Pakistan J. Zool., pp 1-4, 2024. DOI: https://dx.doi.org/10.17582/journal.pjz/20240114032649

Short Communication

Characteristics and Temporal Patterns of Testes and Ovaries Development in the Hybrid Snakehead of *Channa argus* (♂) × *Channa maculate* (♀)

Aiguo Zhou[1], Shaolin Xie[1], Yanfeng Chen[2], Huijuan Tang[1] and Jixing Zou[1]*

[1]*College of Marine Sciences, South China Agricultural University, Guangzhou 510642, Guangdong, China*

[2]*School of Life Science and Engineering, Foshan University, Foshan 528231, Guangdong, China*

Article Information
Received 14 January 2024
Revised 09 March 2024
Accepted 26 March 2024
Available online 12 August 2024 (early access)

Authors' Contribution
JZ, SX, and AZ: Methodology, formal analysis, writing-original draft, funding acquisition, and samples collection and pretreatment. YC and HT: Writing-review and editing, Data curation.

Key words
Hybrid snakehead, Ovary, Testis, Gonadogenesis

ABSTRACT

This study presents histological observations of hybrid snakehead ovaries and testes at different stages, as well as ultrastructural observations of sperm. The ovaries of hybrid snakehead exhibit a batch asynchronous type of development from stage III to stage VI, with oocytes in stages I, II, and III present. Histological sections of stage IV ovaries reveal the presence of oocytes in phases I, II, III, and IV, indicating a multiple egg laying per year pattern. Furthermore, the testis of hybrid snakehead exhibits a typical radiating type with seminiferous lobules arranged in a leaf-like manner from ventral to dorsal sides. Notably, male cells show significant asynchrony during development. The sperm structure consists of three parts: head, middle piece, and tail; characterized by an acrosome-type morphology. The flagella's central axis displays a typical "9+2" microtubular structure. This study provides valuable insights for exploring reproductive performance enhancement through artificial sex regulation and improving breeding yield in hybrid snakehead.

The hybrid snakehead (*Channa argus* (♂) × *Channa maculate* (♀)) has emerged as a pivotal aquaculture species with significant economic value in Southeast Asia. In China alone, the annual breeding yield of snakeheads exceeds 500,000 tons, reaching 548,481 tons in 2021 and further increasing to 553,196 tons in 2022 according to the China Fishery Statistical Yearbook for 2022 and 2023. Our research reveals pronounced sexual dimorphism among snakeheads during the breeding process: Male individuals exhibit accelerated growth rates, larger body sizes, and lower feed coefficients compared to their female counterparts (Jiang *et al*., 2013). Consequently, there is growing attention and research focus on the development and cultivation of all-male varieties (Chen *et al*., 2020; Huang *et al*., 2023; Niu *et al*., 2024).

Gonad development is a fundamental biological process that significantly influences the growth, differentiation, and metabolism of organisms (Nishimura and Tanaka 2014; Adolfi *et al*., 2023). Our study demonstrates that the primary oocytes appear on the 27th day after hatching while the primary spermatocytes appear on the 34th day after hatching, respectively (Zhou *et al*., 2018). However, the histological developmental characteristics of male and female gonads in hybrid snakehead remain unclear, and further research is urgently needed. Therefore, this study aims to investigate the spatiotemporal patterns and histological features of gonadal development in hybrid snakehead, to provide valuable data support for sex control breeding strategies, and genetic enhancement of *Channas*.

Materials and methods

Specimens of the snakehead were cultured in Tan's Aquatic Breeding Farm located in Madong Village, Xingtan Town, China (113°10'26.641"E, 22°44'50.860"N). The healthy fish were collected and dissected in batches to obtain ovarian and testis tissues at different developmental stages. The ovarian and testis tissues were fixed with Bouin's solution and stored at room temperature.

* Corresponding author: zoujixing@scau.edu.cn
0030-9923/2024/0001-0001 $ 9.00/0

Paraffin sections were prepared following our previously established method (Zhou *et al*., 2018). Simultaneously, sexually mature male hybrid snakeheads were selected for dissection to obtain their testes. Subsequently, the testes were crushed, homogenized, and semen was extracted for air drying before performing HE staining. All the slides and sperm morphology and structure were observed and documented under the LEICA DM2500 optical microscope. Preparation and observation of transmission electron microscope slices followed the method of Madhavi *et al*. (2015).

Results and discussion

The mature female hybrid snakeheads possess paired cylindrical cystic ovaries situated on both sides of the midline within the body cavity behind the digestive tract. These ovaries are connected to the body wall by ovarian mesentery. Based on anatomical observations of ovarian development stages in hybrid snakeheads (Fig. 1), six distinct stages can be identified. During stage I ovary development, it is challenging to differentiate between males and females due to its transparent linear appearance. The peritoneum of the stage I ovary closely adheres to both swim bladder as well as body cavity walls. A membrane covers its surface which can be clearly divided into two layers: an outer layer consisting of peritoneum while an inner layer composed of white connective tissue membrane. Large blood vessels are present between these inner and outer membranes. During this period, the cytoplasm and nucleus of the phaseIoocyte undergo an increase in size, resulting in irregular cell morphology. The nucleus, approximately 10-20 μm in diameter, is relatively large and centrally located within the cell (Fig. 1A). The stageIIovaries exhibit a slender shape with microscopic egg grains, making it challenging to differentiate between male and female individuals. Additionally, connective tissue from the inner layer of the ovarian capsule extends into the ovary along with primitive germ cells, forming multiple layered ovarian lamellae densely populated by phase II oocytes and a small number of phaseIoocytes (Fig. 1B). Stage III ovaries have increased volume compared to stageIIovaries and appear as elongated elliptical cylinders containing visible circular egg granules predominantly composed of phase III oocytes alongside concurrent presence of phase I and II oocytes. In these phase III oocytes, the nucleus remains relatively large with a wavy nuclear membrane while exhibiting abundant nucleoli (approximately 20-25) distributed near the nuclear membrane (Fig. 1C). Stage IV ovaries rapidly expand in volume occupying most of the abdominal cavity; they contain visible golden yolk granules covered by a tough elastic ovarian membrane lined with blood vessels on its surface. Phase IV oocytes are predominant at this stage accompanied by simultaneous presence of phases I, II, and III oocytes (Fig. 1D). Eggs within stage V ovaries have matured into a flowing state where gentle pressure on or extraction from parent fish results in their release through ejaculatory orifice. These eggs possess a golden yellow coloration with transparent elastic membranes. At this stage, primary type of oocyte is phase IV accompanied by varying numbers of phases I-IV oocytes. Nuclear membrane dissolution occurs along with disappearance (Fig. 1E). The stage VI ovary exhibits a loose cystic morphology, characterized by the elimination of most mature eggs and the presence of wrinkled and empty follicles. Additionally, there is thickening of the follicular membrane in some expelled phase I, II, and III oocytes, which fills the gaps between the eggs (Fig. 1F).

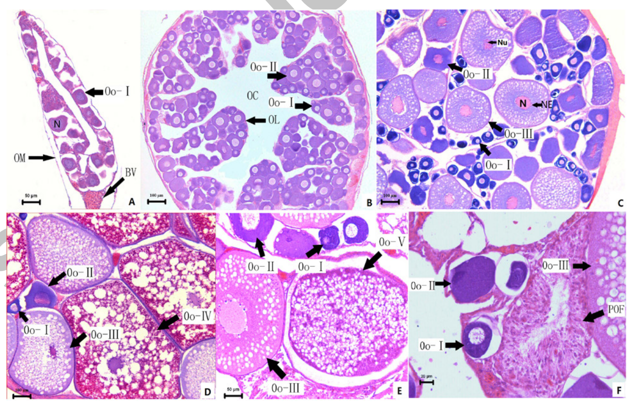

Fig. 1. Histological observation of ovarian development in the female hybrid snakehead. A, The stage I ovary (× 400). B, The stage II ovary (× 100). C, The stage III ovary (× 100). D, The stage IV ovary (× 100). E, The stage V ovary (× 100). F, The stage VI ovary (× 400).
OM, ovarial membrane; BV, blood vessel; OC, ovarian cavity; OL, ovarial lamellae; N, nucleus; Nu, nucleolus; NE, nuclear envelope; POF, postovulatory follicle; Oo-I, the phaseIoocyte; Oo-II, phaseIIoocyte; Oo-III, phase III oocyte; Oo-IV, phase IVoocyte; Oo-V, phase Voocyte.

The paraffin sectioning results reveal that a pair of testes is present in the hybrid snakehead, which is positioned posterior to the swim bladder and on both sides of the digestive tract. This pair of testes is connected to the body wall through the testis mesentery. The testicular wall extends backwards to form vas deferens, with a Y-shaped connection at its rear end leading to an ejaculatory orifice. Sexually mature testes exhibit a milky white color. The structure of these testes consists of two parts: outer membrane (OM) and parenchyma (P). The outer membrane extends into the parenchyma forming numerous septa that divide it into several seminiferous lobules (SL). Based on their characteristics, these hybrid snakehead fish possess radiation type testes (Fig. 2A). Observations indicate that male primordial cell development in hybrid snakehead can be categorized into five stages: Spermatogonia, primary spermatocytes, secondary spermatocytes, sperm cells, and mature spermatozoa. Spermatogenic cells are dispersed throughout the testes as circular or elliptical cells representing one of largest cell types involved in spermatogenesis process with an approximate diameter ranging from 9-11 μm. These cells possess prominent nuclei located centrally within each cell measuring approximately 4-6 μm in diameter while exhibiting prominent nucleoli situated at their centers measuring around 1-2 μm in diameter (Fig. 2B). Primary spermatocytes originate from differentiation processes undergone by spermatogonia; they appear circular or elliptical with smaller diameters compared to spermatogonia (about 6-8 μm) without any observable nucleoli (Fig. 2C). The secondary spermatocytes are derived from primary spermatocytes through meiosis, exhibiting a smaller volume compared to primary oocytes and a cell diameter of approximately 4-5 μm (Fig. 2D). Sperm cells originate from secondary spermatocytes via mitosis, displaying a circular shape with reduced volume and a diameter of around 2 μm. The nucleus is large, circular, and deeply stained (Fig. 2E). Subsequently, sperm cells undergo a series of metamorphoses to develop into mature spermatozoa. On the sperm smear, the darkly stained round nucleus on the sperm head measures about 1 μm in diameter. Additionally, the slender light red-stained tail extends approximately 75-82 μm in length (Fig. 2F).

Ultrastructural observations of hybrid snakehead sperm reveal that it consists of three distinct parts: the head, midpiece, and tail. In hybrid snakehead spermatozoa, the nucleus constitutes the main structure with minimal cytoplasmic content. The chromatin within the nucleus appears dense and evenly distributed while adopting an elliptical shape featuring a central nuclear fossa (NF). The surface of the sperm is not very smooth, and the nuclear membrane appears wavy without acrosome (Fig. 3A). The midpiece structure of hybrid snakehead sperm consists of a centriole complex and a sleeve. The centriole complex comprises three components: Proximal centriole (PC), distal centriole (DC), and intercentriolar body (IB). Below the nucleus, there are 2-3 single-layer mitochondria (M) arranged in an orderly manner. A shallow sleeve is located below the distal centriole, with the narrow cavity between the flagella and the distal centriole serving as its space (S) (Fig. 3B). The tail of hybrid snakehead sperm possesses slender flagella (Fig. 3C). Its anterior end connects to the posterior end of the substrate, while its starting point lies at the junction between the sleeve cavity and substrate. Inside the flagella, there exists a prominent axon composed of nine sets of double microtubules on its periphery and two central microtubules, forming a characteristic "9+2" microtubule arrangement (Fig. 3D).

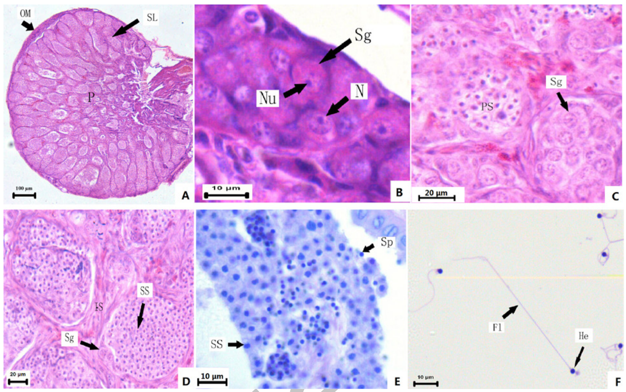

Fig. 2. Histological observation of spermatogenesis in the male hybrid snakehead. A, The testis of the hybrid snakehead (×100). B, The spermatogonia (Sg) (×1000). C, The primary spermatocyte (PS) (×400). D, The second spermatocyte (SS) (×400). E, The spermatozoa (Sp) (×1000). F, The spermatozoa (×1000). SL, seminiferous lobules; OM, outer membrane; P, parenchyma; N, nucleus; Nu, nucleolus. He, head; Fl, flagellum.

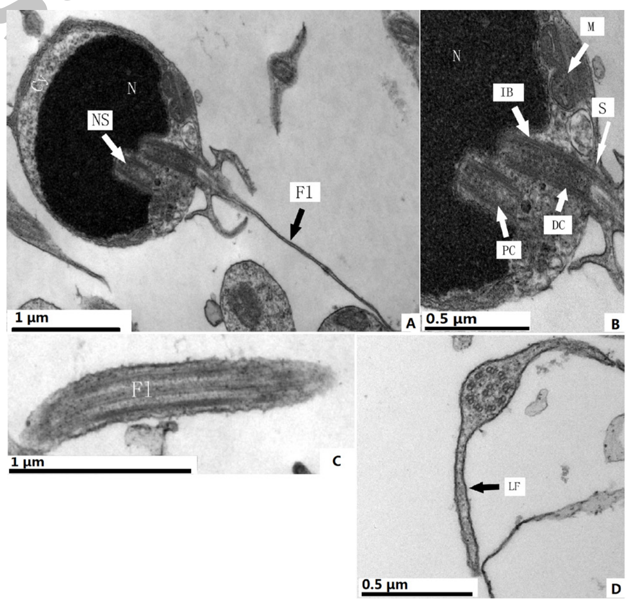

Fig. 3. The ultrastructural observation of the hybrid snakehead sperm. A, The sagittal section of sperm head (×2900). B, The midpiece of hybrid snakehead sperm (×6800). C, The longitudinal section of the hybrid snakehead sperm(×2900). D, The cross section of the hybrid snakehead sperm flagellum (×9300). N, nucleus; NF, nuclear fossa; Fl, flagellum; PC, proximal centriole; DC, distal centriole; IB, intercentriolar body; S, space of sleeve; M, mitochondria; LF, lateral fin; "9+2" micro-tubular structure.

DECLARATIONS

Acknowledgments

We also wish to express our appreciation to our anonymous reviewers for providing valuable comments on the manuscript. The authors thank Dr. X.L. Zhuo for his assistance in samples collection, experimental processing and data analysis.

Funding

This work was Funding by Science and Technology Projects in Guangzhou (202201010470); Science and Technology Planning Project of Guangdong Province (2017A020225035); Fund Fostering Talents for Young Scholars of South China Agricultural University (201707N025).

IRB approval

This study was approved by Guangzhou Basic Research Program.

Ethics statement

The animal study was approved by the Animal Care Committee of South China Agricultural University (Guangzhou, China).

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Adolfi, M.C., Depincé, A., Wen, M., Pan, Q. and Herpin, A., 2023. *Sex. Dev.*, **2023**: 1-15.

Chen, B., Yin, J., Chen, K.C., Ou, M., Liu, H.Y., Luo, Q., Jiang, X.W. and Zhao, J., 2020. *J. Aquacult.*, **41**: 21-24.

Huang, L., Zhang, G., Zhang, Y., Li, X., Luo, Z., Liu, W., Luo, F., Liu, H., Yin, S., Jiang, J., Liang, X. and Cao, Q., 2023. *Fishes*, **8**: 426.

Jiang, L.,Wang, Z.W., Zhou, L. and Gui, J.F., 2013. *Acta Hydrobiol. Sin.*, **2013**: 1174-1178.

Madhavi, M., Kailasam, M. and Mohanlal, D.L., 2015. *Anim. Reprod. Sci.*, **153**: 69-75.

Nishimura, T. and Tanaka, M., 2014. *Sex. Dev.*, **8**: 252-261.

Niu, J.S., Wang, T., Li, Z., Wang, Z.W., Ding, M., Wang, M.T., Lian, Z., Mei, J., Wang, Y., Zhou, L., Zhang, X., Gui, J.F. and Li, X., 2024. *Aquaculture*, **578**: 740023.

Zhou, A., Xie, S., Wang, Z., Chen, Y., Fan, L., Wang, C., Wang, M. and Zou, J., 2018. *Aquacult. Res.*, **49**: 2345-2348.