



A HISTOLOGICAL STUDY ON *PAMPUS ARGENTEUS* (EUPHRASEN 1788) OFF RATNAGIRI COAST, MAHARASHTRA STATE, INDIA

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ABSTRACT

In these situations, it is quite advantageous that apart from the size, gross appearance and overall histological picture, the status and stages of the germinal tissue must be taken into account. The seasonal changes in the testis of *Pampus argenteus* are described on the basis of gonadal volume and histological changes in the testis. The testicular cycle was divided into gametogenesis, maturity, spawning and recovery. The testicular cycle reported for pomfrets *P. argenteus* (Family: Stromateidae and Order: Perciformes) is not typical of teleosts. This is characteristic of this family is not known as no other species from this family has been studied so far. This means that the presence or absence of sperms alone in the testes is anecdotal information.

KEY WORDS

Preeclampsia, Antihypertensive Drugs, Labetalol, Nifedipine and Tertiary Care Hospital.

Introduction

Brown-Peterson et al. (2002) has suggested that the traditional concepts of gonad classification be revised and newer histological techniques like plastic embedding, selective staining and histochemistry be explored to define the stages of development in fish gonads. By using these concepts, a whole array of new stages for the classification of the fish gonads will be defined based on more quantitative histological techniques. However, in a lobule different spermatocysts can be at different stages of maturity. The spermatogonia divide by mitosis for a certain number of cycles (range from 6-14 times). This number seemed to be constant for a species but very few species have been studied to confirm this claim (Ewing, 1972, Danio rerio; Ando et al., 2000, Hucho perryi, Oncorhynchus masou, Oryzias latipes; Miura et al., 1991, Anguilla japonica; Billard, 1986, *Poecilia reticulata*; Utoh et al., 2004, *Conger myriaster*). After a certain number of mitotic divisions, the spermatogonia begin the first meiotic division and become Primary

spermatocytes. These cells begin the second meiotic division and become secondary spermatocytes which generally referred to as spermatocytes. The spermatocytes divide and end up as spermatids. After this stage the cyst ruptures, spermatids are released into the lobule lumen and the process of spermiogenesis starts in which fully mature sperm with their characteristic tails appear and are ready to fertilize the egg at the time of spermiation. The testicular structure and spermatogenesis in *P. argenteus* fish is of the lobular type. The fact is that in *P. argenteus* testis contained sperm in the lobule lumen in both November-December and March-April months of the year.

1. Materials and Methods

All the white pomfrets (*Pampus argenteus*) in the size range 25-35cm were preferred from PFZ validation advisory, one trawler fishing boat hired and operated in PFZ areas and on the part of other operated in outside PFZ away from 2-3km in the available fish catch. Each

male and female pomfret fishes were sorted out distinctly inboard fishing vessels. After landing they were brought into the laboratory in order to perform experiment during the study tenure from 2006 to 2012. The matured males and females were classified microscopically their permanent slides were checked very accurately to recognize each part of the tissue is to be identified.

2. Result

During this study, it is appeared in November-December, the testis size was 7.3 cm in length from within PFZ and while for outside it was observed PFZ 6.2cm in length and occupied about in the body cavity. On the other side in early pre-spawning the testis size were 6cm for within and for outside PFZ 5.2cm when they are in mature condition. The lobule wall consisted of nests of germ cells. From November, onwards the lobule size began to increase and greater number of spermatogonia was formed by December. The lobules were mainly composed of two types of spermatogonia. Apparently, the gonads in this plight were during (March-April) and gradually increased in size to occupy in the body cavity upto spawning. Spermatogonia are formed in relative abundance. Primary and secondary spermatocytes initially increased in number but later decreased and spermatids and spermatozoa greatly increased. In March-April i.e. early pre-spawning the lobules further produced spermatogonia and there were many spermatocytes by this month. In pomfret fishes with lobular testis, spermiation corresponds to the release of spermatozoa from the lumen of the lobules into the spermaduct. This probably occurs after a rise in the hydrostatic pressure inside the lobule. The spermaduct has two parts: One is adjacent to the testis and corresponds to the opening of the lobule; the other is a simple duct connecting the posterior part of the testis to the genital papilla.

In the *P. argenteus* male fish, the reproductive organs consist of a pair of testes situated on either side ventral to the kidneys in the abdominal cavity. Testes are elongated 7.3 to 6.2cm in length, flattened structure, attached to the body wall by mesorchia. The testes show indentations along their margin and may or may not be equal in size. The two sperm ducts run posterior and join to open into the urinogenital papilla. The entire testis may be functional serving to produce sperms. At the anterior and middle region of the testis germ cells

in various stages of development are usually present. Seminal vesicles are secretory in nature and produce a fluid whose function appears to be nourishing the sperms keeping them in active and viable condition.

3 Discussion

In white pomfret a lobular type of testis occurs which consists of a large number of seminiferous lobules bound together by means of a thin layer of connective tissue. These lobules are highly convoluted tubules that are separated from each other by thin connective tissue stroma. The lobules of the testis may be surrounded by lobule boundary cells that resemble connective tissue cell. The lobules open behind into a spermatic duct lined with secretory epithelium. The space between neighbouring lobules contains connective tissue cells, blood capillaries and interstitial cells. The lobule of the testis is not lined by a permanent germinal epithelium, but primary spermatogonia are present which undergo division to form cysts that finally produce sperms. During the growth of the testis the resting germ cells become active and divide to form sperm mother cells or nests of spermatogonia. Each spermatogonium is a large spherical cell containing a large, round, central nucleus with a distinct nucleolus. The spermatogonia divide and give rise to primary spermatocytes which are smaller in size and contain a darkly staining nucleus. These divide and multiply to form secondary spermatocytes, which are still smaller in size, and their nuclear material is seen as a thick clump on one side. These divide further to produce smaller spermatids having a deeply stained elliptical nucleus. The spermatids undergo metamorphosis to produce sperms, and the process is called "spermiogenesis". Eventually the seminiferous tubules are seen full of sperms. The pomfret also exhibit cyclic changes in the testis and various plights in the annual cycle in late post-spawning and early pre-spawning can be described as under:

1. Maturing Phase Testis show increase in weight and volume. They become opaque and show increase in vascularisation. In a section, a large number of primary and secondary spermatocytes are visible and intense spermatogenesis can be observed.

2. Mature Phase There is increase in the weight and volume of the testis which become turgid and opaque white in colour. Histologically the seminiferous tubules show increase in size and are full of sperms. The

spermatogonia are reduced in number while all other stages of spermatogenesis can be seen in the tubules.

3. Spent Phase Once again the testis is partially becoming thin and translucent. The weight and volume of the testis is reduced due to discharge of the sperms. In a section empty and collapsing seminiferous tubules are seen, some of which may contain residual sperms. The testes enter a brief resting period after which the cycle of maturation starts again.

Spermatogonia are the only germ cells present during the resting phase and are called 'resting germ cells'. Generally, males are smaller than females. In late post-spawning and early pre

spawning season the ovary for within and outside PFZ areas were 7cm and 6cm in size and subsequently 7.5cm and 5.10cm in length respectively. The ovaries of *Pampus argenteus* are paired, broad in compact structure lying in the body cavity ventral to the kidney. They are attached to the dorsal coelomic wall by a thin membranous mesovarium. Both the lobes of Lshaped ovary are closely opposed to each other throughout their length. Posteriorly the two ovaries are fused together to form a common, short and muscular oviduct opening to the exterior through a common urinogenital aperture immediately behind the anus. The anterior free ends of the ovaries are relatively broader and rounder than the posterior end. There will be somewhat more or less right and left lobe of the ovary distinct.

Maturity stages of ovary: At the size of 25-30cm (total length) the females can be distinguished from males by the presence of yellow opaque gonads occupying in the body cavity. In males, the testis makes their appearance as two white opaque complex structures, flattened occupying in the body cavity. Based on the size, shape, colour and texture of the ovaries three arbitrary stages of maturity have been identified.

Stage I (Maturing virgins and Recovering spent): Each ovarian lobe at this stage becomes turgid in size and appears yellowish opaque; the two lobes are more or less unequal in length. Maturing virgins and recovering spents which cannot be distinguished macroscopically but histologically a recovering spent ovary is characterized by having a thick ovary wall and often containing residual oocytes.

Stage II (Ripening or Mature): Pair of distinctly yellowish and opaque ovarian lobes, the length of ovary varies 7cm to 6.2cm from within and outside PFZ in

November-December whereas for both PFZ 7.5cm to 5.10cm in length in March-April. The right lobe shows somewhat a maximum length than the left lobe. The entire gonad occupies in the body cavity which are almost fully packed with yolky oocytes. The ovarian wall appears as dark bodies under the microscope.

Stage III (Ripe): The massive opaque yellowish ovaries which are fully packed with ripe eggs fill the half body cavity with numerous blood vessels ramifying over their surface.

Ovary

The ovaries of silver pomfret *P. argenteus* are paired, L-shaped and opaque yellow in structure. They are attached to the viscera on one end and kidneys on the other. The two lobes are enveloped in a transparent sheath which brings the two lobes in close proximity to each other. Towards the distal end the two lobes unite and form a very short oviduct that opens to the outside and is used to expel the ripe eggs to the exterior at the time of spawning. Ovaries can be easily classified according to the standard terminology; some stages were recognized based on the gross structure of the ovaries. The stages are developing, maturing, ripening, partially spawned and spent. The ovarian maturity cycle of the Silver pomfret female is described by stages below.

Stage 1 (Developing):

There was an increase in the size of the gonad. The girth of the ovary increased. The colour of the gonad was yellow and the blood vessels were quite visible on the surface of the ovary. The texture of the ovary was compact and hard. Histologically, the primary oocytes dominated, but some early stages of secondary oocytes in the yolk vesicle stage were also seen. The size of the oocytes increased because of yolk vesicle deposition. In the secondary oocytes, zona radiata was now established. However, this size distribution was season-dependent. Late post-spawning in the season ovaries had oocytes in the smaller range but during early pre-spawning months the females in this stage had bigger oocytes than small oocytes.

Stage 2 (Maturing)

The weight of the ovaries increased linearly in both the season from within and outside PFZ. The ovarian texture was still solid but blood circulation had increased. The entire surface of the ovary was covered with big and small arteries. The colour of the ovary became dark yellow because of the yolky oocytes.

Histologically three types of oocytes could be seen. A very small group of primary oocytes was present between the crevices made by the growing oocytes. Some were in the secondary stage, but by far, the largest group was of tertiary-stage oocytes. These oocytes were characterized by a well-developed zona radiata. This was further divided into zona externa and zona interna. The theca and granulosa cells were seen around the zona externa. The tertiary-stage oocytes had well developed, true yolk granules and some fat droplets. The nucleus had nucleoli in it. The tunica was thinner than in the previous stages and the yolked oocytes could be seen through the tunica.

Stage 3 (Ripening)

The gonads continued to increase. The ovaries at this stage exhibited a softer, yellow speckled appearance when seen through the surface because the tunica was thin, completely stretched and transparent. The overall colour was yellow with a spotted appearance of yellow. The blood supply was at its peak and the surface was completely covered with the thick arteries and their capillaries. Histologically only two types of oocytes could be seen: the primary oocytes present in the crevices and the tertiary stage oocytes. This stage was characterized by the movement of the (nucleus) germinal vesicles. Along with this there are oil droplets and the yolk granules. This stage is a clear cut indication of the final maturity of the oocytes. The oocytes of this type were tightly packed and were at the same stage of development.

Stage 4 (Partially spawned)

The ovary further in part evacuated with the oocyte decreases in this stage. This stage was generally seen in the early pre-spawning season. The tunica is wavy and oocytes could be seen through them. Histologically the ovarian structure is very similar to that of above three stages may be seen.

Stage 5 (Spent):

In general, the ovary decreases further as this type of ovary is found in the last part of the early pre-spawning season. The ovarian outline is loose and baggy and the tunica is thick. Nothing can be seen through it. Histologically only the primary cells predominated in the ovarian cavity. There were a lot of empty spaces present in the ovary. Stroma was developing again.

4.Conclusion:

In March-April when the water temperature and photoperiod started increasing there was an increase in the ovary size. This means that real changes in the development of the ovaries started in the last days of April. This was the time when endogenous vitellogenesis had already started and early phase of exogenous vitellogenesis could also be seen. Endogenous vitellogenesis means that the oocyte's cytoplasm is directly involved in the synthesis and deposition of the yolk nucleus. Yolk vesicles and related materials in the cortical cytoplasm of the developing oocyte. In this stage, the oocyte's own nuclear genes that are actually taking part in the transcription and translation of the genetic material into yolk proteins. This material stains very deeply with basic stains. This blue-coloured material is sometimes referred to as Balbiani bodies. This initiation of the whole process is dependent to a large extent on the water temperature. The increase in the water temperature also causes the abundance in food and an increase in the foraging behavior of the fish. The present detailed study on the pomfret species from both within and outside PFZ upon validation of PFZ advisory is interesting from viewpoint that it describes an integrated account of the gross structure of the ovary, seasonal cycle and oogenesis cycle for the benefit of the fishery biologists.

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