Observations on formation and development of primary germinal tissue of cultured Chinese sturgeon, *Acipenser sinensis*

By Xihua Chen[1,2], Qiwei Wei[1,2], Deguo Yang[1,2], Yongjiu Zhu[1]

(1. Key Laboratory of Freshwater Fish Germplasm Resources & Biotechnology of Agriculture Ministry of China, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jingzhou 434000, China;2.Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China)

E-mail: chenxh@yfi.ac.cn

Summary

Formation and development of primary germinal tissue of cultured *Acipenser sinensis* were studied by light microscope. The primordial germ cell was firstly seen between the nephridium area and yolk sac of fish, 1.7cm T.L., on the third day after hatching. By the 11th day, the genital fold formed, which was composed of several cells. With the primordial gonad growing, the blood vessels appeared in it when fish was 2 months old, with a average T.L. of 7.1 cm. The adipose fatty tissue appeared by the age of 7 months, average T.L.59 cm. Histological sex differentiation of the gonads occurred at the age of 9 months, 0.6~1.1kg B.W., and the gonads entered stage 1, during which mitosis of the spermatogonia and oogonia occurred. With the primary spermatocytes or oocytes appearing, the testes or ovaries reached stage 2. The testes reached stage 2 when fish was 22~26 months old, 1.55~5.6kg B.W., and the ovaries reached stage 2 when fish was 30~36 months old, 3.1~8.2 kg B.W.. After the age of 3 years, the gonads, in succession, became what could be sexed by gross examination, and at age 5 years, 18~35.5kg B.W., all of the gonads could be sexed by gross examination and still remained in stage 2, with the oocyte diameter of 60~240μm. Besides, the results inferred that in the Yangtze River, all of the fingerlings arriving at the estuary had gonads with no anatomical sex differentiation and most of them no histological sex differentiation.

Introduction

Sexual maturation is delayed in sturgeons and paddlefishes. Gametogenesis and gonadal cycles were elucidated in cultured *Acipenser baerii*(Le Menn and Pelissero, 1991), the hybrid *Huso huso* × *A.ruthenus*(Amiri,et al., 1996a;b), *A. schrenckii*(Zhang et al., 2002), and *A. transmontanus*(Doroshov et al., 1997). Nevertheless, when primary germinal tissue formed and when sex differentiation of the gonads occurred remain nearly unknown. Chinese sturgeon, *Acipenser sinensis*, one of Chinese National Glass Ⅰ Protected Animals, is an anadromous fish, with the maturity ages of 8~18 in males and 14~26 in females. The artificial spawning of this fish below Gezhouba Dam has been performed since 1983, and in 1997 the aquaculture on large scale began in China. Yet, knowledge of the gonadal development of the fish remains rare (Anonymous,1988).

Material and methods

The animals used in this study were taken from the offsprings of the Chinese sturgeon broodfish captured below Gezhouba Dam and reared at the hatchery of Yangtze River Fisheries Research Institute at the water temperature of 18~22℃ for larva and fingerlings and 8~30℃ for larger individuals. Fish, except larva, were fed with artificial diet.

225 specimens were sampled. Larva and fingerlings of several hours to 1 month old after hatching were sacrificed every 12 hrs. to 10 days. Fish of few months to 1 year old were sacrificed every 15 days to 3 months. Fish over 1 year old were sampled twice or 3 times every year.

Trunks of larva, body walls of fingerlings and gonadal tissues of large individuals were fixed with Bouin's fluid. Gonadal tissues of fish above 2 years old were sampled by biopsies. All materials fixed were then embedded in paraffin. Serial sections 7 μm thick were stained with haematoxylin and eosin for light microscope studies.

Results

We classified formation and development of primary germinal tissue of the fish as gonadal origin and gonadal differentiation.

Gonadal origin

On the paraffin sections of larva 1~2 days old after hatching, nothing related to gonad origin were found by light microscope. The primordial germ cell, PGC, was firstly seen in the larva with an average T.L. of 1.7cm, on the third day after hatching. It was a large cell with a diameter of about 20μm, located between the nephridium area and yolk sac. It had a large light-stained nucleus and a small dark-stained granule (**Fig. 1-A**).

On the 11th day, the genital fold formed when the average T.L. of fish was 2.5cm and the yolk sac was disappearing. On the section, the genital fold was composed of several epithelium cells. On some sections, PGC seemed to be moving to the genital fold (**Fig.1-B-1**), and on the others, to have joined it (**Fig.1-B-2**).

By the 18th day, the section of the genital fold had enlarged up to a club-shaped protuberance, with a length of 40~50μm (**Fig.1-C**). When fish was 1 month old, the protuberance was 90~130μm long, with uncountable cells. The genital fold was to become primordial gonad.

When fish was 2 months old, with an average T.L. of 7.1cm, the blood vessels appeared in the primordial gonad, the section of which was now 130~180μm long and 80~100μm wide (**Fig.1-D**). When fish was 4 months old, with an average T.L. of 20.9cm., length of the section of the primordial gonad reached 300~320μm, and the diameter of the blood vessel was now about 50μm.

When fish was 7 months old, with an average T.L. of 59cm, the adipose fatty tissue appeared in the end of the gonad on the section. The section was now 950~1400μm long. Besides the fatty tissue, the gonad was composed of germinal tissue, germinal portion, on one side and connective tissues and blood vessels on the other (**Fig.1-E**).

By the age of 8 months, the gonads had been yet invisible, nor could be sexed by light microscope. According to Hochleithner & Gessner(1999), we classified the gonadal stage with no histological sex differentiation as stage 0.

Gonadal differentiation

Histological sex differentiation of the gonads occurred at age of 9 months, when fish weighed 0.6~1.1kg. The gonads now assumed a transparent strip. They didn't show any sexual dimorphism except under the light microscope. In the light microscope, there were two kinds of gonads. One was testis in which the germinal portion assumed a compact half-cycle on the section and consisted of cysts with spermatogonia. The diameter of the half-cycle was 380~410μm (**Fig.2-A**). In 18 specimens at age of 9 months, about a half were testes. The other kind of the gonads was ovary in which the germinal portion assumed a 1000~1100μm long strip with waving sides on the section and consisted of follicles with oogonia. The follicles were irregularly arranged. Between the follicles were blood vessels and connective tissues (**Fig.2-B**). In the testis or ovary, mitosis of the spermatogonia or oogonia occurred (**Fig.2-C**). The gonads at this stage were thought to enter stage 1.

When fish were 22~26 months old, weighing 1.55~5.6kg, the testis enlarged up to a strip with a width of 0.7~1.5mm and a thickness of 300~370μm on the section. The spermatogonia had ceased mitosis and become primary spermatocytes. The testis was thought to have reached stage 2 (**Fig.2-D**). The ovary of fish of such sizes still stayed stage 1.

When fish were 30~36 months old, weighing 3.1~8.2 kg, the ovary reached stage 2 (**Fig.2-E**), while the width of the testis reached 1.4~2.5mm and the thickness 350~1000μm on the section. The ovary on the section exhibited lobes and foliages. Some of the oogonia had grown up to primary oocytes, which were round or polygonal. They were characterized by a diameter of 60~110μm and dark alkal-stained cytoplasm. Their nucleus were large, with a few nucleoli close to the nuclear envelope (**Fig. 2-E-1**). In some specimens of fish at this stage, the primary oocytes, with the maximal diameter of 160μm, clustered and took up most space of the ovary. These ovaries were thought to represent the more developed ovaries (**Fig.2-E-2**). In all specimens 22~36 months old, it was difficult to distinguish between testes and ovaries by gross examination.

After the age of 3 years, the gonads, in succession, became what could be sexed by gross examination. In 44 specimens 41~58 months old, weighing 13.5~32kg, 7 testes and 13 ovaries were identified by gross examination. The testis had a white color, smooth surface and rigid texture. The ovary had a light yellow color, flexible surface through which the oocytes loomed up. At the age of 60~67 months, weighing 18~35.5kg, all of the gonads could be sexed by gross examination and still remained in stage

2, with the oocytes having a diameter of 60~240μ m and no yolk granules (**Fig.2-F**).

Discussion

Accurately, the gonadal development of fish begins at as early as gonadal orgin, or formation of primary germinal tissue. This early stage of gonadal development, as well as sex differentiation of gonads, has been poorly investigated in Acipenseriformes. The genital fold of Chinese sturgeon formed on the 11th day after hatching, when the yolk sac was disappearing, much later than formation of rudiments of somatic organs like excretory, nervous organs. This pattern of development is also utilized by other teleostei. In *Clarias lazera*, the genital fold forms on the 11th day after hatching (Liu, 1991).

Histological sex differentiation of Chinese sturgeon gonads occurred at the age of 9 months, much later than that of teleostei gonads. In *Clarias lazera*, histological sex differentiation gonads occurred when fish was 1 month old (Liu, 1991). The delay of sex differentiation in Chinese sturgeon could be thought to contribute partly to long duration of sexual maturation. In our findings, the testes of Chinese sturgeon reached stage 2 earlier than the ovaries. This kind of difference could be thought to contribute partly to diversities of sexual maturation between sexes in this species.

Anatomical sex differentiation of Chinese sturgeon gonads was completed after the age of 3 years. As all of the gonads of Chinese sturgeon at ages of 5 years, 18~35.5kg B.W., could be sexed by gross examination, fish of this sizes were the good candidates for sexing by gross examination. In white sturgeon, *A. transmontanus*, the appropriate sizes for sexing by gross examination were 3~4 years old, 7~9 kg B.W.(van Eenennaam,et al.,2001).

In the Yangtze River, the fingerlings of Chinese sturgeon arrive at the estuary when they are 6~9 months old, with T.L. of 7~38cm. According to our findings, fish of such sizes have gonads with no histological sex differentiation or gonads that have just completed histological sex differentiation. It can be concluded that not only gonadal development of fish but also sex differentiation is ready to be finished after fish enter the sea.

References:

Amiri B M, Maebayashi M, Adachi S, et al. Testicular development and serum sex steroid profiles during the annual sexual cycle of the male sturgeon hybrid, the bester. J Fish Biol, 1996, 48:1039-1050.

Amiri B M, Maebayashi M, Adachi S, et al. Ovarian development and serum sex steroid and vitellgenin profiles in the female cultured hybrid, the bester. J Fish Biol, 1996, 48: 1164-1178.

Anonymous. The biology of the sturgeons in Changjiang and their artificial propagation. Chengdu: Sichuan Scientific and Technical Publishing House,1988, 92-93(in Chinese).

Doroshov S I, Moberg G P, van Eenennaam J P . Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. Environmental Biology of Fishes, 1997,48: 265-278.

Le Menn F, Pelissero C. Histological and ultrastructural studies of oogenesis of the Siberian sturgeon. P Williot Ed. *Acipenser*, CEMAGRAEF Publ, 1991:113-117.

Liu S J. Studies on the origin and migration of the primordial germ cells and gonad differentiation in *Clarias lazera*. Acta Hydrobiol Sin, 1991,15(1):1-7(in Chinese).

Hochleithner M, Gessner J. The sturgeon and paddlefishes of the world: biology and aquaculture. Austria, AquaTech Publications, 1999. 81-82

Zhang L Z, Zhuang P, Zhang T, et al. Gonadal development of cultured Amur sturgeon *Acipenser shrenckii*. J Fish Sci China, 2002, 9(4)：321-327(in Chinese).

Van Eenennaam J P, Bruch R, Kroll K. Sturgeon sexing, staging maturity and spawning induction workshop. 4th International Sturgeon Symposium, Oshkosh,Wisconsin USA, 2001, July 8-13.

Key words: *Acipenser sinensis*; germinal tissue; gonadal differentiation

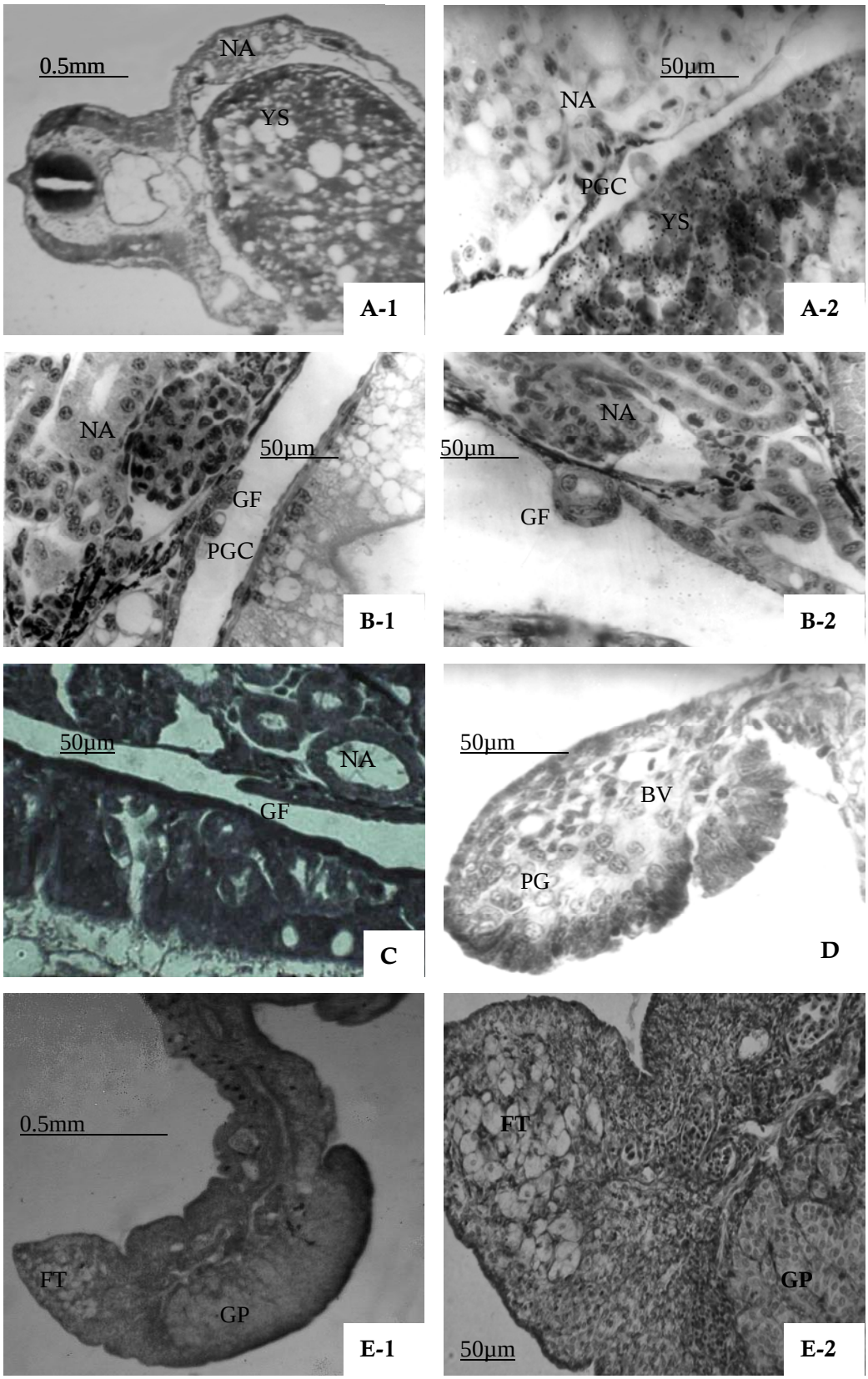

Fig. 1. Paraffin sections of larva trunks or fingerling body walls of cultured *Acipenser sinensis*, showing formation of primary germinal tissue. A: 3 days old after hatching; B: 11 days; C: 18 days; D: 2 months; E: 7 months. NA: Nephridium area; YS: Yolk sac; PGC: Primordial germ cell; GF: Genital fold; PG: Primordial gonad; BV: Blood vessel; FT: Fatty tissue; GP: Germinal portion

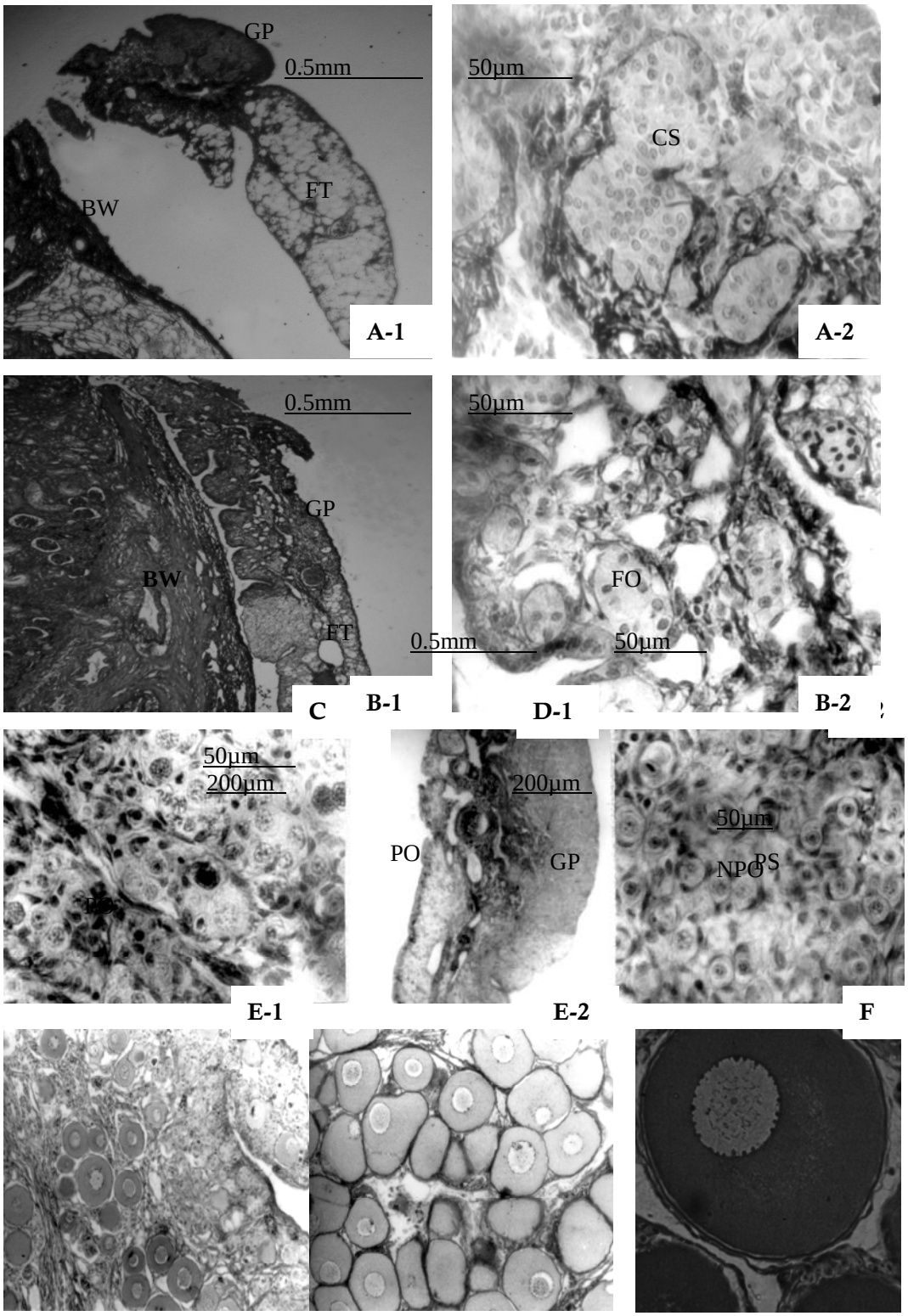

Fig.2. Paraffin sections of gonad tissues of cultured *Acipenser sinensis*, showing formation of primary germinal tissue. A: 9 months old after hatching, testis; B: 9 months, ovary; C: 1-2 years, showing mitosis of the spermatogonia or oogonia; D: 22-26 months, testis; E: 30-36 months, ovaries; F: 5 years, ovary.
GP: Germinal portion; FT: Fatty tissue; BW: Body wall; CS: Cyst with spermatogonia; FO: Follicle with oogonia; PS: Primary spermatocyte; PO: Primary oocyte; NPO: Nucleus of primary oocyte