Mansoura Veterinary Medical Journal

Manuscript 1245

Early Post-Hatching Development of the Grass Carp Pancreas

Eman Fiala

Mahmoud B. Shoeib

Safwat Ebada

Ahmed M. Abdellatif

ORIGINAL ARTICLE

Early Post-hatching Development of the Grass Carp Pancreas

Eman Fiala, Mahmoud B. Shoeib, Safwat Ebada, Ahmed M. Abdellatif*

Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

Abstract

INTRODUCTION: The pancreas is a mixed gland that plays indispensable roles in digestion and glucose uptake. Grass carp [*Ctenopharyngodon idella* (*C. idella*)] is a cyprinid fish used in aquaculture, medicine, and research. Knowledge about the early posthatching development of grass carp pancreas is lacking.
OBJECTIVE: The present study aimed to elucidate the morphogenic events involving the pancreas of *C. idella* during the early posthatching period.
METHODS: Developmental steps involving the pancreas of *C. idella* during the early post-hatching period were studied using histological, histochemical, and morphometric techniques. Larvae were collected at 1-day posthatching (1 dph), 4 dph, 10 dph, and 20 dph and stained with hematoxylin and eosin and periodic acid-Schiff stains.
RESULTS: The pancreas of *C. idella* displayed progressive developmental changes throughout the study period. At 1 dph, the pancreatic primordium was located dorsal to the gut and caudal to the hepatic primordium. Zymogen granules were detected at 4 dph and appeared clearer onward, suggesting the initiation of pancreatic digestive activity at this age. At 10 dph, the pancreas appeared to be in close contact with the liver and spleen. At 20 dph, the pancreas significantly increased in size, was infiltrated by patches of adipose tissue, and housed a large number of pancreatic acini containing well-developed zymogen granules within the apical portions of their cytoplasm. Endocrine cells were detected among pancreatic acini at 20 dph and appeared to be more concentrated in the vicinity of the blood vessels.
CONCLUSIONS: The present study reports age-related structural changes in the pancreas of grass carp during the first three weeks of post-hatching life and will help to understand the pancreatic biology of the studied species.

Keywords: Development, Grass carp, Histochemistry, Pancreas, Zymogen granules

1. Introduction

Fish pancreas is a mixed gland consisting of exocrine and endocrine components [1]. The exocrine portion is composed of pancreatic acinar cells that produce digestive enzymes. These enzymes are transported to the intestine through a well-developed duct system. The endocrine pancreatic portion is represented by islets of Langerhans. The latter structures consist of various types of hormone-producing cells that release their secretions directly into the bloodstream [2,3].

Cyprinidae is a large family of teleost fish that comprises more than 3.000 species [4]. Grass carp [*Ctenopharyngodon idella* (*C. idella*)] is a Cyprinidae member with specialized herbivorous behavior. Grass carp was originally native to China and has spread to several parts of the world including Egypt [5]. Grass carp are regarded as a green tool against aquatic weeds because of their ability to ingest enormous amounts of aquatic flora. *C. idella* also serves as a research model due to its ability to withstand a wide range of temperature fluctuations and oxygen deprivation [6].

The pancreas of most vertebrates originates as two buds, dorsal and ventral, from a predetermined area of the foregut just caudal to the liver [7]. At the hatching stage, the pancreas is not distinguishable

Received 29 February 2024; revised 21 June 2024; accepted 21 June 2024.
Available online 30 July 2024

* Corresponding author at: Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.
E-mail address: abdellatif_ma@mans.edu.eg (A.M. Abdellatif).

https://doi.org/10.35943/2682-2512.1245

and appears as a mass located dorsal to the gut tube [8]. In zebrafish, the dorsal and ventral buds fuse at the time of hatching [9]. While the post-hatching morphogenic features of the pancreas have been investigated in various fish species including Nile tilapia (*Oreochromis niloticus*) [10] and zebrafish (*Danio rerio*) [11], the early development of the pancreas and its differentiation in grass carp have not been fully studied. The present study aimed to characterize the morphogenic steps in the pancreas of grass carp (*C. idella*) during the early posthatching period. Grass carp were collected at 1, 4, 10, and 20 days post-hatching (dph) and subjected to histological and histochemical analyses.

2. Material and methods

2.1. Collection of grass carp larvae

One hundred grass carp larvae (*C. idella*, Valenciennes, 1844) were collected from the Central Laboratory for Aquaculture and Fish Hatchery (El-Abbassa, Abu-Hammad, Sharkia Governorate, Egypt) at 1 dph. The complete taxonomy of the used species (NCBI:txid7959) is shown in Fig. 1. Within 2 h of collection, the larvae were brought in air-filled plastic aquaria to the Anatomy Laboratory at the Faculty of Veterinary Medicine, Mansoura University, Egypt. The present study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (M/106). The study was conducted by the ARRIVE guidelines and the National Institutes of Health Guide for Use of Animals in Research (NIH Publications No. 8023, revised 1978).

2.2. Acclimatization of grass carp larvae

Twenty 1 dph larvae were initially sampled at the hatchery. Following arrival at the laboratory, the remaining 1 dph larvae were allowed to acclimatize to laboratory conditions in an air-circulated freshwater tank. Sampling was subsequently performed

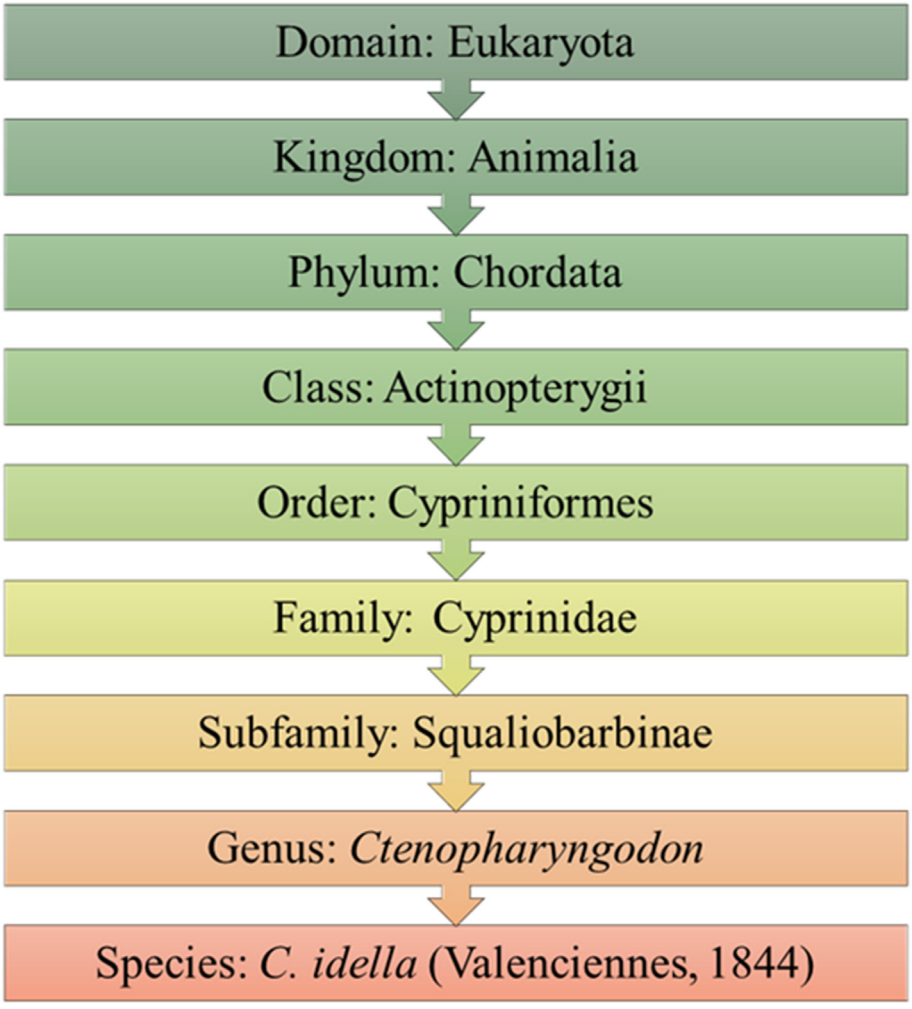

Fig. 1. Taxonomic classification of Ctenopharyngodon idella (Valenciennes, 1844).

Table 1. Fish adaptation strategy.

Larval age	Laboratory conditions
1 dph*	No feeding on the first day. Water tank at temperature of 23–25 °C. Oxygen and pH at 85% and 7.7, respectively. The photoperiod was kept at 12:12 h light dark cycle
2–4 dph	Feeding on egg yolk solution twice daily. The water was partially (10%) replaced daily
5–10 dph	Feeding on scrambled commercial fish ration (30% protein). The water was partially (10%) replaced daily
10–20 dph	Feeding on fine pelleted commercial fish ration (30% protein). The water was partially (10%) replaced daily

* dph, day posthatching.

at 4, 10, and 20 dph. Details of the fish adaptation strategies used in our laboratory are shown in Table 1.

2.3. Histological analysis

The whole larvae were fixed in 10% neutral buffered formalin for 72 h following their anesthesia in freshwater containing clove oil (30 μl/l) [12]. Clove oil is regarded as a powerful natural anesthetic agent for fish anesthesia [13]. Loss of tail response and cessation of movement were considered as signs of complete anesthesia. The formalin-fixed specimens were processed for paraffin embedding using routine histological methods. A 4-μm thick sections were cut, attached to glass slides, and stained with hematoxylin and eosin and periodic acid-Schiff (PAS) stainings as described elsewhere [14–16]. Briefly, paraffin sections were deparaffinized in xylene for 15 min and rehydrated in four descending grades of ethanol (absolute, 95, 80, and 50%) for 20 min. For hematoxylin and eosin staining, deparaffinized slides were immersed in Harris hematoxylin solution for 8 min, differentiated in 1% acid-alcohol solution (1% HCl in 70% ethanol) for 30 s, and finally immersed in 0.5% eosin Y solution for 1 min. Next, the slides were dehydrated in ascending grades of ethanol (95% and absolute) for 15 min, cleared in xylene for 15 min, and coverslipped using a synthetic mounting medium. For PAS staining, deparaffinized slides were oxidized in 0.5% periodic acid solution for 5 min, stained with Schiff's reagent for 15 min, and counterstained with Mayer's hematoxylin for 2 min. The slides were then dehydrated, cleared, and covered with coverslips.

2.4. Immunohistochemistry

Paraffin sections of 4 μm thickness were prepared for 3, 3′-diaminobenzidine (DBA) immunohistochemistry as previously described [14]. Briefly, sections were microwaved in sodium citrate (pH = 6) for 20 min. A solution containing 5% bovine serum albumin in PBS was used to mask the nonspecific epitopes for 1 h. Glucagon monoclonal antibody (14–9743-80; Invitrogen, MA, USA) was employed for 3 h. Biotinylated donkey anti-mouse secondary antibody (715-065-140; Jackson ImmunoResearch, PA, USA) was then added for 30 min, followed by the VECTASTAIN Elite ABC kit (PK-6100, Vector Laboratories, CA, USA) for another 30 min. The final immunoreaction was induced using a fresh DAB solution (SK-4103, Vector Laboratories). The sections were counterstained with Mayer's hematoxylin for 2 min, dehydrated, cleared, and covered with coverslips.

2.5. Morphometric analysis

The morphometric analysis in the present work included the diameter of pancreatic acini and the intensity of zymogen granules. Five microscopic images captured at 400× magnifications were analyzed for each larval age. All images were initially calibrated using ImageJ software (NIH, Bethesda, MD, USA) to convert the pixels to a micrometric scale before conducting the analysis. The diameter of pancreatic acini was estimated using the measure tool of the ImageJ software. The intensity of zymogen granules per pancreatocyte (10 randomly selected pancreatocytes per microscopic

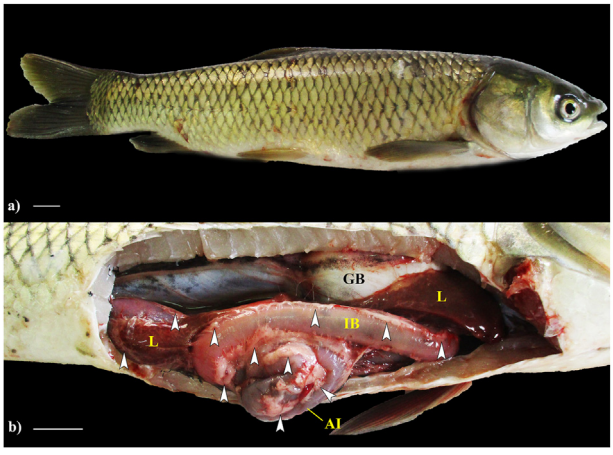

Fig. 2. Gross appearance of the pancreas in adult grass carp. a) Intact adult fish. b) Dissected adult fish; the pancreas (mesenteric form) is denoted by arrowheads. AI, anterior intestine; GB, gas bladder; IB, intestinal bulb; L, liver. Scale bar = 10 mm.

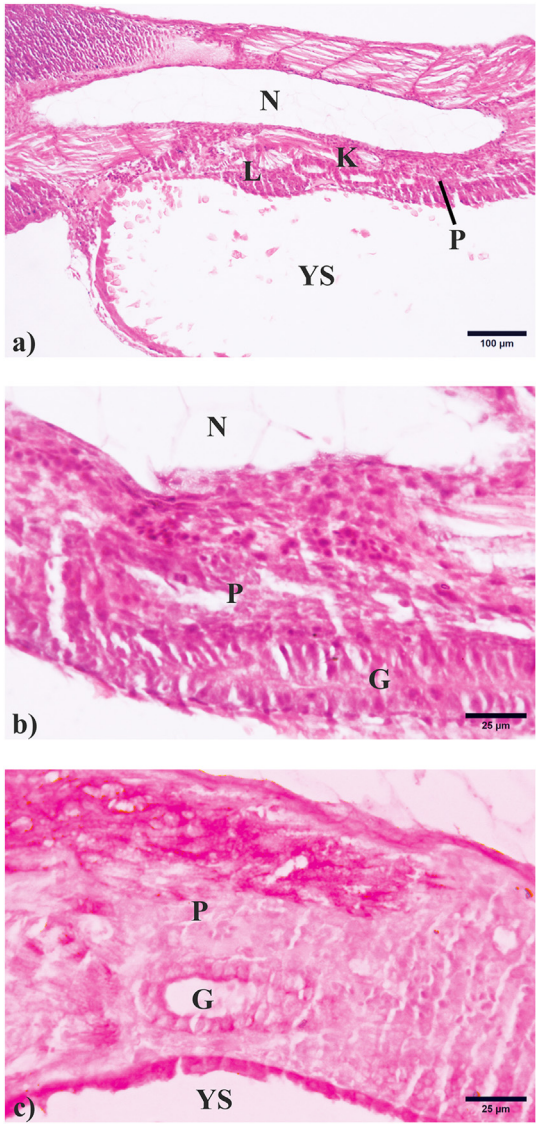

Fig. 3. Light microscopic appearance of the developing pancreas in grass carp larvae at 1-day post-hatching (1 dph). (a) The pancreatic primordium (P) of 1 dph grass carp is located dorsal to the developing gut tube (G) at an area caudal to the developing liver (L). Hematoxylin and eosin stain. (b) High magnification of Fig. 3a showing the pancreas (P) consisting of undifferentiated cells. (c) The pancreatic anlage displayed a weak reaction to PAS staining. PAS stain. K, kidney; N, notochord; YS, yolk sac.

field) was calculated using the color histograms of the same software.

2.6. Statistical analysis

Data were analyzed using GraphPad Prism 7 (GraphPad Software, CA, USA) and presented as mean ± SD. The differences in acinar diameter and zymogen granules intensity between various larval ages were determined using one-way analysis of variance followed by Tukey's multiple comparison tests. *P* values less than 0.05 were used to indicate statistical significance.

3. Results

3.1. Overview of the gross appearance of the pancreas in adult grass carp

Grossly, the pancreas of adult grass carp appeared as yellowish glandular masses of variable sizes interspersing the celomic cavity and filling the spaces between the celomic viscera, including the liver, intestinal bulb, and intestines (Fig. 2).

3.2. Early developmental microanatomy of the grass carp pancreas

The pancreas of grass carp (*C. idella*) revealed progressive developmental changes during the early post-hatching life (Figs. 3–6).

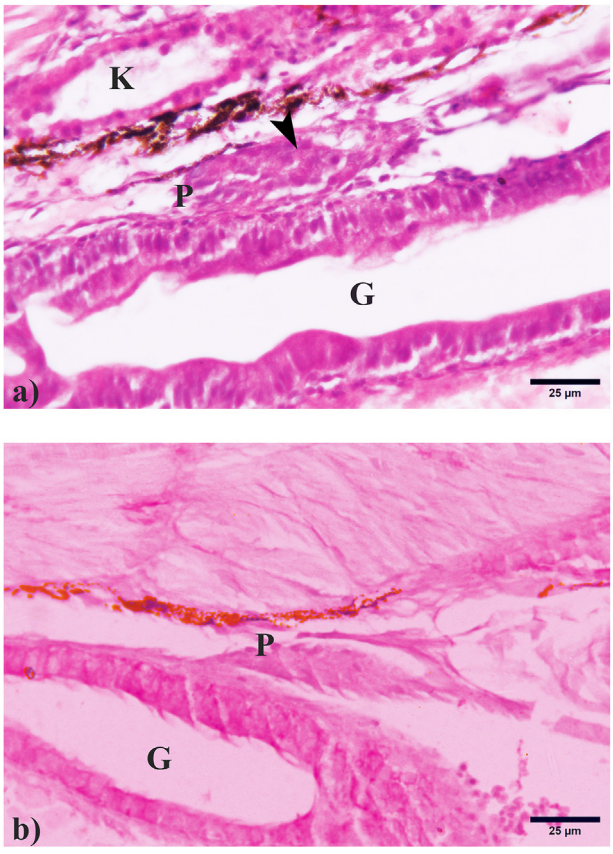

Fig. 4. Light microscopic appearance of the developing pancreas in grass carp larvae at 4-days post-hatching (4 dph). (a) The pancreas (P) of 4 dph grass carp is located dorsal to the gut tube (G) and ventral to the kidney (K). Note the granular cytoplasm of pancreocytes indicating the formation of zymogen granules (arrowhead). Hematoxylin and eosin stain. (b) The cytoplasm of pancreocytes exhibited a moderately positive reaction to PAS staining. PAS stain.

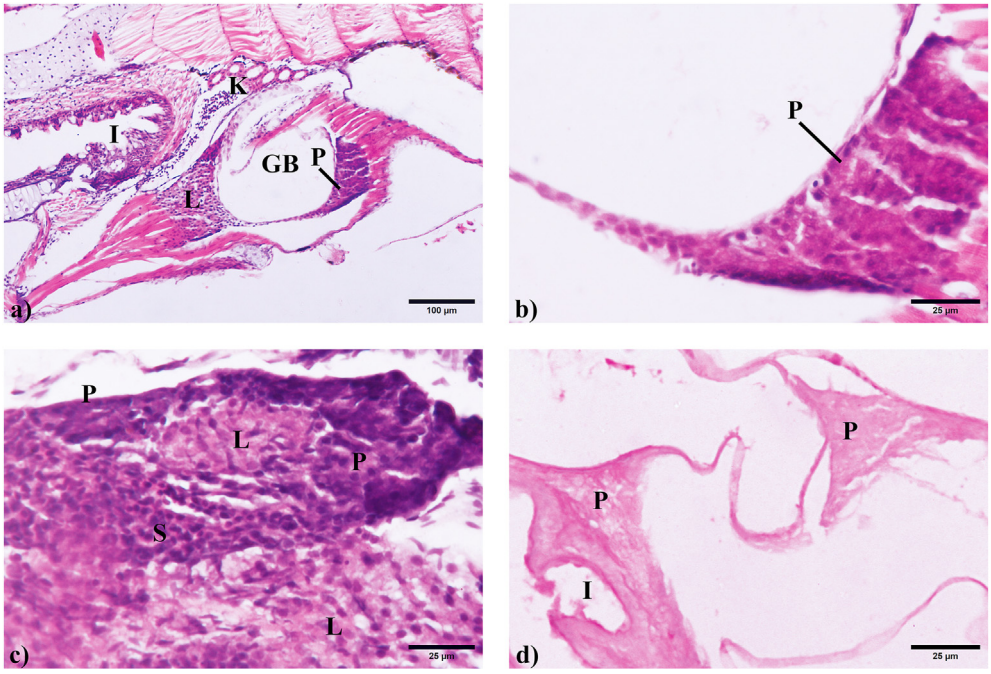

Fig. 5. Light microscopic appearance of the developing pancreas in grass carp larvae at 10-days post-hatching (10 dph). (a) The pancreas (P) of 10 dph grass carp is located caudal to the liver (L) and gas bladder (GB). Hematoxylin and eosin stain. (b) High magnification of Fig. 5a showing the pancreas (P) consisting of several pancreatic acini. (c) The pancreas is closely related to the liver (L) and spleen (S). Hematoxylin and eosin stain. (d) The mesenteric portion of pancreas exhibited a moderately positive reaction to PAS staining. PAS stain. I, intestine; K, kidney.

At 1-day post-hatching (1 dph), the primordium of the pancreas was observed dorsal to the developing gut tube, which appeared potentially closed by this age, in an area caudal to the liver (Fig. 3a and b). No evidence of zymogen granule formation was detected at this age (Fig. 3b). The pancreatic cells showed a weak reaction to PAS staining at this age (Fig. 3c).

At 4 dph, the pancreas was seen on the dorsal aspect of the gut tube ventral to the gas bladder and developing kidney (Fig. 4a). The cytoplasm of the pancreocytes revealed a granular eosinophilic appearance indicating the formation of zymogen granules by this age (Fig. 4a). The cytoplasm of the pancreocytes showed a moderately positive reaction to PAS staining (Fig. 4b).

At 10 dph, the pancreas increased in size and was found caudoventral to the gas bladder and caudal to the liver, to which it was connected by a narrow strip of cells (Fig. 5a and b). The number of pancreatic acini was remarkably higher than that at 4 dph (Fig. 5b). A group of these acini was in close contact with the liver and spleen (Fig. 5c). A number of pancreatic acini were seen attached to the intestine and mesenteric pancreas and exhibited a moderately positive reaction to PAS staining (Fig. 5d).

At 20 dph, the pancreas revealed a significant expansion in its dimensions, infiltrated by patches of adipose tissue, and appeared intimately related to the liver and spleen (Fig. 6a). A group of pancreatic acini surrounded the branches of the portal vein as they entered the hepatic parenchyma (Fig. 6b). The mesenteric pancreas showed progression in size, where several pancreatic acini were clustered within the spaces in-between the intestinal coils (Fig. 6c). The pancreatic acini contained well-developed zymogen granules within the apical portions of the cytoplasm (Fig. 6d). Exocrine pancreatic acini displayed a strong positive reaction to PAS staining (Fig. 6e). Small clusters of endocrine cells with round nuclei and pale stained cytoplasm were observed among the pancreatic acini in close position to small blood vessels (Fig. 6f and g). The cytoplasmic expression of glucagon by these endocrine cells was evident immunohistochemically (Fig. 6h). The main histological and histochemical findings related to early pancreatic development in grass carp observed in this study are summarized in Table 2.

3.3. Morphometric analysis of grass carp pancreas

The morphometric study involved the diameter of the pancreatic acini and the intensity of zymogen granules (Table 3). The diameter of pancreatic acini

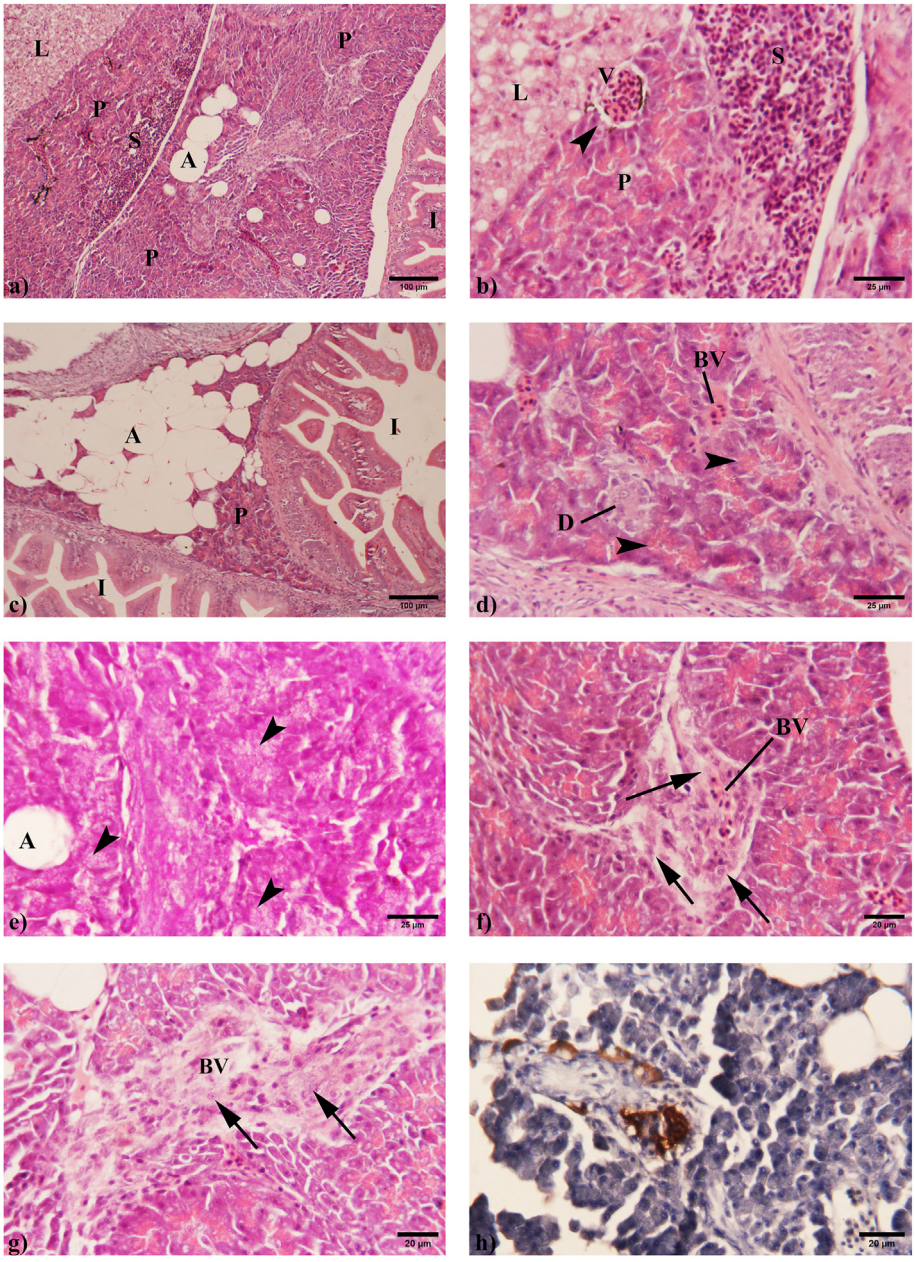

Fig. 6. Light microscopic appearance of the developing pancreas in grass carp larvae at 20-days post-hatching (20 dph). (a) The pancreas (P) of 20 dph grass carp is remarkably expanded to fill the spaces between the liver (L) and spleen (S). Hematoxylin and eosin stain. (b) A group of pancreatic acini encircled a portal vein branch as it enters the liver (V; arrowheads). Hematoxylin and eosin stain. (c) The mesenteric portion of pancreas (P) increased in size and is disseminated within a mass of adipose tissue (A). Hematoxylin and eosin stain. (d) High magnification of Fig. 6c showing the exocrine pancreas consisted of pancreatic acini containing visible zymogen granules with eosinophilic staining (arrowheads) and an intrapancreatic duct (D). (e) Strong positive reaction of zymogen granules (arrowheads) to PAS staining. PAS stain. (f, g) Endocrine cells (arrows) are detected in the vicinity of an intrapancreatic blood vessel (BV). Hematoxylin and eosin stain. (h) Glucagon immunoreaction (brown color) within grass carp pancreas is shown within a subset of endocrine cells. DAB immunohistochemistry. A, adipose tissue; BV, blood vessel; I, intestine.

significantly increased with larval age (12 ± 1.6 μm at 4 dph; 19 ± 1.75 μm at 10 dph; 29.5 ± 2.7 μm at 20 dph, $P < 0.05$ for all). In the same way, the rise in the intensity of zymogen granules was age-dependent (110 ± 5.0 at 4 dph; 133 ± 8.0 at 10 dph; 182 ± 15 at 20 dph, $P < 0.05$ for all).

4. Discussion

The pancreas contributes significantly to both digestion and glucose homeostasis. Grass carp larvae hatch at an advanced stage as the digestive tract and its associated glands are formed, but are

Table 2. Summary of the main findings related to the early development of grass carp pancreas.

Larval age	Position of pancreas	Area of expansion	Zymogen granules	Endocrine cells
1 dph*	Dorsal to the developing gut and caudal to the liver	Narrow strip of small undifferentiated cells	Not detected; weak reaction to PAS staining	Not detected
4 dph	Along the dorsal aspect of the gut ventral to the kidney	Narrow strip of differentiated cells	Detected; moderate reaction to PAS staining	Not detected
10 dph	In close contact to the liver, spleen, and intestinal coils	Partially filled the available spaces between nearby viscera.	Detected; moderate reaction to PAS staining	Not detected
20 dph	Intimately related to the liver, spleen, and intestine	Almost completely filled the available spaces between nearby viscera	Detected; strong reaction to PAS staining	Detected as small cellular clusters among the acini of the exocrine pancreas

* dph, day posthatching.

Table 3. Morphometric analysis of the developing grass carp pancreas.

Item	Larval age		
	4 dph*	10 dph	20 dph
Acinar diameter (μm)	12 ± 1.6^{a}	19 ± 1.75^{b}	29.5 ± 2.7^{c}
Zymogen granules intensity	110 ± 5.0^{a}	133 ± 8.0^{b}	182 ± 15.0^{c}

Different superscript letters indicate statistical significance. *P* less than 0.05.

* dph, day posthatching.

still not fully functional [17]. The present study examined progressive changes in the structure of the developing pancreas at 1, 4, 10, and 20 dph. The pancreatic anlage appeared as an elongated structure at the dorsal aspect of the gut caudal to the liver in 1 dph grass carp larvae. This finding is in line with those of Morrison *et al.* [18] in Nile tilapia (*Oreochromis niloticus*) and Kumari *et al.* [19] in Indian walking catfish (*Clarias magur*) in which the pancreas was formed at the time of hatching.

At 1 dph the gut of the developing larvae is still closed, as the larvae depend totally on endogenous feeding from the yolk sac at this stage. The digestive activity of the teleost's pancreas is usually gained soon after hatching [10,20]. Posthatching maturation of exocrine cells usually involves their ability to secrete and store digestive enzymes into specialized secretory granules termed zymogen granules in their cytoplasm [21]. The enzymatic contents of zymogen granules are released into the lumina of pancreatic acini as a starting point of their journey toward the intestinal lumen. In the present study, the cytoplasm of pancreatic acinar cells of grass carp appeared remarkably granular at 4 dph, suggesting the onset of formation of a massive amount of zymogen granules. The appearance of zymogen granules is accompanied by marked regression in the size of the yolk sac and a possible transition of grass carp from endogenous to exogenous feeding [22]. In line with our findings, zymogen granules were seen inside the pancreatic acinar cells at 2 dph in golden mahseer (*Tor putitora*) [23], 3 dph in longfin yellowtail (*Seriola rivoliana*) [24] and Indian walking catfish (*Clarias magur*) [19], and 5 dph in European catfish (*Silurus glanis*) [25]. Taken together, these data point to the first week posthatching as a critical time window for acquiring the digestive activity of the fish pancreas.

The present study revealed an increase in pancreatic size from 1 to 20 dph. At 20 dph the pancreas appeared more disseminated in the mesentery, and parts of it surrounded the hepatic and splenic blood vessels. Similar age-related expansion of the pancreas has been reported in Nile tilapia (*Oreochromis niloticus*) [18]. The increase in pancreatic size possibly represents an age-related adaptation of the larvae to the progressively increasing digestive and metabolic demands.

The pancreatic acini of 20 dph grass carp larvae appeared at the periphery of the liver adjacent to the intestinal bulb. Many pancreatic acini appeared to enter the hepatic parenchyma via encircling the venous tracts entering the liver at various points. These findings were similar to those reported by Sousa *et al.* [26] in guppy fish. Pancreatic acini were also detected within the intestinal mesentery at 20 dph with several pancreatic cells in-between them. These findings are correlated with those described by Morrison *et al.* [18] in *Oreochromis niloticus*.

The endocrine pancreatic portion secretes several hormones that are essential for glucose uptake and energy production. The endocrine cells of grass carp pancreas were identified as small, dispersed cells with round nuclei surrounded by a pale stained cytoplasm at 20 dph by the present work. Glucagon expression in these endocrine cells was confirmed immunohistochemically using specific antibodies. In agreement with our findings, endocrine cells were observed at 20 dph in longfin yellowtail (*Seriola*

rivoliana) [24] and 19 dph in European catfish (*Silurus glanis*) [25] using routine histological techniques. However, Morrison *et al.* [18] reported the presence of a single principal islet, positive for insulin, glucagon, somatostatin, and pancreatic polypeptide, in the pancreas of Nile tilapia (*Oreochromis niloticus*) from the first-day posthatching. The latter study may suggest species-specific differences in the timing of the appearance of pancreatic endocrine cells and define the immunohistochemical technique as more reliable compared to conventional histological methods for approximate identification and localization of each type of endocrine pancreatic cells.

5. Conclusions

The present study revealed several aspects of the pancreas during early post-hatching development in grass carp. Further studies are needed to identify mechanisms governing the proliferation and differentiation of each cell type.

Ethics approval

The current research work was permitted to be executed according to the standards of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (M/106).

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

This work is part of the MSc thesis by E.F.E.F. and A.A. performed the practical study, A.A. wrote the initial draft, and A.A., M.S., and S.E. finalized the manuscript. All authors supervised the findings of this study and approved the final version of the manuscript for publication.

Availability of data and materials

The study data are available upon request from the corresponding author.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank all members of the Department of Anatomy and Embryology, Faculty of Veterinary Medicine at Mansoura University (Egypt) for their help during the accomplishment of this work.

References

[1] Mokhtar DM. Histological, histochemical and ultrastructural characterization of the pancreas of the grass carp (Ctenopharyngodon idella). Eur J Anat 2015;19:145−53.
[2] Chanet B, Schnell NK, Guintard C, Chen W-J. Anatomy of the endocrine pancreas in actinopterygian fishes and its phylogenetic implications. Sci Rep 2023;13:22501.
[3] Heller RS. The comparative anatomy of islets. In: Islam MS, editor. Islets of langerhans. Dordrecht: Springer Netherlands; 2015. p. 1−18.
[4] Kuang Y-Y, Zheng X-H, Li C-Y, Li X-M, Cao D-C, Tong G-X, et al. The genetic map of goldfish (Carassius auratus) provided insights to the divergent genome evolutions in the Cyprinidae family. Sci Rep 2016;6:34849.
[5] Shen Y, Wang L, Fu J, Xu X, Yue GH, Li J. Population structure, demographic history and local adaptation of the grass carp. BMC Genom 2019;20:1−16.
[6] Fan Z, Deng Y, Yuan Q, Liu X, Shi H, Feng C, et al. Effect of total dissolved gas supersaturation on the tolerance of grass carp (Ctenopharyngodon idellus). Environ Sci Eur 2020;32:1−10.
[7] Bastidas-Ponce A, Scheibner K, Lickert H, Bakhti M. Cellular and molecular mechanisms coordinating pancreas development. Development 2017;144:2873−88.
[8] Lavergne A, Tarifeño-Saldivia E, Pirson J, Reuter A-S, Flasse L, Manfroid I, et al. Pancreatic and intestinal endocrine cells in zebrafish share common transcriptomic signatures and regulatory programmes. BMC Biol 2020;18:1−19.
[9] Kimmel R, Meyer D. Zebrafish pancreas as a model for development and disease. Methods Cell Biol Elsevier 2016;134:431−61.
[10] del Valle JC, Zanazzi AN, Rodriguez YE, Haran NS, Laitano MV, Mallo JC, et al. Morphological changes, peptidase activity, and effects of exogenous enzymes in the early ontogeny of Nile tilapia, Oreochromis niloticus. Aquacult Int 2022;30:1645−58.
[11] Guerrera MC, De Pasquale F, Muglia U, Caruso G. Digestive enzymatic activity during ontogenetic development in zebrafish (Danio rerio). J Exp Zool B Mol Dev Evol 2015;324:699−706.
[12] Fernandes IM, Bastos YF, Barreto DS, Lourenço LS, Penha JM. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz J Biol 2017;77:444−50.
[13] Aydõn B, Barbas LAL. Sedative and anesthetic properties of essential oils and their active compounds in fish: a review. Aquaculture 2020;520:734999.
[14] Abdellatif AM, Farag A, Metwally E. Anatomical, histochemical, and immunohistochemical observations on the gastrointestinal tract of Gallinula chloropus (Aves: Rallidae). BMC Zoology 2022;7:61.
[15] Layton C, Bancroft JD. Carbohydrates. In: Suvarna KS, Layton C, Bancroft JD, editors. Bancroft's theory and practice of histological techniques E-book. Philadelphia: Elsevier; 2019. p. 176−97.
[16] Abdellatif AM, Lashen S, Kandyel RM, Shoeib MB, Madkour FA. Age-related morphological and ultrastructural changes in the palate and pharyngeal masticatory apparatus of grass carp (Ctenopharyngodon idella) juveniles. Tissue Cell 2024;86:102264.
[17] George AE, Chapman DC. Embryonic and larval development and early behavior in grass carp, Ctenopharyngodon idella: implications for recruitment in rivers. PLoS One 2015;10:e0119023.
[18] Morrison CM, Pohajdak B, Tam J, Wright Jr JR. Development of the islets, exocrine pancreas, and related ducts in the Nile tilapia, Oreochromis niloticus (Pisces: Cichlidae). J Morphol 2004;261:377−89.

[19] Kumari R, Sharma P, Sarma D, Siddaiah GM, Dubey MK, Mir IN, et al. Ontogeny and development of the gastrointestinal system in Indian walking catfish (Clarias magur) during its early development. Fish Physiol Biochem 2021;47:1033−52.
[20] Zheng X, Du C, Gao X, Ni J, Wang Y, Hou C, et al. Changes in the histology and digestive enzyme activity during digestive system development of silver pomfret (Pampus argenteus). Aquaculture 2023:739905.
[21] Gorelick FS, Pandol S, Jamieson JD. Structure-function relationships in the pancreatic acinar cell. In: Physiology of the gastrointestinal tract. Cambridge, Massachusetts: Elsevier; 2018. p. 869−94.
[22] Kossakowski M. The influence of temperature during the embryonic period on larval growth and development in carp, Cyprinus carpio L., and grass carp, Ctenopharygodon idella (Val.): theoretical and practical aspects. Archwum Polskiego Fish 2008;16:231−314.
[23] Sharma P, Akhtar MS, Singh AK, Das P, Sarma D. Histomorphological changes in digestive tract of golden mahseer (Tor putitora) during different developmental stages. Fish Physiol Biochem 2016;42:1681−98.
[24] Teles A, Salas-Leiva J, Alvarez-González CA, Gisbert E, Ibarra-Castro L, Urbiola JCP, et al. Histological study of the gastrointestinal tract in longfin yellowtail (Seriola rivoliana) larvae. Fish Physiol Biochem 2017;43:1613−28.
[25] Kozarić Z, Kuzir S, Petrinec Z, Gjurcević E, Bozić M. The development of the digestive tract in larval European catfish (Silurus glanis L.). Anat Histol Embryol 2008;37: 141−6.
[26] Sousa S, Rocha MJ, Rocha E. Characterization and spatial relationships of the hepatic vascular-biliary tracts, and their associated pancreocytes and macrophages, in the model fish guppy (Poecilia reticulata): a study of serial sections by light microscopy. Tissue Cell 2018;50:104−13.