Research Article

Development of liver and pancreas in *Acipenser persicus* (Borodin, 1897): A histological study

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Abstract: In the present study, different stages of the ontogeny of the liver and pancreas in the *Acipenser persicus* were illustrated from hatching until 36 days post hatching (dph) by histological techniques. The histological sections (sagittal plane) were made by a microtome at a thickness of 5 µm and stained with Hematoxylin and Eosin, Periodic Acid-Schiff and Masson's trichrome. Based on the results, in 2 dph, liver stroma was formed and stellate hepatocytes included numerous vacuoles with nuclei situated parametrically. On 10 dph, the pancreas was observed between the liver and anterior part of the intestine for the first time. Exocrine pancreatic cells were organized in acini. At 18 dph, the central veins and hepatic sinusoids were remarkably apparent in the middle of the liver parenchyma. The pancreas grew as a basophilic organ and it was situated in the posterior intestine on the 22-dph, bile duct, portal vein and hepatic artery was acquiring their structure in connective tissue. On day 36, the liver was characterized by an enlarged and organized form. Formation of the hepatopancreatic tissue is completed within 16 to 36 days after hatching.

Keywords: Persian sturgeon, Liver, Pancreas, Ontogeny, Histology.


Introduction

The Persian sturgeon, *Acipenser persicus* (Acipenseriformes; Acipenseridae), is the most common sturgeon species in the Caspian Sea (Billard & Lecointre 2001; Eagderi et al. 2017). The digestive system of *A. persicus* consists of seven anatomical parts, including pharynx, esophagus, stomach, intestinal tract, liver, pancreas, and rectum (Çinar & Şenol 2005). The liver of *A. persicus* with two lobes is a dense parenchymal organ located centrally in the cranial portion of the body cavity, and it is composed of the hepatocytes, sinusoids, biliary ducts, lymphatic vessels and Kupffer cells (Sheybani & AdibMoradi 2002; Akiyoshi & Inoue 2004). In sturgeons, pancreatic tissue is formed along with the liver parenchyma and the combined hepatic and pancreatic tissues are collectively called the hepatopancreas (Pousty & Sedigh-Marvasti 2000; Alboghobeish & Khaksar-Mahabady 2005).

From a commercial point of view, larval fish rearing is a critical issue, and a well-developed digestive system plays a crucial role in successful larval rearing (Yousefian & Najafpour 2011). In other words, the establishment of modern and optimal methods in aquaculture requires a firm understanding of the digestive system ontogeny and alternation pattern of this system. One of the essential points in the aquaculture is starting active feed (Ostaszewska et al. 2011). A proper understanding of the ontogeny of the digestive system, including the liver and pancreas attached to the gastrointestinal tract, is essential to obtain information about the developing abilities of the larvae to begin feeding on the external food i.e. establishing an appropriate
Rearing strategy requires knowledge on changes in the gastrointestinal tract and its associated processes of digestion and absorption of food during the development of larvae. The most important information in this regard is recognition of the moment when feeding should begin exogenous feeding (Ostaszewska et al. 2011) that will help to design an appropriate diet.

Development and changes of the digestive system in sturgeon occur at the embryonic stage. Differentiation begins after fertilization and ends with hatching (Sanz et al. 2011). During the digestive differentiation after fertilization to hatching, the endoderm gives rise to the hepatoblast (Pousty & Sedigh-Marvasti 2000). In the next step, when the yolk sac is completely absorbed, the larvae enter a new stage of life, and their active nutrition begins, which is called the free-living embryonic period (Sanz et al. 2011).

After absorbing the yolk sac by active feeding, there will be a series of structural and morphological changes in the digestive system that play a vital role in the growth especially regarding larvae survival (Gawlicka et al. 1995). Although various researchers have studied the ontogeny of sturgeons (Babaei et al. 2011; Ghasemi et al. 2020), there are still gaps about the development of the pancreas and liver tissue histology of these fishes need to be addressed (Wegner et al. 2009; Ostaszewska et al. 2011). Zambonino-Infante et al. (2008) found that although the organ development process in the larvae of most fish follows a similar pattern, the rate of organ development depends on the biology of the species. Despite similarities in the basic stages of the development and evolution of various organs in the teleost fish, there are differences in the relative timing of developmental stages. The histological ontogeny and structural properties of the gastrointestinal tract of Persian sturgeon are investigated in the previous study (Batebi et al., 2015). However, the purpose of the current study is to define the ontogeny and developmental changes of major digestive accessory glands such as liver and pancreas in the Persian from hatching up to 36 days post-hatching (dph) by histology and histochemical staining.

**Materials and Methods**

A total of 80 samples of larvae from two to thirty-six days after post-hatching (including 2, 7, 10, 12, 18, 20, 32 and 36 dph) were collected from north of Iran, Agh-Ghala Shahdi Marjani Hatchery Gorgan, Golestan Province, Iran. Juveniles were obtained from the Shahid Rajaie proliferation and culture center, Sari, Mazandaran Province. The specimens were transported in aerated tanks to the laboratory. Experimental protocols were performed in accordance with the Iranian animal ethics framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and Shiraz University Research Council (IACUC no: 4687/63).

Initially, the larvae were classified into 1 to 36 dph and then euthanized by immersion in unbuffered MS222 solution (250 mg/L; 25 to 30°C). Then the larvae was fixed in neutral buffered (phosphate buffer) formalin 10%, dehydrated in a graded series of ethanol (70–96%), embedded in paraffin wax molds at 55–60°C, and cut in serial sagittal sections with a thickness of 5µm with a microtome (Rotary microtome). Finally, the sections were stained with hematoxylin-eosin (H&E), periodic Acid-Schiff Reaction (PAS) (Lipscomb et al. 2020), and Masson’s trichrome (Ozawa & Sakaue, 2020), and images were observed by a camera attached to the light microscope (Model CX22; Olympus, Tokyo, Japan).

**Results**

On day 2 dph, the liver primordium was visible locating in the anterior region of the stomach. At this time, the liver appeared as a dense tissue, and stroma was formed with spaces between the plates of the hepatocytes in some parts of the liver’s parenchyma (Fig. 1). The stellate hepatocytes contained numerous vacuoles, and their nuclei were located peripherally. The liver sinusoids became apparent, but pancreas morphogenesis was not initiated; therefore, no islets
of the Langerhans were detectable. On day 7, the pancreas was not yet observed. In 10 and 12 dph, the pancreas was visible between liver and intestine (Figs. 2, 3). Compared to the 2 dph, the size of the liver was increased due to hepatocyte differentiation. Multiple clusters of the basophilic pancreatic cells (exocrine pancreas) were detected and exocrine pancreatic cells were regulated in acini. At this time, the hepatocytes nuclei were round, dense and numerous lipid vacuoles was observed in the hepatocyte cytoplasm.

Eighteen days after hatching, the central veins and sinusoids were distinguishable among liver tissue

Fig.1. 2 dph, left: formation of liver. At this time, liver appeared as a dense tissue. (H&E, X100). Right: formation of liver stroma and sinusoids (The arrows). Numerous lipid vacuoles observed in hepatocyte cytoplasm (H&E, X400).

Fig.2. 10 dph, left: Stomach and intestine acquired their shapes. The first day of pancreas formation between liver and intestine. (Masson's trichrome, X100), right: The pancreas organ located near liver. Exocrine (Exo p) and endocrine (Endo P) part of Pancreas tissue showed (H&E, X400).

Fig.3. 12 dph. Stomach(s), intestine (i) and pancreas (p). (PAS, X100).
(Fig. 4). By larval development, the pancreas grew as a basophilic organ and it was situated in posterior-dorsal of the intestine (Fig. 5). As the larvae grew older (following the stages of larval development), subsequent pancreas differentiations occurred but no distinct histological changes appeared in the liver (Fig. 6). On 20-22 dph, the hepatocytes were appeared, whereas their nuclei demonstrated eosinophilic and euchromatic characters.

On 32 dph, the central veins, hepatic arteries, and sinusoids were obviously visible (Fig. 7), which were less at the beginning of the development. These veins and arteries increased in the following days. On day 36, the liver was detected as an expanded and extended organ. Although the liver structure was similar to that of the previous period, the number and
size of sinusoids and central veins were increased (Fig. 8). The central vein’s prominent character was an accumulation of the red blood cells without a typical vessel structure. On 20 dph, the number of fat vacuoles gradually decreased and the hepatocytes and sinusoids increased in size. Following the increase in liver tissue volume, the hepatocytes are treated as more coherent tissues. The cytoplasm of these cells is acidophilic and contains a basophilic central nucleus. Liver tissue was still full of lipid vacuoles and no typical lobule was observed and this will not occur until 20 dph. Pancreas grew together with the liver tissue as a result of which the structure called hepatopancreas appears, and it began to form between 16 and 36 dph.

**Discussion**

An ontogeny survey is a comprehensive overview which can present essential results for more practical researches (Banaee & Naderi 2014; Abdali & Eagderi 2015; Faustino et al. 2018; Ghasemi et al. 2020). The present study was performed on *A. persicus*, and the results of the liver and pancreas development were relatively similar to other sturgeon.
species (Buddington & Christofferson 1985; Wegner et al. 2009). However, because of differences in biological and behavioral characteristics of *A. persicus* with other sturgeon species, the histological results are to some extent different. These differences are due to the developmental and ontological stages of liver and pancreatic tissues. The liver is the largest accessory organ located in the anterior part of the digestive system. As in other vertebrates, liver and pancreas of *A. persicus* are of endodermal origin (Ostaszewska 2005). Other researches indicated that at the hatching no liver and pancreas were visible in green and white sturgeon larvae (*A. medirostris* and *A. transmontanus*) (Buddington & Christofferson 1985), whereas Adriatic sturgeon larvae (*A. naccarii*) displayed both liver and pancreas at a similar developmental phase (Boglione et al. 1999).

In the present study, the histological investigation was done from 1 to 36 dph in which many of the most critical developmental changes have occurred. However, the alimentary canal did not acquire its shape on the second dph and liver appeared as a dense tissue and not a complete organ. On 7 dph compared to 2 dph, size of the liver was increased, which is related to the differentiation of hepatocytes. Wegner et al. (2009) found that the first symptoms of the hepatic and pancreatic activity were observed between the 6-8 dph in the sterlet (*A. ruthenus*). These different results could have been the result of within-species variation which requires more comparative studies. In the present work, 10 dph, the stomach and intestines acquired natural forms, the pancreas was visible, hepatocytes nuclei were complete, and cytoplasm was full of lipids.

Larvae of many fish species have three feeding stages, including endogenous feeding, mixed and individually exogenous feeding (Mani-Ponset et al. 1996). Ostaszewska et al. (2011) stated that *A. oxyrinchus* larvae began feeding on exogenous food and therefore at the beginning of exogenous feeding displayed entirely developed digestive glands, including the liver and pancreas. Alimentary glands must be activated and dynamic gastrointestinal (GI) system ready for digestion and absorption processes in the exogenous feeding stage in these animals.

Similar to Wegner (2009), distinguished histological alterations occur as soon as the exogenous feeding of larvae begins. Developmental changes between 12-18 dph are explained in detail in the results section and significant changes were related to liver central vein and sinusoids. In subsequent days, no significant histological changes
occurred and only growth and development of digestive tract glands were remarkable. Similar to other studies on sturgeon species (Wegner 2009), the present work indicated that the organization and discrimination of diverse digestive system structures in *A. persicus* are analogous to other *Acipenser* species and larvae at the beginning of exogenous feeding were adequately enlarged to utilize food correctly. The results also showed that the growth and development of various appendages of the gastrointestinal tract have occurred simultaneously with the change of the fish diet. Therefore, with the development of the digestive system, the efficiency of digestion and digestion will also increase.

**Conclusion**

The present finding on digestive system development may help improve rearing techniques for *A. persicus*. Our results showed that a morphologically functional hepatopancreas is established in the early phase of the larval period. It may be essential to feed *A. persicus* larvae under rearing conditions because a practice could, by reducing cannibalism, improve survival above the increases realized by improved nutrition.

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**References**


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مقاله پژوهشی

رشد و تکامل کبد و پانکراس در تاس ماهی ایرانی (Acipenser persicus) (Borodin, 1897)

مطالعه بافت‌شناسی

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چکیده: در مطالعه حاضر، مراحل مختلف آنتوژنی کبد و پانکراس در تاس ماهی ایرانی (Acipenser persicus) از تفریخ تا روز 36 پس از تفریخ به وسیله روش‌های بافت‌شناسی به تصویر کشیده شده است. اسلام‌ندازی از 5 میکروتوم با ضخامت 5 میکرومتر تهیه و با هم‌نوسیال‌های هماتوکزیل و اوزین، پریودیک اسید-شیف و تریکروم ماسون رنگ‌آمیزی شده است. در روز دوم پس از تفریخ، استرومای کبدی تشکیل شده و سلول‌های کبدی شمایل تعدادی با کولوی با هم‌نوسیال‌های پریودیک اسید-شیف مشاهده شده است. در روز 10 پس از تفریخ، پانکراس بین کبد و بخش قدامی روده مشاهده شده است. سلول‌های پانکراس به صورت کلی گروه‌های ترشحی توزیع شده‌اند. در روز 18 پس از تفریخ، عروق مرکزی و سینوس‌های کبدی به طور مشخصی در بخش میانی پانکراس چسبند. در روز 22 پانکراس به عنوان یک انداز فیبری به صورت رشد کرده است. سلول‌های پانکراس به صورت کلی مشاهده شده است. در روز 36 پس از تفریخ صورت می‌گیرد.

کلمات کلیدی: تاس ماهی ایرانی، کبد، پانکراس، تکامل بافت‌شناسی.