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Gonadal development and differentiation of germ cells during larval growth of *Cyprinus carpio*

S.L. Gaikwad, T.S. Pathan, M.V. Gaikwad, P.M. Davne, D.K. Hivrale, Y.K. Khillare*
Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad- 431 004. India

**Corresponding Author, Email: tanvirforu@rediffmail.com*

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Abstract
Gonadal development and differentiation of germ cells was studied during larval growth of *Cyprinus carpio*. The larval stages (immature) collected from fish breeding center, at Paithan. The first fish sampled after 15 days of hatching. The anatomical and histological studies have been carried out form fry to fingerling. The gonadal tissue showed its presence at very early stages of life. It is found that the gonadal tissue formation started at early fry stage where they attached to the coelomic wall by means of chord of mesovarium. A correlation has been noticed between the length and weight of fish to the development of gonad. In early stages, the gonad was differentiated with few germ cells. On 4th week the fish was measured 10mm and showed development of gonad stared form anterior region and proceeded in posterior direction. The gonads were filled with large number of somatic cells, few surrounding Cyst cell stained dark blue with Haematoxylin. Further, at fingerling stage the cells were differentiated into male and female germ cells.

1. Introduction

In most organisms, sex is determined during early stages of development. The studies on the germ line and development of the gonads have been studied in several fish species. The morphological characters of early germ cells and developing gonads studied using light microscopy by many researchers [1-6], as well as electron microscopy has also been used to study the ultra structure of early germ cells [7-10] & gonadal differentiation [11,12] in a number of Teleosts.

Up till now there exist only a few morphological descriptions of gonadal development and germ cell differentiation in Cyprinids. Stromsten [13] investigated germ cell differentiation in the gold fish. Few data on physiology of developing gonads and germ cell differentiation are available. Sexual differentiation in fish (the physical translation of the genetic sex) may occur within a few weeks of hatching, as occurs in salmonids [14], or alternatively, it may occur much later in development, even a few months after hatching, as occurs in some Cyprinid fish. [15]

Today it is generally accepted that cell membrane determinants responsible for cellular differentiation and play a significant role in regulation of intra & inter cell processes. The presence of these determinants on membrane of differentiating germ cells during development of male and female carp using immunofluorescence technique has been done [16]. In fishes, genetic (Chromosome – based) as well as environmental mechanisms have been implicated in sex determination. For example, in Medaka and some Poecillid fishes, sex chromosomes can be distinguished from autosomes [17]. However, even fish with established sex chromosomes shows strong dependent on environmental cues, the prominent of which is temperature & other such as pH, pollution & social effects have been shown to influence sex determination as well [18].

In the present study we observed the course of anatomical differentiation of gonads in relation to body growth in *Cyprinus carpio* during the development of larval stages on the basis of determination of their shape and microscopic structure as well as cytological development.

2. Materials and Methods

2.1. Collection of Animals

For the experimental studies the larval stages of common carp (*Cyprinus carpio*) from early fry (8-9 mm) to fingerling were collected from near fish breeding center located at Paithan (MS) India. The fry feed with Rice brane and the naturally available foods like Phytoplankton & Zooplanktons. The length and weight of the fishes wear measured for records (Fig.5).

2.2. Histological process

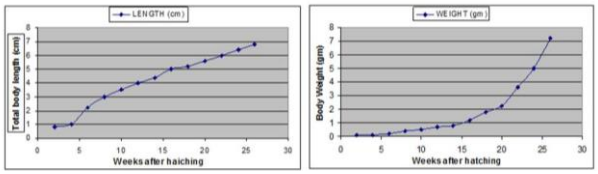
The larvae from each stage were sampled and collected samples were immediately fixed in the Bouins solution for about 24 hours. The tissues were passed through the series of ethanol concentrations for dehydration process and impregnated in the paraffin wax. The sections were cut are 6µ and stained with the Harris haematoxylin and Eosin treated with DPX mounted and observed under light microscope.

3. Results

3.1. After 1- 2 weeks post hatching (9-10 mm)

After first week of hatching the initial total length of larvae was 8-9 mm total weight was 0.10 g. There is a chord of somatic cells present in an anterior-posterior direction at the dorsal body wall. The immature gonad located at the right side dorsal to the liver, posterior to the developing swim bladder & just above to the gut. After 15 days of hatching the metamorphosis from post larva to fry stage took place (Fig.1).

Fig: Graphs showing growth of larvae in length and weight



3.2. After 4-6 weeks post hatching (10-20 mm)

The average lengths of larvae are measured 10-22 mm in length. The fish was said to be in fry stage. The gonad was somewhat increased in size. The development of gonad started form anterior region and proceeded in posterior direction. There was a short & thick band of somatic cells which attaches gonads to the peritoneum. In most fishes it was also observed that the development of right lobe of gonad was more than the left gonad. The histological observation shows that around this stage the formation of gonads started (Fig.2). The slide shows that there was no formation of gonad cells but the tissue must be filled with large somatic cells and single (1-2) primordial germ cells (PGC's). Compared to somatic cells, PGC's were larger, marked border between the cytoplasm and somewhat eccentrically located nucleus surrounded by deeply staining somatic cells (Fig.1).

3.3. 7-9 weeks post hatching (3.0-3.5 cm)

After 7th week the fry attained the length of 3.0 cm and resembles just like an adult in all respect except sexual maturity. The gonadal tissues extended from the posterior third of the swim bladder to the hind gut. The gonads were increased in size and filled with stroma cells and rapid proliferation of germ cells was found. In the 9th week old larvae the germ cells gradually accumulated in the gonad and no sex differentiation was distinguished.

Fig. 1: Immature gonad with chord of mesovarium.
Fig. 2: Primordial germ cell (PGC) surrounded by somatic cell. Note Cytoplasmic space & surrounding cells stained dark blue with Haematoxylin.
Fig. 3: Fingerling testis showing seminiferous tubules.
Fig. 4: T.S. of fingerling Testis showing irregular sperm ducts.

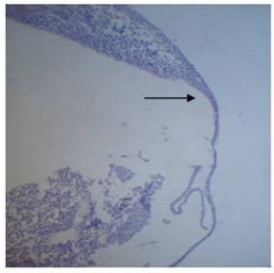


Fig. 1

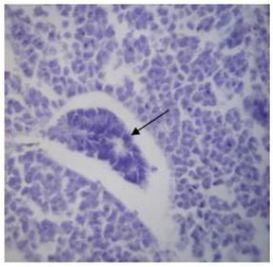


Fig. 2

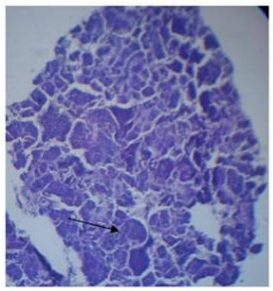


Fig. 3

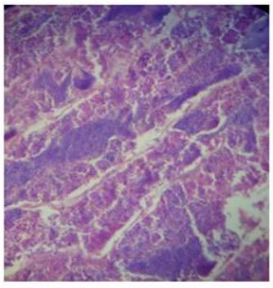


Fig. 4

3.4. 10- 16 weeks post hatching (3.5-5.0 cm)

After ten weeks of hatching the average size of common carp fry measures above 3.5cm & 0.5g. During this period the gonads were in different stages

of development & can be distinguished on some anatomical characteristics. In some fishes the gonads were undifferentiated while in others it is slender, club-shaped or narrow shaped attached to the coelomic wall and hanging freely in the coelomic cavity. These gonads largely filled with stroma cells and blood vessels.

Moreover some gonad was flattened sickle shaped in form and attached at two sides to the coelomic wall at the mid-dorsal side. The gonad filled with blood vessels, stroma cell and few germ cells.

3.5. 20-24 weeks post hatching (5.2-6.4 cm)

The fish with this length can be considered as Fingerling. Now onwards the fish can be distinguished in male and female on microscopic observations of gonads. In case of male fish the testis shows gradual formation of cyst cells and primary as well as secondary spermatogonia (Fig. 3). The spermatogonia are larger in size than PGC's. The ovaries are well vascularized with blood vessels. They showed primary Oogonia and a group of Secondary Oogonia. The Nucleus and cytoplasm are visible in some larger Oogonia.

On 30th week fingerling measured 6.8 cm in length showed prominent characters of male and female gonads. The testis gradually obtains the shape of adult organ [16] and consists of irregularly shaped tubules, separated by interstitial tissue with blood vessels. In some fishes primary spermatogonia and secondary spermatogonia are also found, sometimes the spermatids found but no mature spermatozoa found in this stage (Fig.4).

4. Discussion and Conclusion

It is already known that in common carp at day three after fertilization the primordial germ cells were present at the dorsal wall of the coelomic cavity at the site of future gonadal ridge. After one week duration the gonad located posterior to the developing swim bladder, at the right side dorsal to the liver and posterior to the gut. Gonads remain the same for 3-4 weeks and later on it shows gonad formation. The term Primordial germ cells (PGC) were proposed by Nieuwkoop & Sutasurya [19] for all stages in the formation of germ cells. We followed the same term for the cells developed after early morphological differentiation in male and female gonads. The

Proliferation of PGC's observed at the age of seven weeks and gonad differentiation in male and female found 10 weeks onwards. The initial PGS's is low and their numbers remain low for up to six weeks after which rapid proliferation started. The gonad germ looks alike in both sexes. After the penetration of PGC's into gonads, we can observe the differences in the size and shape of the sex glands that appear in vascularisation and localization of the PGS's [20], Persov [21] reported that the primordial germ cells were present from the earliest stage of embryonal development to the beginning of their first mitotic divisions. The author also stated that only the PGC originated other sex cells, but Khoo [22] claimed that sex cells could also be formed in the later period of ontogenesis (e.g. from cells of epithelium folliculi as a result of their metamorphosis).

Morphological sex differentiation characteristic of the most primitive multi cellular animals is associated with the genetic material of the parents. The development of fish embryo (including means of sex determination) is theoretically predetermine by genetic factors and includes sensitization of the bipotential gonads to endogenous endocrine factors prior to, during and even after commitment to maleness of femaleness. However, young fish are relatively vulnerable to a host of environmental (physical and chemical) factors can affects the endocrinology or even overriding the putative development pathway [23].

Description of gonad development revealed that considerable differences exist between different fish species [24,25]. Yamamoto concluded that in gonochoristic species two types of gonad development can be distinguished: an undifferentiated type, in which during development all gonads pass through a female phase containing follicular oocytes and a differentiated type in which the gonads differentiate directly into a female or male gonad. Besides these two forms of gonochorism, various type of hermaphroditism was found in fishes [26]. Here in this study we found that the gonadal development in carp is of differentiated type. The same results obtained by Stromsten [13] for the gold fish (*Carassius auratus* L.) and Timmermans &Taverne [27] for the rosy barb (*Barbus conchonius* L.) However, our results differ from Davies & Takashima [28] who reported that carp gonads develop through an initial female phase. In the present study we found the same gonad differentiate into male and female.

Summarizing it can be concluded that the Primordial germ cells are in fish at very early stage of growth. Primarily the future gonads are in the form of very thin chord like structures growing horizontal to the body. Early gonad has only PGC's for about 3-6 weeks and later on the gonad formation started. The differentiation of gonads occurred at fingerling

stage onwards. The primary and secondary spermatogonia are found in male fingerling while in case of female fish some stages of Oogonia are observed.

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