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Original Article

Gonadal histology of the tiger barb *Puntius tetrazona* (Cyprinidae)

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Abstract

The ornamental fish industry constitutes a big portion of the economy in many countries of the world. Thousands of attractive fish species are traded annually. The tiger barb *Puntius tetrazona* is one of the most charming freshwater aquarium species worldwide. The present paper aimed at expanding the knowledge of the reproductive biology of this tropical fish. The ovary and the testis tissues of the tiger barb were embedded in paraffin following routine histological processes, stained with hematoxylin and eosin and Mallory's trichrome techniques, and investigated by light microscopy. Histological examinations confirmed asynchronous-type ovaries resembling four different developmental stages including primary growth, cortical alveolar, vitellogenic and maturation. In the testis, primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa were identified. All oogenetic and spermatogenetic phases and mature germ cells occurred simultaneously in the tiger barb. This feature allows this tropical fish to breed rapidly and helps expanding its global trades.

Keywords: *Puntius tetrazona*; tiger barb; ovary; testis; histology; gonadal stages

1 | INTRODUCTION

Fish are attractive organisms kept by humans for hobby or aesthetic purposes. Ornamental fish may be both freshwater and marine species in many sizes, shapes, and colours that are reproduced and traded widely for the last several decades (Sales and Janssens 2003). For many countries, ornamental fish trade comprises a big part of the economy (Rodrìguez 2006). It is estimated that over one billion ornamental fish including hundreds of freshwater and marine species are traded annually (Whittington and Chong 2007).

Tiger barb or Sumatra barb (*Puntius tetrazona*) is a tropical freshwater ornamental fish that is native to Southeast Asia throughout the Malay Peninsula, Sumatra, Borneo, and Cambodia (Barik *et al.* 2018). They belong to the family of Cyprinidae and are characterised by reddish or brownish body with four vertical black stripes located across the eye, in front of the pelvic base, above the anal fin and at base of the caudal part. This colouration pattern gives rise to take the name of 'tiger' (Kottelat 2013). Tiger barb are very popular and commercially important aquarium fish worldwide due to their strikingly bright colouration, active lifestyle and easy maintenance (Norazila and Patimah 2002; Galib and Mohsin 2010; Galib *et al.* 2013; Roosta and Hoseinifar 2016).

It is necessary to reveal the reproductive biology and behaviour of fishes to increase the effectiveness of fishery resources (Brewer *et al.* 2008; Grandcourt *et al.* 2009; Muchlisin *et al.* 2010). Several authors reported the reproductive biology features such as sex ratio, macroscopic and microscopic gonadal development, gonadosomatic index, and fecundity rate of a range of freshwater tropical fishes (Nasution 2005; Nasution *et al.* 2007; Muchlisin *et al.* 2010).

Determining and identification of the gonadal development is considered a key parameter for revealing the effective and healthy reproduction in reproductive studies (West 1990; Montchowui *et al.* 2012; Dopeikar *et al.* 2015). Histological examinations serve as a powerful technique to evaluate the gonadal structure and developmental stages of fishes (West 1990; Paugy and Lévêque 1999; Blazer 2002; Montchowui *et al.* 2012). The aim of the current study was to investigate the histology of ovary and testis of *P. tetrazona* with a view to expanding the knowledge of reproductive biology.

2 | METHODOLOGY

Ten adult tiger barb (five females and five males, 1.89 – 2.78 g in weight and 3.8 – 4 cm in length) were obtained from a commercial supplier. They were maintained in a 40 L glass aquarium with well-aerated aged tap water at 26±2 °C for two weeks, at 14 : 10 (light : dark) photoperiod. They were fed twice daily with frozen blood worm and brine shrimp.

Fish were euthanized with MS-222 (250 mg L^{-1}), ovary and testis tissues were removed and fixed in Bouin's fluid for 48 h at room temperature. Specimens were dehydrated in a series of ethanol solutions (70%, 96% and 100%); cleared in xylol and embedded in paraffin. 5 µm-thick cross-sections were stained with hematoxylin and eosin (H&E) or Mallory's trichrome (MT) and investigated by light microscopy. Microphotographs were taken with Zeiss Axio Scope. A1 equipped with Zeiss AxioCam ERc5s camera.

3 | RESULTS

The ovary was a paired organ located bilaterally between the abdominal wall and swim bladder. Histological examinations showed that all specimens of *P. tetrazona* had an asynchronous-type ovary. Four different developmental stages such as primary growth, cortical alveolar, vitellogenic and maturation were observed (Figure 1). In addition, follicular cells (Figures 2b, 2c, 2d & 2g), zona radiata (vitelline envelope) (Figures 2b, 2c, 2e & 2g) and atretic oocytes (Figures 1 & 2h) were identified.

In the primary growth stage, oocytes were smaller and spheric. The nucleus was in the centre of the oocyte while the nucleoli were in the peripheral zone. Ooplasm was more intense in comparison to the other stages. In this stage zona radiata was not observed (Figure 2a).

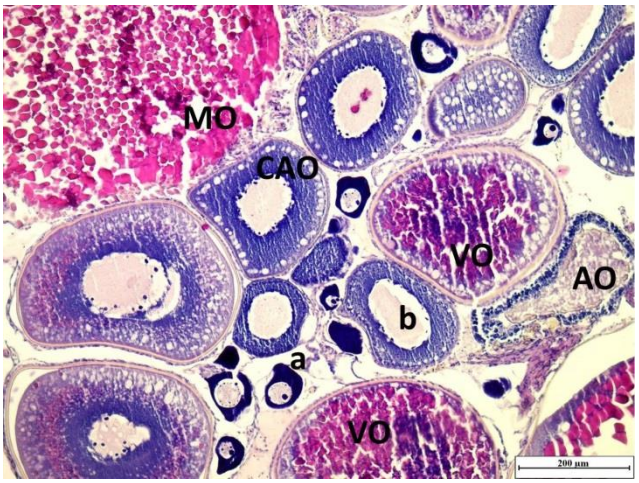

FIGURE 1 Ovarian histology of *Puntius tetrazona*. *a*, early phase of primary growth stage; *b*, late phase of primary growth stage; CAO, cortical alveolar oocyte; VO, vitellogenic oocytes; MO, mature oocyte; AO, atretic oocyte; H&E stain, scale bar 200 µm.

The cortical alveolar stage was identified with the cortical alveoli that started to form from the periphery of the oocyte. These alveoli gradually grew, increased in number, migrated to the centre and filled the oocyte. Zona radiata was observed in this stage for the first time (Figures 2b & 2c).

Yolk granules were determined firstly in the middle of the growing oocyte in the vitellogenic stage. Cortical alveoli decreased in number and observed only peripherally. Zona radiata was thickened. Because of the intense accumulation of yolk, the area of ooplasm was narrowed (Figures 2d, 2e & 2f).

In the maturation stage, oocytes were filled by vitellus droplets, cortical alveoli were noticed at the peripheral area and a thin ooplasm layer was noted. Yolk granules merged and became bigger than the cortical alveoli in size. In this stage, nuclei could not be identified (Figure 2g). Finally, atretic oocytes were observed with disrupted vitellus (Figure 2h).

The testis was a paired and elongated organ. The structural organization of the testis was shown in Figure 3. It was surrounded by outer tunica albuginea. It contained numerous seminiferous tubules covered by a thin basement membrane and interstitial tissue among these tubules was observed. Leydig cells (Figure 4b) were noticed in the interstitial area. Large solitary primary spermatogonia, secondary spermatogonia (Figure 4a) in small clusters and Sertoli cells (Figure 4b) with their irregular nuclei were identified at the luminal side of the basement membrane which bounds the tubules. Primary spermatocytes were discerned with their homogeneously distrib-

uted chromatin content. Secondary spermatocytes were spheric shaped, basophilic and they had condensed chromatin material. Spermatids were also spheric and basophilic cells smaller than the secondary spermatocytes. Spermatozoa were the smallest germ cells filled the lumen of the tubule (Figure 4a).

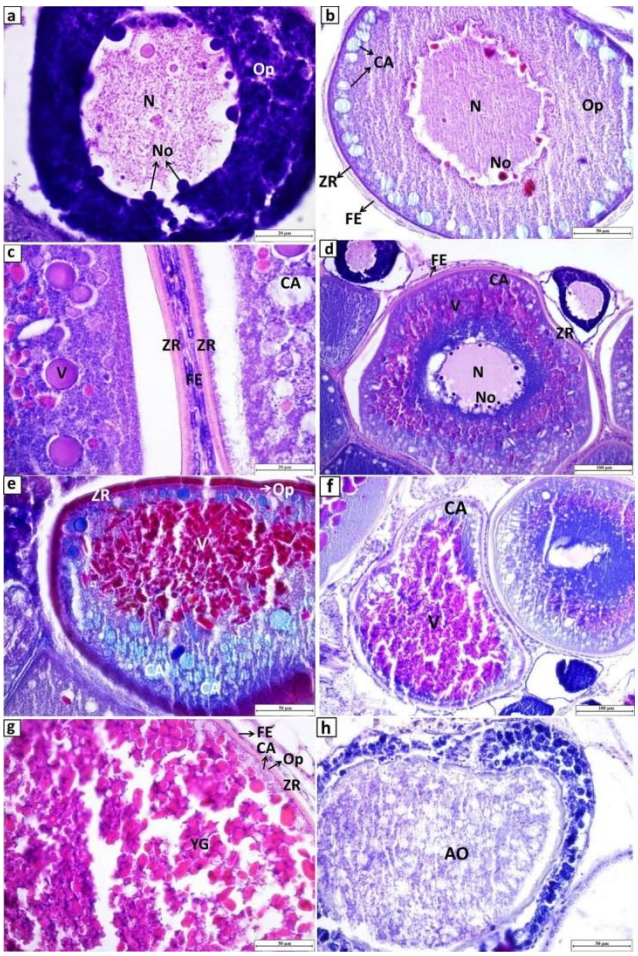

FIGURE 2 Different stages of oocyte development. *a*, primary growth stage (*N*, nucleus; *No*, Nucleoli; *Op*, Ooplasm; H&E stain, scale bar; 20 µm); *b*, cortical alveolar stage (*N*, nucleus; *No*, nucleoli; *Op*, ooplasm; *CA*, cortical alveoli; *ZR*, zona radiata; *FE*, follicular epithelium; MT stain, scale bar 50 µm); *c*, detailed structure of zona radiata (ZR), follicular epithelium (FE) and vitellus droplets (V) of an oocyte at cortical alveolar (CA) stage; H&E stain, scale bar 20 µm; *d*, early vitellogenic stage (*N*, nucleus; *No*, nucleoli; *V*, vitellus droplet; *CA*, cortical alveoli; *ZR*, zona radiata; *FE*, follicular epithelium; H&E stain, scale bar 100 µm); *e*, late vitellogenic stage (*V*, vitellus droplets; *CA*, cortical alveoli; *Op*, ooplasm; *ZR*, zona radiata; MT stain, scale bar 50 µm); *f*, late vitellogenic stage (*V*, vitellus droplets; *CA*, cortical alveoli; MT stain, scale bar 100 µm); *g*, mature oocyte (*YG*, yolk granules; *Op*, ooplasm; *CA*, cortical alveoli; *ZR*, zona radiata; *FE*, follicular epithelium; H&E stain, scale bar 50 µm); and *h* showing an atretic oocyte (*AO*) (H&E stain, scale bar 50 um).

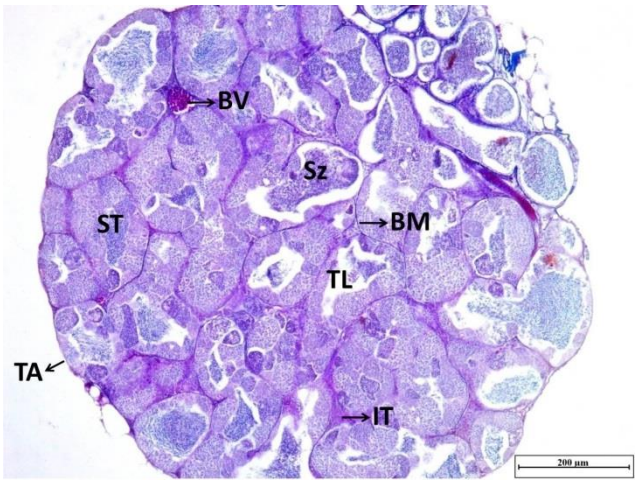

FIGURE 3 Testicular histology of *Puntius tetrazona*. *TA*, tunica albuginea; *BM*, basement membrane; *ST*, seminiferous tubules; *IT*, interstitial tissue; *BV*, blood vessel; *TL*, tubule lumen; *Sz*, spermatozoa; MT stain, scale bar 200 µm.

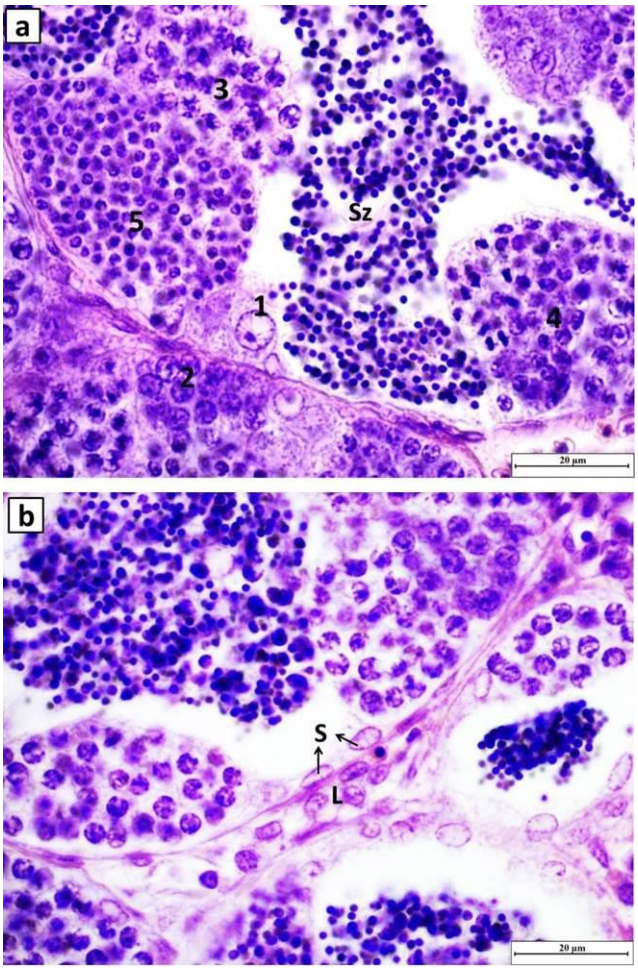

FIGURE 4 Above, different stages of spermatogenesis (*1*, primary spermatogonia; *2*, secondary spermatogonia; *3*, primary spermatocytes; *4*, secondary spermatocytes; *5*, spermatids; *Sz*, spermatozoa; H&E stain, scale bar; 20 µm), and below, Sertoli (*S*) and Leydig cells (*L*) (H&E stain, scale bar 20 um).

4 | DISCUSSION

Cyprinidae is one of the most important teleost groups due to various reasons including its economic importance. Cyprinids constitute a remarkable part of human food sources as well as very attractive ornamental aquarium species. There have been several studies concerning the developmental stages of both female and male gonads of cyprinid fish such as *Labeo cylindricus* (Booth and Weyl 2000), *Cyprinus carpio* (Smith and Walker 2004), *Barbus neefi* (Vlok 2005) and *Danio rerio* (Çakıcı and Üçüncü 2007; Koc *et al.* 2012). In this paper, the tiger barb was chosen due to its tremendous popularity in aquarium trade. Revealing the reproductive features of the fish requires focusing on the gonadal development stages. Histological examinations have been the most precise method besides oocytes size measurements, gonadic indices determination, and macroscopic examinations for evaluating the developmental stages (Paugy and Lévêque 1999; Montchowui *et al.* 2012).

Our results showed that *P. tetrazona* had an asynchronous-type ovary represented by four main different developmental stages. In the asynchronous ovary, all stages of developing oocytes are available without a dominant population in the same period. In tropical cyprinid *D. rerio* asynchronous oocyte with similar development phases were also observed (Çakıcı and Üçüncü 2007; Koc and Akbulut 2012). It was reported that the ovaries of *L. cylindricus* and *B. neefi* were synchronous iteroparous (Booth and Wely 2000) but its seasonal developmental pattern in *B. neefi* did not match with the pattern observed in *L. cylindricus* (Vlok 2005). The six oocyte developmental stages (chromatin-nucleolus oocytes, perinuclear oocytes, primary yolk vesicle oocytes, secondary yolk vesicle oocytes, tertiary yolk vesicle oocytes and atretic oocytes) were observed in both *L. cylindricus* and *B. neefi*. Another synchronous iteroparous *L. parvus* samples showed five oocyte development stages including chromatin-nucleolus oocytes, perinucleolus oocytes, yolk vesicle oocytes, vitellogenic oocytes and mature oocytes (Montchowui *et al.* 2012). Rutaisire and Booth (2004) investigated the ovulation in *L. victotarius* and identified eight discrete stages including oogonia, chromatin nucleolar oocytes, perinucleolar oocytes, primary yolk vesicle oocytes, secondary yolk vesicle oocytes, tertiary yolk vesicle oocytes, post-ovulatory follicles and atretic oocytes. The ovary of *Varicorhinus gerlachi* was reported as non-synchronous and it began to spawn in April and the peak periods were from May to July (Zou *et al.* 2011). Six different stages of ovary development in *V. gerlachi* was reported, original differentiation, yolk nucleus stage, vacuolated oocyte, differentiated oocytes, formed eggs and degenerated oocytes (Zou *et al.* 2011). Smith and Walker (2004) indicated six oocyte development stages in *C. carpio*; these are perinucleolar, yolk vesicle, primary yolk, secondary yolk, tertiary yolk and migratory nucleus. However, Shabanipour and Hossayni (2010) divided the oogenesis of *C. carpio* into immature, primary growth, cortical alveoli, vitellogenesis and maturation stages. It has also been pointed out that the gonadal development is continuous with optimum light and temperature regime and spawning occurred asynchronously in the population (Smith and Walker 2004).

In the current study, *P. tetrazona* testis showed four main stages of spermatogenesis as spermatogonia, spermatocytes, spermatids and spermatozoa simultaneously. In *D. rerio* all these stages were observed in separate cell groups in the same seminiferous tubules (Koc *et al.* 2012) similar to *P. tetrazona*. These stages were also observed in *L. cylindricus* (Booth and Weyl 2000) and *B. neefi* during all four seasons (Vlok 2005). *Leuciscus cephalus* testes were immature in December with spermatogonia; in April, spermatogenetic activity was recorded with spermatocytes, spermatids, and spermatogonia; in June, sperms were formed and finally, post-reproductive testes were observed mainly with spermatogonia in August (Guerriero *et al.* 2005). It was revealed that in *C. carpio* spermatogenesis was continuous and spermatogonia, spermatocytes, spermatids and sperms all were present during both spawning and non-spawning seasons (Smith and Walker 2004).

It has been reported that tiger barbs are serial spawners meaning that they spawn more than once during the spawning season and proper conditions induce females to spawn every two-week (Munro *et al.* 1990). On the contrary to seasonal breeders, continuous reproduction is a result of simultaneously occurring all phases of oogenesis and spermatogenesis and mature germ cells. This may be one of the main reasons why the tiger barb bred rapidly resulting in wider trade over the world.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SA laboratory work & manuscript preparation;
SİÜ supervised the study

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