The Development of the Pronephros in Sailfin Fish *Poecelia Latipinna*

Ruqaa Badr Reda[1*], Mukhtar Khamis Haba[2]

Abstract

This study included the development of the pronephros kidney in one invasive species in Iraq, the sailfin fish *Poecelia latipinna* which spread in the aquatic surfaces particularly the marshes which situated in the south portion of Iraq. This study depends on the length of the embryos. The bilateral progenitor pronephric cells appear in the mesodermal plate in embryo 1.5 mm in long. In embryo 2mm in long, the histological sections showed the occurrence of the progenitor cells with the precursors of the blood cells near the notochord and dorsal aorta. The undifferentiated pronephric tubules formed in the embryo 4mm in long and the pronephric kidney started to surround with connective tissue cells. In embryo 5mm in long, the histological sections appeared that the pronephric tissue completely surrounded by connective tissue to form capsule, the tubules differentiated to proximal and distal tubules and the glomeruli appeared as well, also the pronephric ducts were completed in this stage to fuse with the cloaca.

Key Words: *Poecilia latipinna*, Pronephros Embryogenesis, Progenitor Cells.

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Introduction

In vertebrates, the kidneys are classified into three types Pronephros, Mesonephros and Metanephros, all of which are derived from Archinephros which consist of Nephrons, the urinary units (Balinsky, 1981) (1). The development of kidney included four main stages, (i) Cell commitment as an undifferentiated mesodermal nephrogenic fate, (ii) Formation of primordial organ and epitheliazation of pronephric ducts, which will form as collecting system, (iii) The formation of nephrons with the events of cell patterning, which coincides with the emergence of cells related to the formation of both glomeruli and renal tubules.(vi),(Drummond, 2000)(2). The pronephros is the first kidney form during embryogenesis, in teleost fish and amphibians, the pronephros considers as the functional kidney of early larva life (Tytler *et al*, 1996; Vize *et al*, 1997) (3)(4). According to the fate map, the pronephros of Zebra fish develop from bilateral stripes of renal progenitor cell derived from intermediate mesoderm precursors, from a region of the ventral mesoderm that is close to and somewhat intermingled with blood and vascular progenitors (Sadler, 2004)(5). (Gerlach and Wingert, 2013) (6), explained in their study that the cells destined to generate the pronephros which appear to overlap with cells destined to form blood. A pronephric primerdium appears during early somitogenesis, under the anterior somites as a mass of intermediate mesoderm (Chimenti and Accordi, 2011; Wingert *et al*, 2007; Drummond, 2010) (7) (8) (9), Given that this set of cells gives rise to the future pronephros, its crucial to know where these cells came from in the embryo. The most rostral part of nephron procursors produces podocytes, which form the glomerulus (Anzenbergerb *et al*, 2006; Stickney *et al* 2007) (10) (11).

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The remaining rostal central and caudal parts contain nephron precusors, which form a series of tubule segments that are followed by a duct (Wingert *et al*, 2007; Drummond, 2010) (8) (9). At the most cranial end of the mesodermal nephrogenic cord, the pronephros develops from mesenchymal buds of pronephric primordia or nephrotomes (Cochard, 2002; Chimenti and Accordi, 2011) (12) (7). The pronephric tubules are formed when the buds of pronephric primordial hollow down (McCrory, 1974) (13). The following functional units make up a typical pronephric nephron, which can be found in adult teleosts (Hamilton *et al*, 1972) (14). The external glomerulus or glomus is a vascular component that filters wastes, which serves as a waste collecting unit, A ciliated nephrotomes connects to the pronephric tubule which empties into the pronephric duct (Cho *et al*, 2011) (15), the glomerulus is a filtering vascular structure that is one body segment long, A glomus on the other hand is a vascular structure that spreads over many body segments (Pole *et al*, 2002) (16). Relatively the development of the glomerulus occurs late after the completion of the development of the renal tubules (Drummond *et al*, 1998; Tytler, 1988) (17) (18). During early somitogenesis stages, the gene expression studies emphasized that the renal progenitor cell located with cells that produce a mixture of angioblasts and primitive blood precursors. In zebra fish gastrula embryos, cell labeling demonstrated that the cells which destined to form the pronephros overlapping with cells fated to form blood (Kimmel *et al*, 1990) (19). Following the early patterning of the mesoderm, the tight connection of nephrogenic cells with blood producing cells. the stage markers of blood cell development and expressed in a cell population that overlaps a pressing presumptive kidney cells population at the end (Wessely, and Tran, 2011) (20). The blood island where erythropoietin production occurs later in development is located ventral to the developed pronephric duct (Chimenti and Accordi, 2011) (7). Teleosts have a pair of kidneys that include the renal tubules that are directly connected to the renal glomeruli, as well as a pair of renal ducts that carry waste to the outside of the body (Hill *et al,* 2004) (21),through Cloaca, (Naylor *et al*, 2016) (22) revealed that the Renal progenitor mesenchymal precursors undergo physical and morphological changes as development progresses past the 15 somite stage in Zebra fish, resulting in the formation of a pair of nephrons that are similarly located on either side of the trunk in parallel tracks. The progenitor of the mesenchymal nephron becomes epithelial and, undergoes tubulogenesis. Fresh molecular evidence suggests that the tubular epithelium of the zebra fish pronephros is actually separated in to two proximal tubule segments (proximal convoluted tubule and proximal straight tubule and two distal tubule segments (distal early and distal late) that are similar to mammalian nephron segments in many aspects (Wingert and Davidson, 2008) (23). Kidney primordial initiates pronephric ducts on both two sides of the embryo by caudal cell migration ventral to the somites, forming the collecting system after epithelization and fusion at the cloaca (Drummond, 2000) (2).

Aim of Study

To identify the embryonic stages of the pronephros in sailfin fish *Poecilia latipinna*, one of the invasive species in Iraq.

Materials and Methods

Samples Collection

The samples were collected from the Al-Ghazil market in Baghdad from April to October 2020, fish were placed in a special aquariums equipped with an air pump with a filter, and special heater to stabilize the temperature ranges between (23-25˚c), fish were fed with commercial paper food, they were fed twice daily (morning and evening). Embryo lengths ranging from (1.5-5 mm) were obtained from pregnant fishes.

Fish Dissecting

Fishes dissected according to (Billet and Wild, 1975) (24), by making a fold in the fish body wall by cutting it from the ventral midline up to the front of the trunk. After dissection, the ovary containing the embryos is removed using fine forceps and placed in a saline solution (0. 7%) to obtain the required embryos. The lengths of the embryos are measured under the Dissecting microscope and using a Petri dish equipped with a ruler at its base.

Fixation

Embryos are preserved with 10% formalin solution after making a hole in the gas bladder for developed embryos (Al-Adhami, 1976) (25). The samples are washed after fixation with 70% ethyl

alcohol several times to remove the largest amount of excess fixative, and leave in this solution until use.

Microscopal preparations: According to (Bancroft and Stevens, 2012) (26).

After fixation and for dehydration the samples were passed through a series of ascending concentrations of ethyl alcohol (70%, 80%, 90%, 100%). The samples were putting in xylene for half an hour to make them more clear. The samples were placed in a mixture of xylene and paraffin wax with a melting point (56-58ºc) for a quarter of an hour, and then transferred to a new molten paraffin wax that was replaced three times (half an hour in each time), the embedded with paraffin wax using a special mold, then left to solidify at room temperature and kept in the refrigerator. The samples were cut to a thickness of (5 µm) sequentially by using microtome, the sections were mounted on slides smeared with Mayers solution. The slides left to dry until staining process. Sections were stained by hematoxylin–eosin stains. Canada Balsm was used for mounting the sections, then the sections were covered with cover slip.

Photography

Dissecting Microscope (Roma) was used to photograph the phenotypic characteristics of the embryo, and Compound Microscope (Meji) provided with Digital camera (canon) used to photograph the sections on the slides.

Results and Discussion

In this study, the embryonic stages were divided according to the length and external phenotypic characteristics of the embryos.

1 - Embryo 1.5 mm Long (Tail Bud Stage)

The embryo appears as a transparent arc on the surface of the yolk sac. At this stage, the mesoderm plate has been identified, and the embryo shows the beginning formation of the forebrain and Beginning of the vascular formation (Fig. 1). This plate contains many cells that will form many organs such as the heart, muscles, kidneys, and blood vessels, (Prummel *et al*, 2020) (27), emphasized that this plate harbors precursor cells for distinct organ systems.

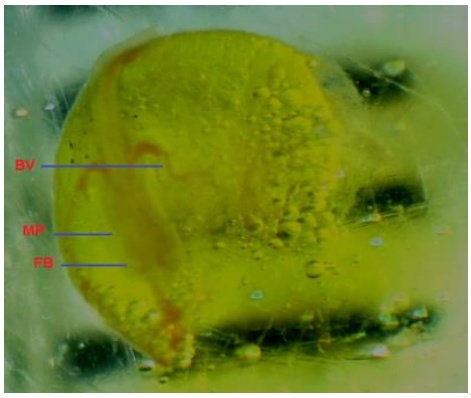

Fig. 1. Showed the Mesodermal plate in embryo 1.5mm long. FB=Fore Brain, MP=Mesodermal Plate, BV=Blood Vessels

This result was similar to what was reached by (Gerlach and Wingert, 2013) (6), when they studied the embryonic development in the zebra fish, they relied in their study on the gene expression technique and determined the presence of the mesoderm plate in the tail bud stage, also they divided the mesoderm into three regions: Lateral Plate Mesoderm, Intermediate Mesoderm and Plate Mesoderm.

2 - Embryo 2 mm Long (Optical Cup Stage)

The embryo is characterized by the appearance of the first somite, which represents the dorsal part of the intermediate mesoderm, as well as the beginning formation of the optic cup. The histological sections of this stage showed the appearance of progenitor cells with increased formation of blood vessels (Fig. 2,3).

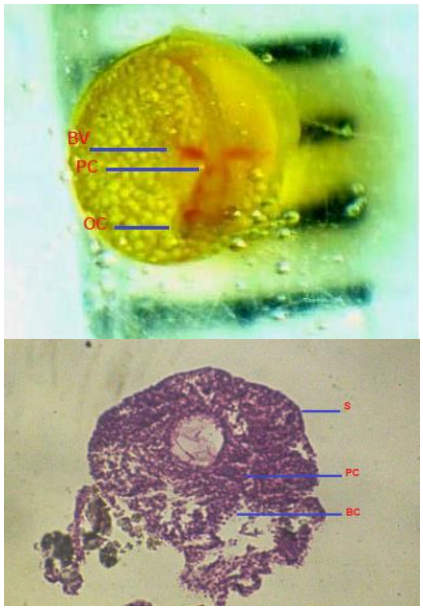

Fig. 2. Showed embryo 2mm long.OC=Optic Cup Fig 3: showed the transvers section in embryo 2mm long. PC=Progenitor Cells, BV=Blood Vessel S=Somite, PC=Progenitor Cells, BC=Blood Cells. (H&E 40X)

Several previous studies described this stage according to the stages of embryonic development of the fish that were used in their studies. Iwamatsu, 2004 (28), showed that the pronephric kidney appears near the first somite segment in the form of an aggregation of cells on bilateral sides of the notochord. In Zebra fish, embryonic cells which destined to form the pronephose are derived from ventral mesoderm overlapping with cells fated to form blood (Kimmel *et al*, 1990) (19). Gerlach and Wingert, 2013 (6), During their study, they emphasized the occurrence of the progenitor cells with blood precursors in Zebra fish by using genetic expression, the researchers were able to divide the mesoderm into three fields from the inside out, which are: Angioblasts, Primitive blood field and Renal progenitor cell field. Here, the researchers showed that precursor cells are formed in the mesoderm during the process of formation of fats, which are located near the area of cells that will be the mixture of hematopoiesis and blood precursors (Drummond *et al,* 2010) (9).

3 -Embryo 3mm Long (Heart Appearance Stage)

At this stage, the heart's development is complete so that the blood is distributed to all parts of the embryo's body, particularly the pronephric kidneys, which continue to include blood cells coming from the dorsal aorta. Another phenotypic characteristic is the increased growth of the optic cup with the beginning of the lenses development (Fig 4).

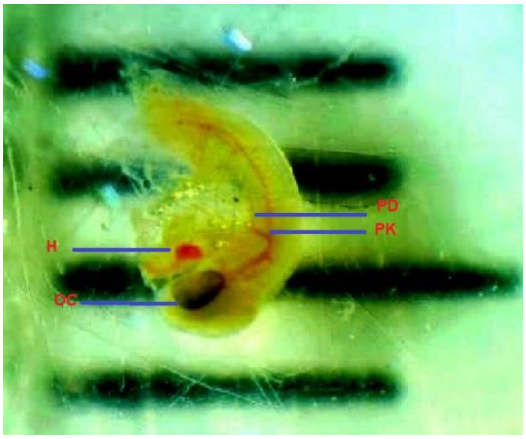

Fig. 4. Showed embryo 3mm long. OC=Optic Cup, H=Heart, PK=Pronephric Kidney, PD=Pronephric Duct.

4 - Embryo 4 mm Long (Lens Stage)

The phenotypic characteristics of this stage are, the completion of the lens growth and the differentiation of the pronephros with the proliferation of some pigment cells in the head and trunk region, appearance of the dorsal and pectoral fins with extension of the pronephric ducts. The histological sections of this stage showed the borders of the pronephric kidney are differentiated by surrounding them with connective tissue cells that will form the capsule after the completion of the assembly of the progenitor cells and blood precursor cells (Hematopoietic cells) which began to produce blood cells, also the histological sections showed the beginning of the formation of undifferentiated tubules at this stage (Fig 4,5).

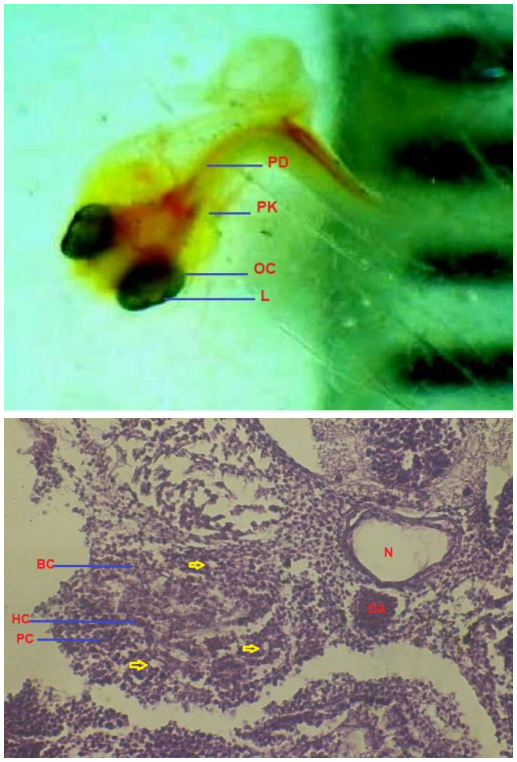

Fig. 4: Showed embryo 4mm long. L=Lens, Fig 5: Showed transvers section in embryo 4mm long. OC=Optic Cup, PK=Pronephric Kidney, N=Notochord, DA=Dorsal Aorta, PC= Progenitor PD=Pronephric duct. HC=Hematopoietic cells, arrow= Undifferentiated nephric tubules. (H&E 40X)

Teleost fish can produce differentiated cells similar to the blood lineages produced in mammals (Rowley *et al*, 1988) (29). Embryonic hematopoiesis in bony fishes develops early for the first time within the lateral mesoderm and then the cells migrate to the middle to form the so-called Intermediate Cell Mass (ICM) which is associated with the dorsal aorta (Al-Adhami and Kunz, 1977; Detrich *et al*, 1995; Zon, 1995) (30) (31) (32). The mechanism of gene expression that (Rodaway *et al*, 1998) (33) applied in zebra fish showed that some genes confirmed that haematopoietic, endothelial and pronephric duct progenitors in the lateral

mesoderm.

5 -Embryo 5mm Long (Caudal Fin Stage)

In this stage, the dorsal and pectoral fins increase in growth with the appearance of the caudal fin, increased proliferation of head and trunk pigment cells (Fig 6). Histologically results, it follows:

1. The primary kidneys are completely surrounded by the connective tissue that makes up the entire capsule (Fig 7).
2. The appearance of pronephric glomeruli (Fig 7).
3. Complete differentiation of the proximal and distal tubules with the completion of the pronephric ducts that extend posteriorly to connect with the Cloaca (Fig 8).

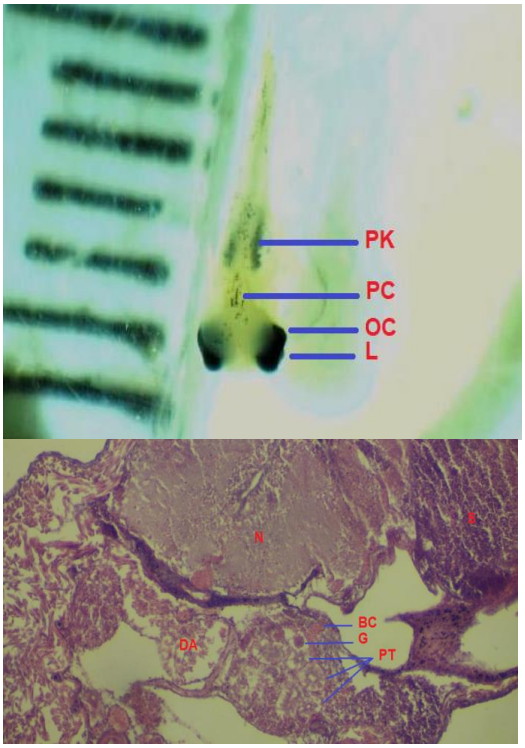

Fig. 6. Showed embryo 5mm long. L=Lens, Fig 7: Showed the transvers section in embryo 5mm OC=Optic Cup, PC=Progenetor cells, PK= N=Notochord, DA=Dorsal Aorta, PT= Pronephric Kidney Differentiated pronephric tubules (H&E 20X)

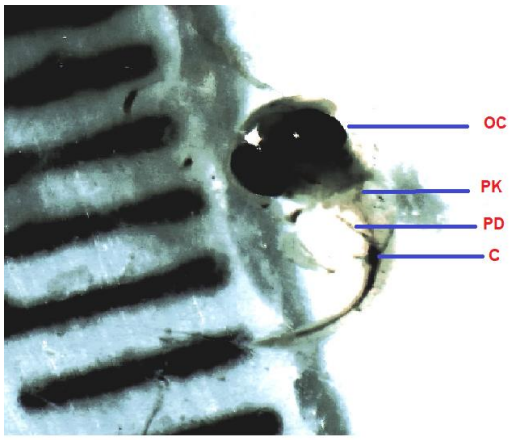

Fig. 8: Showed the fusion of the pronephric ducts with the cloaca. OC= Optic Cup, PK= Pronephric Kidney, PD=Pronephric Duct, C=Cloaca

In fish, the pronephros considered as the first kidney which growing and succeeded to form the mesonephros, which is represent the adult kidney. Generally, teleost fishes possess a pair of functional pronephroi, which include three anatomical subunits (glomerulus, pronephric tubule, and pronephric duct) (Drummond *et al*, 1998)(17). The structure of the glomerulus types in vertebrates depend on different development and homeostatic requirements (Ichimura *et al*, 2007; Ichimura *et al* 2009) (34)(35). The results of the current study were in agreement with many previous studies, despite the limitations of the techniques used in this study. Naylor *et al*, 2016 (22) showed a new view for the pronephric formation during the necessity of the movement and proliferation of embryonic cells to determine the size of the pronephric kidney. In Zebrafish, the pronephros composed two nephrons that fused rostrally at a common glomerulus and posteriorly fused with cloaca (Basu *et al*, 2003) (36). Wingert e*t* al, 2007 (8) concluded that the proximal renal tubules are divided into two segments (proximal convoluted tubule; PCT and proximal straight tubule; PST), and the distal tubules are divided into two segments (distal early; DE and distal late; DL). In order for the pronephric urine to be excreted, the DL segment must fuse with the cloaca. The DL segments will becomes fused with cloaca via its caudal most ends during the pronephric development, so the DL progenitors should be included in this domain and therefore they must be situated close to the site of the cloaca. Other studies explained that a small amount of migration cells needed to fuse the DL segment with the cloaca. (Naylor *et al*, 2016) (22). (Serluca *et al*, 2001; Slanchev *et al*, 2011).

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