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ORIGINAL ARTICLE

Study on the renal development (morphological and stereological) in *H. huso* (*Beluga sturgeon*) larvae

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Abstract The aim of this study was to obtain the information about kidney evolution in *Huso huso*. According to the results on the 1st (day of post hatching (dph)), the pronephros with collecting canal and hemato-poietic tissue was distinguished. On the 3rd (dph), the primordial mesonephric tubules, rounded cell clusters, and a primary coelom cavity were observed. On the 6th (dph), the pronephros was gradually degenerated and changed to mesonephros. On the 15th (dph), the collecting canal was resembled as a small canal, and three parts, head, body, and caudal, could be distinguished in the kidney. The tubules were differentiated to proximal, distal, and collecting tubules. On the 21st (dph), the structure was similar to the 15th (dph), but with decrease of hemato-poietic tissue and developed of renal corpuscles. SPSS ANOVA version 19 was used for statistical analysis. According to stereological results in *H. huso* from the 1st (dph) to 21st (dph), the total volume of kidney was significantly increased ($P < 0.05$).

Keywords Kidney · *Huso huso* · Morphology · Stereology

Introduction

The sturgeon is one of the old fishes that live in freshwater at their beginning life. They can live in both fresh water and saltwater; thus, their kidney has the ability to adapt in different environments. Variation of ray-finned fish's kidney structure occurs independently during development (Northcutt 1978, 1996), and it is reflective of key points in relation to evolution of kidney. *Huso huso* is one of the most long-life species and that their adults usually live in middle-depth sea. Despite all the researches on kidney, there are few studies on kidney development in *H. huso*.

Kidney has two significant functions: removing damage substances from blood and balancing electrolytes and metabolites in the blood circulation. It is contributed substantially to the maintenance of an appropriate balance of the homeostasis and is the significant osmoregulatory organ in vertebrates (Northcutt 1996). Environmental factors play an important role in defining the renal function in fish. In the freshwater habitat, the fish have hyperosmotic relation to the environment and fluids inflow to the body. The role of kidney is similar to a hyperosmoregulator that extracts the extra water from the body (Northcutt 1978, 1996). Marine fish have an internal osmotic concentration lower than the surrounding seawater, so it tends to lose water and gain salt. Most fish are stenohaline, which means that they are adapted to either salt or fresh water and cannot survive in water with different salt concentration. However, some fish show high ability to effectively osmoregulate across a broad range of salinities; fish with this ability are known as euryhaline species (Kimley et al. 2002).

In fish, the kidney is retroperitoneal and fused to the vertebral column. Kidney morphogenesis in fish and other vertebrate is similar and develops by growth of nephrogenic renal vesicle from group cells in the intermediate mesoderm. Vesicle develops to a C-shaped hollow that differentiates to renal corpuscle and renal tissue. Also, the caudal part of vesicle elongates to form the renal tubules (Northcutt 1996).

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Renal corpuscle contains glomeruli with network capillaries and Bowman's capsule. The tubules consist of proximal and distal tubules that fuse to the collecting duct. Generally in vertebrates, three types of kidney are detected as follows: pronephros, mesonephros, and metanephros (Elger and Hentschel 1989).

The pronephros is the first kidney that develops during embryogenesis in fish and remains after post hatching for primary filtration and osmoregulation. Pronephros replaces with mesonephros as the adult kidney (Elger and Hentschel 1989).

Histological studies have been done on the kidney of fish, for example, juvenile *Acipenser persicus* in 2004, juvenile *Acipenser naccarii* in 1995, and Acipenseridae of the Caspian Sea basin.

This research was designed to study histomorphogenes and the volume estimated of kidney in *H. huso* from 1 to 21st (day of post hatching (dph)) by Cavalieri's principle. The Cavalieri's principle is stereological research for quantifying area volumes (Gundersen and Jensen 1987).

Materials and methods

The *H. huso* larvae were obtained on the 1st, 3rd, 6th, 15th, and 21st (dph) from Shahid Marjani Aghghela propagation station in Gorgan. Eight larvae of each age were fixed in 10% neutral formalin, dehydrated with ethanol series to 100%, cleared in xylene, and embedded in paraffin. The serial sections (6 μ) were stained with hematoxylin-eosin for routine histological studies by light microscope. For stereological studies, by random system, the sections were selected and the figures prepared by the camera attached to the light microscope. The point counting grid was used and each area from the kidney was separately counted (Howard and Reed 1998); then, an unbiased estimate of the kidney volume was obtained by using the following formula:

$$V\left(\mathrm{mm}^3\right) = d \times \sum p \times a\,(p)$$

d	interval between sections
$\sum p$	total number of points considered on the area of sections
$a\,(p)$	the area represented of each point in the grid

Data expressed as the mean ± SD. One-way ANOVA by SPSS (version 19; SPSS Inc., Chicago, USA) was used for statistical analysis followed by Tukey's post hoc test. The P value less than 0.05 were assumed as statistically significant.

Results

Histological results

On the 1st (dph), the pronephros with a primary coelom cavity (cav), collecting canal (cc), and hemato-poietic tissue (hp) was distinguished (Fig. 1). It was clearly visible in the posterior area of the yolk sac and consisted of short pronephric tubules (pnt) (Fig. 1). The collecting canal was lined with simple ciliated epithelium. At the first day of post hatching, the pronephric tubule cells contained a large amount of yolk granules (y) (Fig. 1).

On the 3rd (dph), the primordial mesonephric tubules (pmt) were observed as rounded cell clusters (Fig. 2). During their development, mesonephric tubules took on a convoluted shape and they arouse along the pronephric duct (pd). The yolk granules were gradually disappeared in tubule cells. On the 3rd (dph), the small primordial glomeruli (pg) were detected near the tubules (Fig. 2).

On the 6th (dph), the remained pronephros was degenerated and changed to mesonephric tubule (mnt). But the collecting canals (cc) were still remained. Mesonephric tubules had low to high cuboidal acidophilic epithelium and exited to the nephric duct (nd) (Fig. 3). Few renal corpuscles (RCs) in different sizes were formed. They were consisted of a glomerule "dense capillary network" and glomerular (Bowman) capsule. Glomerular capsule had two external (parietal) and internal (visceral) layers. By the 6th (dph), a few hemato-poietic tissues were distinguished in the cranial part of kidney (Fig. 3).

On the 15th (dph), the collecting canals were remained as small canal (Fig. 4). Three parts could be distinguished in the kidney: head (H), body (B), and caudal parts (C) (Figs. 4 and 5). The head consisted of hemato-poietic tissue, few tubules, and renal corpuscles. In body, the hemato-poietic tissue was gradually reduced and the tubules and corpuscles were developed (Fig. 4).

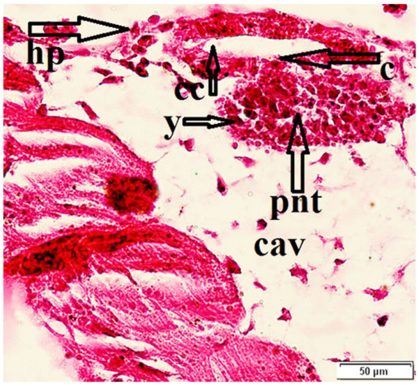

Fig. 1 Frontal section of the kidney (H&E), from 1 day old, *y* yolk granules, *cc* collecting canal, *pnt* pronephric tubule, *c* cilia, *cav* coelom cavity, *hp* hemato-poietic tissue

Fig. 2 Frontal section of the kidney (H&E), from 3 days old, *pg* primordial glomerule, *pd* pronephric duct, *pmt* primordial mesonephric tubule, *In* intestine

In the caudal part, hemato-poietic tissue was significantly decreased and replaced by abundant renal corpuscle and differentiated tubules (Fig. 5). There were many nuclei in the glomeruli that could be capillary endothelial cells, mesangial cells, and podocytes, but up to this age were not recognized from each other. On the 15th (dph), the tubules were differentiated to proximal, distal, and collecting tubules (Fig. 5).

The proximal tubules with simple cuboidal epithelium and spherical nuclei were the longest. These tubules had two types: first proximal (P1) and second proximal (P2) tubules. The first proximal tubule had low cuboidal cells and small central lumen, but the second proximal tubule had high columnar cells with central nucleus and enlarged lumen on the 15th (dph) (Fig. 5).

The distal tubules (D) with simple cuboidal epithelium and faint color cytoplasm were shorter and lesser than proximal tubules. The lumen of these tubules was large (Fig. 5).

The collecting tubules (CTs) with high cuboidal epithelium and centrally nuclei were large and spherical shape. They were longer than two other tubules (Fig. 5).

On the 21st (dph), the development process was similar to 15th (dph), and as the hemato-poietic tissue was decreased, the renal corpuscles were developed (Fig. 6).

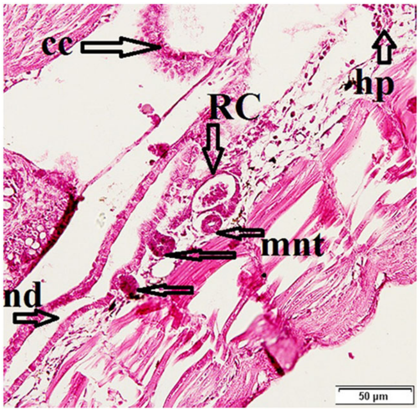

Fig. 3 Frontal section of the kidney (H&E), from 6 days old, *cc* collecting canal, *RC* renal corpuscle, *mnt* mesonephric tubule, *nd* nephric duct

Fig. 4 Frontal section of the kidney (H&E), from 15 days old, *H* head of kidney, *cc* collecting canal, *B* body of kidney, *hp* hemato-poietic tissue

Stereological results

SPSS ANOVA version 19 was used for statistical analysis, and value was expressed in terms of mean and standard deviation (mean ± SD). Stereological characteristics in different parts of the kidney are summarized in Table 1. From the 1st (dph) to 21st (dph), the total volume of kidney had significantly increased ($P < 0.05$) and the tubules compare to other parts had more developed (Table 1). By aging, the volume of tubules and renal corpuscle was increased and the hemato-poietic tissue was decreased significantly; so that on 21st (dph), it was approximately two times more than the first days. The significant difference among different days was $P < 0.05$ (Table 1).

Discussion

Kidney is the principle organ of fish that balances the fluid homeostasis and significantly obtains the post branchial blood (Ojeda et al. 2003). The morphology of kidney in fish species is different (Elger and Hentschel 1989). Chondrostei are a

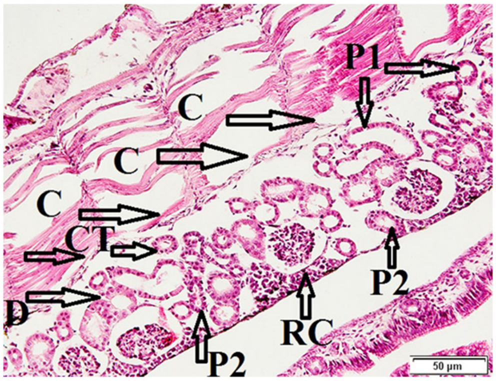

Fig. 5 Frontal section of the kidney (H&E), from 15 days old, *C* caudal part of kidney, *P1* the first proximal tubule, *P2* the second proximal tubule, *RC* renal corpuscle, *D* distal tubule, *CT* collecting tubule

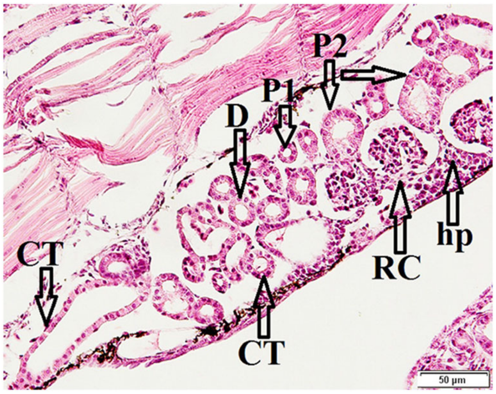

Fig. 6 Frontal section of the kidney (H&E), from 21 days old, *P1* the first proximal tubule, *P2* the second proximal tubule, *RC* renal corpuscle, *D* distal tubule, *CT* collecting tubule, *hp* hemato-poietic tissue

major group of ray-finned fishes, and because of their migration, the kidney requires to adaption in different environments. The kidney of vertebrates is consisted of nephrons that have the same function, structure, and morphology (Mobjerg et al. 2004).

In fish, kidney is made of head "anterior" and caudal "posterior" parts. The head contains of hemato-poietic tissue, a few tubules, and renal corpuscles and the caudal part consists of tubules, renal corpuscles, and to a lesser hemato-poietic tissue.

According to our results, on the 1st (dph), the pronephros with collecting canal, hemato-poietic tissue, and yolk granules was obvious. In fish and amphibians, pronephros is remained after hatching and performs the first filtration and osmoregulation (Vize et al. 1997). In fish, by aging, the pronephros replaces with mesonephros, but in other vertebrates, metanephros is the final kidney. In teleost, pronephros is very simple and composed of two renal corpuscles. They are attached to the midline and their tubules without connection to the abdominal cavity directly join to the renal corpuscle (Tytler et al. 1996).

On the 6th (dph), the pronephros was replaced with mesonephric and contained undifferentiated mesonephric tubules. In Russian sturgeon larvae, the pronephros retains until 8–10 (dph) and then it endures gradually degeneration (Krayushkina et al. 2012). In sturgeon, a mesodermal structure along the pronephric duct differentiates to primordial mesonephric. Also, in Russian sturgeon as *H. huso*, on the third day after hatching, the primordial mesonephric tubules are presented with rounded undifferentiated cell clusters (Krayushkina et al. 2012). In *H. huso*, the mesonephric tubules become longer and obtain slightly thinning shape. The evolving tubules connect to the pronephric duct, and they obtain a convoluted form during their growth (Krayushkina et al. 2012). On the 6th (dph), in *H. huso*, a few renal corpuscles with different sizes were formed. This structure consisted of glomerule and glomerular (Bowman) capsule. In the mostly ray-finned fishes, glomerule is formed on the sixth or seventh day post hatching when the larvae are depended entirely on gill respiration and combined (yolk and exogenous food) feeding. The renal corpuscle in sturgeon is not directly joined to renal tubules and connects freely to the pronephric duct (Krayushkina et al. 2012). The glomerule filters the primary urine into the pronephric duct and pump via ciliated nephrostome shafts into the tubules. Due to progress of secretion and absorption, the composition of the first urine alters in the tubules (Vize et al. 1997).

By the 15th and 21st (dph), in *H. huso* as teleostean and osteichthyans, the kidney had three parts namely head, body, and caudal parts (Hickman and Trump 1969). But in the Cyprinidae, kidney has a uniform structure (Hickman and Trump 1969). The head of kidney, in the most fishes, consisted of endocrine elements and hemato-poietic tissue. In Salmoniformes, as *Salmo trutta fario* and *Coregonus lavaretus,* the head contains lymphoid, endocrine, and hemato-poietic tissues, as well as malpighian structures and nephrons; but in *A. persicus* and other sturgeons, it consists of hemato-poietic and accessory tissues (Krayushkina et al. 1996). The head in *H. huso* larvae consisted of hemato-poietic tissue with few nephrons or tubules.

The body and caudal part functions for excretion and osmoregulation (Anderson and Loewen 1975) and contains a little hemato-poietic tissue, tubules, and renal corpuscles

Table 1 The mean volume and standard deviation in different parts of kidney of *Huso huso* larvae estimated by Cavalieri's principle

Variables	Kidney	Hemato-poietic tissue	Tubules	Renal corpuscle or Primordial glomerule
1 day old	81.74 ± 0.28	60.61 ± 0.1	20.1 ± 0.05	1.03 ± 0.02
3 days old	83.08 ± 0.28	56.01 ± 0.09	23.04 ± 0.05	4.03 ± 0.03
6 days old	94.34 ± 0.29	49.2 ± 0.08	32.1 ± 0.06	3.04 ± 0.06
15 days old	102.96 ± 0.31	32.6 ± 0.07	0.1 ± 0.07	20.26 ± 0.9
21 days old	120.59 ± 0.31	24.07 ± 0.06	60.4 ± 0.09	36.12 ± 0.12
Sig*	0.009	0.011	0.006	0.002

Values are presented as mm^3 ± SD

*Significant difference among different days ($P < 0.05$)

which in *H. huso* were distinguished by the 15th (dph). The kidney tubules divide to three segments: proximal, distal, and collecting tubules, but some of the fresh water fish have no distal tubules (Hickman and Trump 1969).

In *H. huso*, proximal tubule is the longest with simple cuboidal epithelium and similar to other fishes had first and second types, but the *Polyodon spathula* has only one type proximal tubule (Hickman and Trump 1969). Two proximal tubule segments are recognized by various heights of cells and thin brush border. In electron microscopy, several distinguishable characters are determined in each of tubules, as the first proximal tubule presents the large columnar cells with basal or subcentral spherical nuclei and rare mitochondria (Anderson and Loewen 1975). In second proximal tubules, the cells are columnar but higher than the first, with central elliptical nuclei, slight round, or elongated vesicles in the apical cytoplasm and a brush border lower than in the first (Anderson and Loewen 1975). In *A. persicus*, proximal tubule has high epithelium with clearly brush border; the villi increase the membrane surface in interaction with uptake molecules. The proximal tubule cells also have convoluted lateral and basal membranes. In *A. naccarii,* the proximal epithelium is consisted of squamous cells (Cataldi et al.1995).

In *H. huso*, distal tubules with the simple cuboidal epithelium and lighter cytoplasm were detected on 15th (dph) and were shorter and less than proximal tubules. Actually, similar to elasmobranches, distal tubules in sturgeons have cuboidal epithelium without brush border but the basal membranes are folded expansively (Hoar and Randall 1983). Salt water fish have no distal tubule and the other tubules do its function. In Crossoptergii, such as *Latimeria chalumnae*, distal tubule replaces with intermediate segment that is connected to collecting duct (Hoar and Randall 1983).

Collecting tubules in *H. huso* larvae were large with high cuboidal epithelium and centrally nuclei. In other fish as trout, the collecting tubule has an epithelium with some basal smooth muscle cells and connective tissue nearby the basement membrane but its lumen is usually irregular (Anderson and Loewen 1975). In tilapia, the collecting tubule has basally positioned nuclei but in *H. huso* as other sturgeon the epithelium had cells with centrally nuclei. In bony fishes as Polypteriformes, the collecting tubule has two principal and intercalated cells (Anderson and Loewen 1975).

In *H. huso*, renal corpuscle was in different sizes and consisted of a glomerule and a glomerular (Bowman) capsule. In the most fishes, glomerule has a dense network of anastomosing capillaries and this structure is essential for increasing the filtration and absorption. Glomerular capsule has two parietal and visceral layers (Anderson and Loewen 1975). In sturgeon, the function of renal corpuscle layers is still unidentified. The mesengial cells that are essential for fast adaption with environment, in other sturgeons, was described by Gambaryan (1984). The size of glomerulus is depended on various habitats and capacity of species in salinity water. In salt water, the renal corpuscle is smaller and less than freshwater fish (Cataldi et al. 1991). In sturgeons, renal corpuscle is larger than teleost (Elger et al. 2000). In many teleosts, the renal corpuscles are globular and very small. In *A. naccarii*, glomerule is multilobed, but in *A. persicus*, it is cluster shape (Cataldi et al. 1995).

By aging, the volume of tubules and renal corpuscle was increased significantly ($P < 0.05$) and the volume of hematopoietic tissue was decreased ($P < 0.05$), which consider the functional process for osmoregularity and electrolytes balance in this fish. With aging, as other ray-finned fish, the volume of tubules and renal corpuscles was increased; thus, they produce much diluted urine.

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Author contributions All authors contributed equally to this work and are responsible for the data presented herein.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Ferdowsi University, Iran (Animal Use Protocol No. 140).

This article does not contain any studies with human participants performed by any of the authors.

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