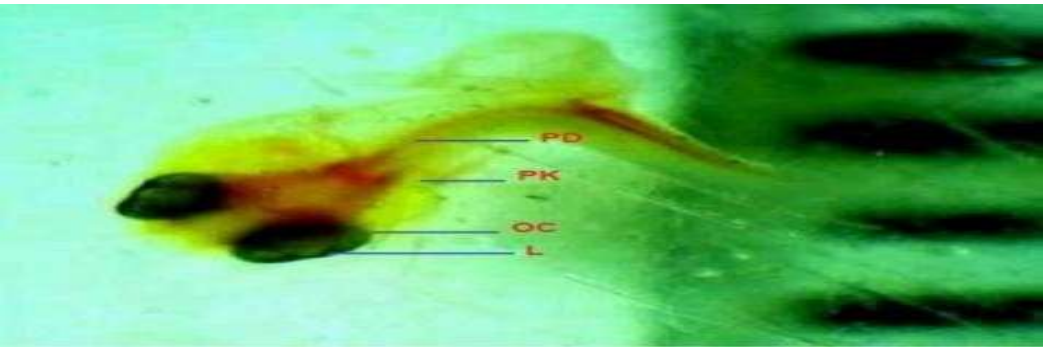


**Figure (3):** Embryo at 4 mm in length showing the formation of lens (L), optic cup (OC), Pronephric Kidney duct (PD).

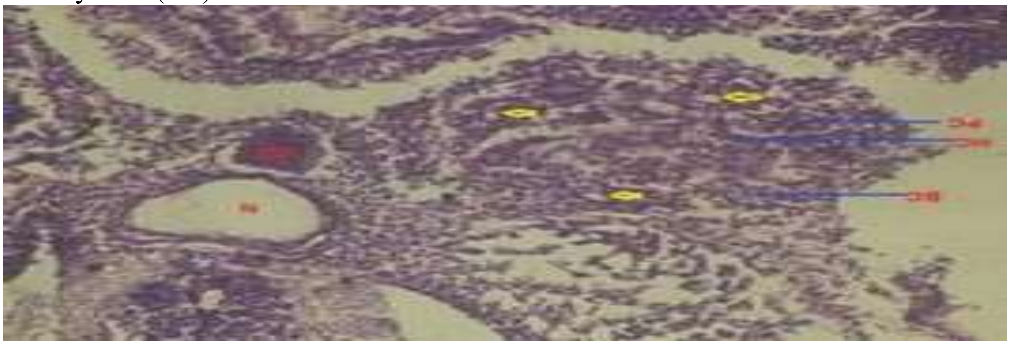

**Figure (4):** A cross-section in a 4 mm embryo in length showing the blood cells (BC), haemangioblast cells (HC), Pronephric Kidney duct (PD), dorsal aorta (DA), and notochord (N), (H&E) (400X).

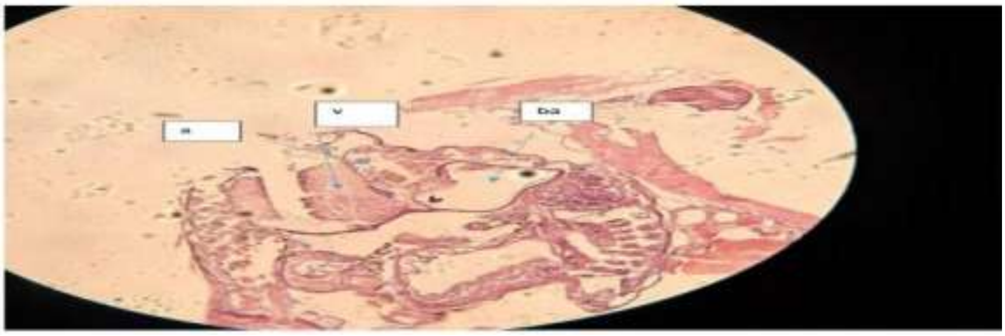


**Figure (5):** A cross section in a 5 mm embryo showing the atrium (a), ventricle (v), and bulbus arteriosis (ba).

**3- Embryo at 7-6 mm in length**

At this stage, histologically it was noticed that the ventricle and atrium are clearer than in the previous stages (Fig. 6). Moreover, it was observed the increases in blood aggregation containing Hematopoietic stem cells and blood cells. Morphologically, it was revealed that the organs appear more distinct, as the eye and its parts are fully developed, in addition to the growth of pelvic fin, dorsal fin, and caudal fin (Fig. 7). These results are in agreement with Liao et al. (1998) study that studied the embryonic formation of the zebrafish, and based on the number of somites, as it was approved that at the 15th somite stage, blood vessel progenitors appear on both sides of the trunk.

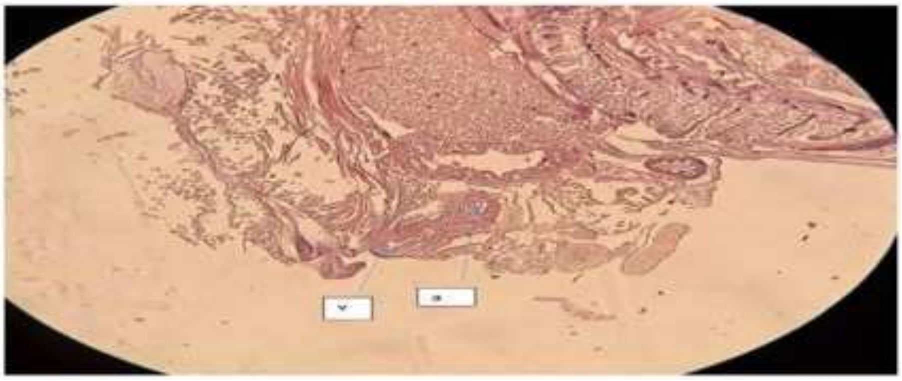


Figure (6): A cross section in a 7 mm embryo showing the atrium (a), ventricle (v).

**Figure (7):** The morphological features of embryo with 6 mm in length.

**4- Embryo at 9 mm in length (Newborn stage)**

This stage included the complete development of all fins and fin rays, and the embryo becomes able to swim after birth, in addition to increase the pigment cells, the scales cover all parts of the body, and the growth development gills and operculum (Fig. 8). These findings are in agreement with Diep et al. (2011) study on the embryonic and larval development of freshwater fish, where at the newborn (birth) stage the embryo length was with approximately 9-10 mm and able to swim and depend on itself to obtain food. However, this result is not consistent with previous phenotypic study of embryonic and larval development in common carp (Cyprinus carpio) which described the stages of embryonic development to the hatching stage where the embryo (newborn stage) with a length of 4.1-3.5 mm, while the length of the newborn embryo is approximately 9 mm in the current study.

**Figure (8):** The final development of the fins and the clarity the scales on the trunk.

**5- Embryo at 10-11 mm in length (Larva stage)**

At this stage, the embryo transformed into the larvae stage, where it is able to swim after the development of the morphological characteristics and its internal systems, as it was noticed the completion and clarity of the heart parts including the atrium, ventricle, bulbus arteriosus, as well as the development of the epicardium and myocardium muscles (Fig. 9). Additionally, the developed shape of the heart was clear (Fig. 10). Morphemically, the larva has fully developed and is able to swim feed. This result is not consistent with the study of the embryonic and larval development in Hemibagrus (catfish), where the heart appeared in somite 25 and the beginning of heart formation, heartbeats, and blood circulation were noticed, although the blood plasma is colorless. Moreover, this result is not agree with Puvaneswari et al. (2009) result where the heart appeared in somite 22 in C. striatus fish. On other hand, a study on zebrafish, it was observed that smooth muscle cells are located at least near the precursors of the heart, and there is a common aggregation within the cardiac fields in the mesoderm (Lazic and Scott, 2011). Mef2cb regulates late myocardial cell addition from a second heart field-like population of progenitors in zebrafish. Developmental biology, 354(1), 123-133.), where at the 14$^{th}$ somite stage, it was appeared that and the cardiac muscle cells moved towards the midline to form the cone-shaped heart, which enabled to detect the cells that contribute to the formation of both the atrium and ventricle (Huang et al., 2003).

**Figure (9):** The formation of the atrium, ventricle, bulbus arteriosus, myocardium muscles and epicardium.


**Figure (10):** The clarification of heart.

## CONCLUSIONS

The heart appears in embryo 3 mm in length. The atrium, ventricle and bulbus arteriosis are formed in a 4-5 mm long embryo. Embryo with 5 mm in length, the atrium and ventricle become more distinct than in the previous stage. At the birth stage, the embryo was with 9 mm in length and is almost fully developed, able to swim and feed, and able to transform into a larva. The embryo becomes a larva with10-11 mm in length, where the formation of the heart and its parts (atrium, ventricle, bulbus arteriosus, epicardium, and myocardium) are developed.

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## التطور الجنيني للقلب في أسماك شراعية الزعانف (POECELIA LATIPINN)

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الخلاصة

تضمنت هذه الدراسة دراسة التطور الجنيني للقلب في سمكة المولي شراعية الزعنفةPoecelia latipinna وهي احدى انواع الاسماك الغازية في العراق والتي تنتشر في سطوح المسطحات المائية وخاصة الاهوار والتي تقع في الجنوب من العراق تم جمع العينات من سوق الغزل في بغداد وثم تشريح الاسماك لإزالة المبيض الذي يحوي على الاجنة بأطوال مختلفة باستعمال ملقط ناعم ووضعت في الفورمالين للتثبيت بعد عمل ثقب في كيس الغاز خاصة في الاجنة المتقدمة بالنمو الغرض الأساسي من الدراسة هو دراسة التطور الجنيني للقلب على اطوال الاجنة المختلفة. في جنين طول 3 ملليمتر اكتمال تكوين القلب ووضوح الاوعية الدموية، في جنين طول 4-5 ملليمتر لوحظ ان الاذين والبطين تطورا بوضوح بالإضافة الى ظهور البصلة الشريانية، إضافة الى ذلك في جنين طول 6-7 ملليمتر لوحظ عدد كبير من التغيرات في كل من الاذين، البطين، والتجمعات الدموية الحاوية على الخلايا المولدة للدم والخلايا الدموية اكثر من المرحلة السابقة، جنين 9 ملليمتر مرحلة الولادة ازداد وضوح القلب واجزائه اكثر من المرحلة السابقة، جنين طول 11 ملليمتر مرحلة اليرقة نلاحظ اكتمال للقلب ووضوح الاذين والبطين والبصلة الشريانية واكتمال النخاب والعضلات القلبية واضحة جدا.