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Seasonal Testicular Cyclicity in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Studies on circaannual changes in reproduction of Rainbow trout (*Oncorhynchus mykiss*), an economically important fish of Kashmir have been carried out. The variations in testicular structure and function of rainbow trout were studied during the present investigation. Morphometric and gravimetric data revealed four distinct reproductive phases viz. (I) Resting or Spent stage (II) Maturation stage (III) Mature stage and (IV) Regression stage. Maximum GSI was recorded during the mature stage and minimum in resting or spent phase (0.747). Histologically, testis revealed different germ cell types which were categorized into primary spermatogonia, secondary spermatogonia, spermatocytes, spermatids and spermatozoa. The primary spermatogonia were the largest cells and were present on the periphery of seminiferous lobules. These cells divide meiotically and form secondary spermatogonia. The secondary spermatogonia were smaller in dimensions than primary spermatogonia. The primary spermatocytes develop from secondary spermatogonia by meiotic division. Second Meiotic division of the secondary spermatocytes produces the spermatids. Finally spermatozoa are formed from the spermatids within the lobule lumen. All these stages were visible at the maturing phase whereas in the mature stage the testicular lobules were filled with spermatozoa only.

Key words: Rainbow Trout · Reproductive Phases · GSI · Germ Cells

INTRODUCTION

Biology of teleosts is receiving a great attention of scientists during the last decade because of economic reasons. Fishes contribute about millions/annum [1]. In fish species the diversity in the gonad structure and reproductive strategies are marked which need to be further explored [2-5]. In teleost the breeding cycle is governed by environmental factors such as photoperiod, temperature, salinity, rainfall and food etc. [6-8]. The relationship of reproduction and seasonality in the fishes can be analyzed by histological studies. During the recent years, much attention has been paid to the seasonal histological changes in the testis. Important contributions on the testicular cycle have been made by Turner [9] in Perch, Van Oordt [10] in *Xiphophorus helleri*, Craig-Bennet [11] in *Gasterosteus aculeatus* and Nair [12] in Siluroid fishes. Despite the highly variable reproductive strategies the basic organization of the testis is notably conserved in fish. In some teleost species, histological changes in the morphology of the testicular germinal epithelium have been used to document a sequence of five reproductive classes during the annual reproductive cycle: regressed, early maturation, mid-maturation, late maturation and regression. This classification is based on the germ cells stages present and the alternation between a continuous and discontinuous germinal epithelium [13-15].

In teleosts, spermatogenesis occurs in the testicular germinal epithelium within spermatocysts. Normally five spermatogenic stages, *i.e.,* primary spermatogonia, secondary spermatogonia, spermatocytes, spermatids and spermatozoa (Sperm) are observed in the testes of fish [16]. For a proper sustainability of a fish species, a thorough study of maturation cycles and alterations of gonads are important, since such a study is aimed in understanding and predicting the annual changes of the population [17, 18]. Rainbow trout constitutes maximum trout fishery of Jammu and Kashmir but it still receives less attention from the fisheries scientist. The information related to its reproductive status from Kashmir waters is still lagging. This is well established exotic fish species it is therefore studies on its development are important to analyze changes in its reproductive performance and seasonality. The information generated by present study shall find applications in enhancing fish seed production.

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MATERIALS AND METHODS

In the present study mature rainbow trout (*Oncorhynchus mykiss*) samples (Length to weight) were analyzed for histoarchitectural analysis. Live samples were obtained from different water bodies of Jammu and Kashmir e.g. Verinag (33.55° N and 75.25° E) and Kokernag (33.69° N and 75.22° E) from January to December 2013. Immediately after the capture of the fish the morphometric and gravimetric analysis were made. Thereafter from the fish the testes were dissected out and were weighed for the Gonadosomatic Index (GSI) and later were fixed in the Bouin's fixative for 24 hours. The samples were subsequently processed through ascending series of ethanol grades for dehydration, then were cleared in xylene and embedded in paraffin wax for sectioning. The sections were cut about 5µm thickness. The slides were stained with hematoxylin and eosin and were examined under light microscopy. The results were analyzed by one way ANOVA.

RESULTS

Gonadosomatic Index (GSI): The present study highlights the seasonal fluctuations in the gonadosomatic index of trout. The value of GSI showed remarkable changes in different seasons. The weight of these gonads varied from 28 grams in mature stage and 4 grams in resting stage. The GSI follows a regular cyclic pattern in the different months of the year. Based on macroscopic and microscopic observations of the testes during seasonal changes and on the gonadosomatic index (GSI), the annual reproductive cycle of male testis was divisible into four stages. (I) Resting or Spent stage (II). Maturation stage (III). Mature stage. (IV). Regression stage. The gravimetric data revealed that the gonadosomatic index was maximum (4) during the mature stage followed by (2.09) in maturation stage it was (1.11) in regression stage while minimum in (0.747) the spent phase (Graph 1).

The testes of Trout are paired organs and are enclosed by an outer thin peritoneum and an inner thick *Tunica albuginea* which eventually composed of dense connective tissue. Each testis is composed of numerous seminiferous tubules surrounded by a lobule boundary wall and containing nests of various germ cells. The sizes of the lobules enlarge during spawning season for the accommodation of spermatozoa and spermatids.

Stage I: Resting or Spent Stage: During the present study it was observed that from early March to late May testis appeared thin, slender and whitish in colour, adhered to the body wall and testis were at resting stage. In this stage, new spermatogonia arise by mitotic division of the quiescent primary spermatogonia, forming groups of secondary spermatogonia close to the testis tubular wall. The mean GSI at this stage was 0.747.

Stage II: Maturating Stage: In this stage the testis were more whitish than previous stage. This stage initiates from mid-June to late September. The testes were thicker and elongated by the end of this stage. Microscopically the seminiferous tubules were larger and contained cysts with spermatocytes. The transformation of the secondary spermatocytes into the spermatids was observed. During the late phase of this the spermatozoa begin to form. The cysts generally showed a higher number of secondary spermatocytes, spermatids and spermatozoa in the lumen of lobules. GSI at this stage was found to be 2.09.

Stage III: Maturation Stage: This stage lasts from early November and ends at the mid of January. In this stage testis showed increase both in volume as well as weight (Figs. 1 & 2). In this stage the milt was released in a large quantity after slight massage to the abdomen. The testis showed intense spermatogenesis in this phase and spermatozoa filled all of the tubules. In few cases the peripheral region of mature testis shows spermatocyte and spermatids. GSI was found to be maximum (4) during this stage.

Stage IV: Regression Stage: This stage starts from mid of January to end of February and the testis shows reduction in weight and volume. In this stage the spermatozoa that were not eliminated were reabsorbed. The testis decreased in size and become almost like stage (I). Light microscopy of the testis revealed total disorganization of the tubular structure with large number of dispersed spermatozoa. Mean GSI was found to be 1.11

Histological Architecture of Testis: In the testis of Trout five types of germ cells viz., spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are identified during different reproductive phases.

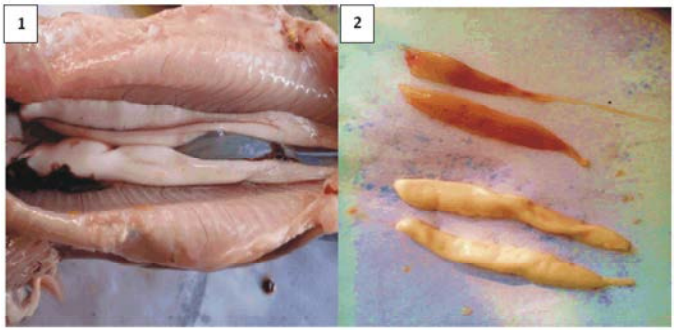
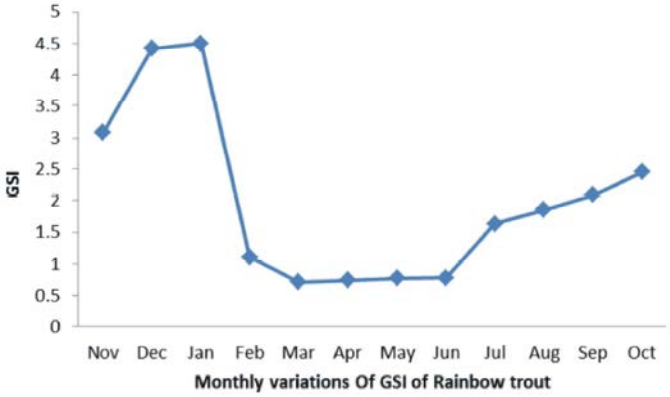
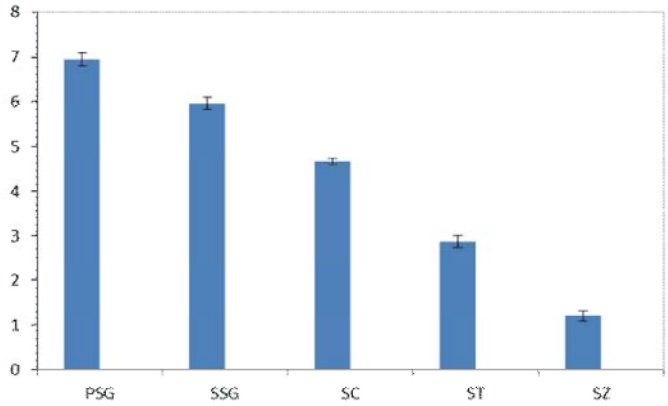


Fig. 1, 2: Shows testis in maturation stage and immaturation stage:



Graph 1: Mean value of GSI through breeding cycle of fish



Graph 2: Showing mean diameter of different types of cells and their standard error

Spermatogenesis: The spermatozoa are formed from the sperm mother cells or spermatogonia through a series of changes collectively referred to as "Spermatogenesis". Through mitotic divisions and growth, the spermatogonia produce the primary spermatocytes. The latter then undergo reduction division to form the secondary spermatocytes. By the successive division of secondary spermatocytes the spermatids are formed, which then change into the motile and potentially functional gametes- the spermatozoa. The spermatogonium is a spherical structure containing a round deeply stained centrally placed nucleus. The cytoplasm does not take much stain. These are larger in size (Diameter6.94±1.53µm), located along the periphery of the lobule. The primary spermatogonia are prominent with cell boundaries and have a large spherical nucleus with prominent and darkly stained nucleolus (Fig. 3). The primary spermatogonia multiply and form a large number of secondary spermatogonia. They were usually smaller than the primary spermatogonia and having less cytoplasm. These cells are slightly smaller (Diameter 5.95±.013 µm) than primary spermatogonia (Fig. 4).

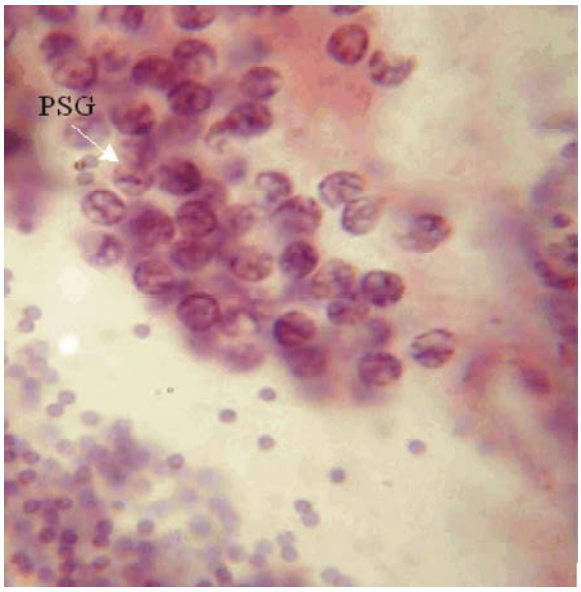


Fig. 3: Photomicrograph of section of testis showing prominent Primary spermatogonia (PSG) with nucleioli. Note small sized spermatids. (X1000)

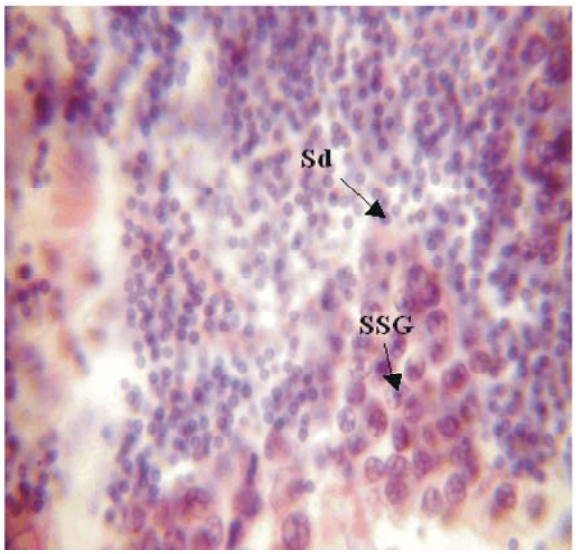


Fig. 4: Photomicrograph of section of testis showing Secondary spermatogonia (SSG) and Spermatocytes (Sd) (X1000)

The primary spermatocytes develop from the spermatogonia by meiotic divisions. Each primary spermatocyte contains a large nucleus as compared to cytoplasm. Primary spermatocytes divided to produce secondary spermatocytes. The size of secondary spermatocytes was found to be smaller (Diameter 4.67±.067µm) (Fig. 5) than that of primary spermatocytes

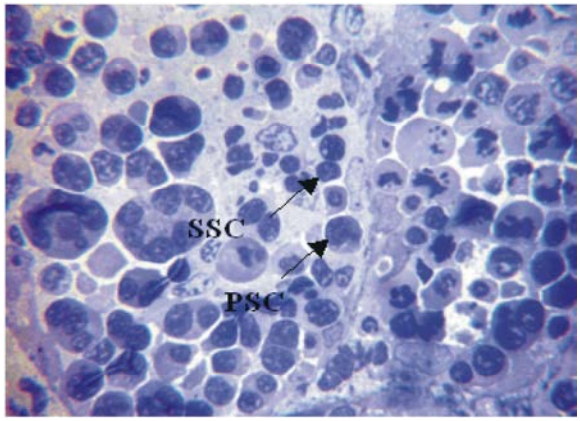


Fig. 5: Primary spermatocyte (PSc) and Secondary spermatocytes (SSc) contain electron-dense chromatin (Ch) in the nucleus (N). (1000X)

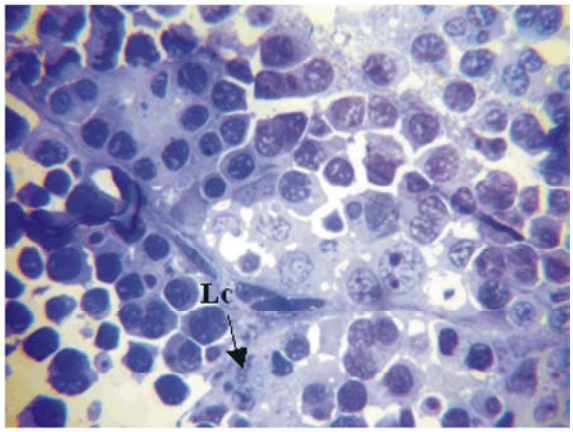


Fig. 6: A portion of maturing stage testis showing cells at various stages of division and maturation. Note Leydig cells in between the lobules (1000X)

(Fig. 6). Second Meiotic division of the secondary spermatocytes produces the spermatids which have no distinguishable cytoplasm. Spermatids are strongly basophilic (Haemotoxylene positive) spherical cells. As they mature, they become smaller and the nucleus is very small, also chromatin becomes uniformly condensed (Diameter 2.88±1.28µm) (Fig. 7 and graph 2). The nest membrane is no longer apparent although these cells remain in dense clusters after detachment from the lobules wall. The spermatids were ultimately transformed into spermatozoa, the smallest cells in the testes and are found in clusters (Diameter 1.20±1.25µm) (Fig. 8). The spermatozoa stained strongly and occurred in the lumen of seminiferous tubules. Sertoli cells are separated from the interstitial tissue by a basement membrane. Some Sertoli cell were located on the tubular basal lamina.

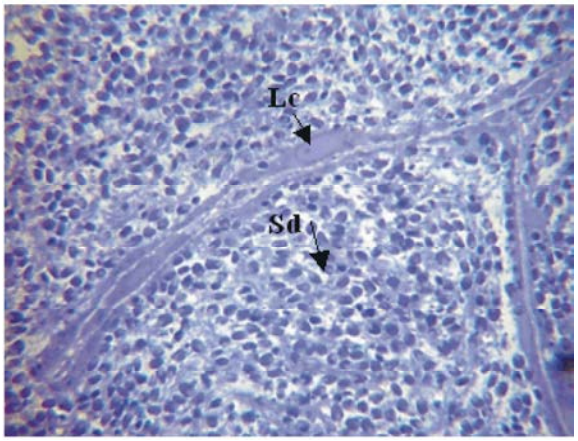


Fig. 7: Photomicrograph of section of testis showing enlarged Leydig cells (Lc)and Spermatids (Sd) (1000X)

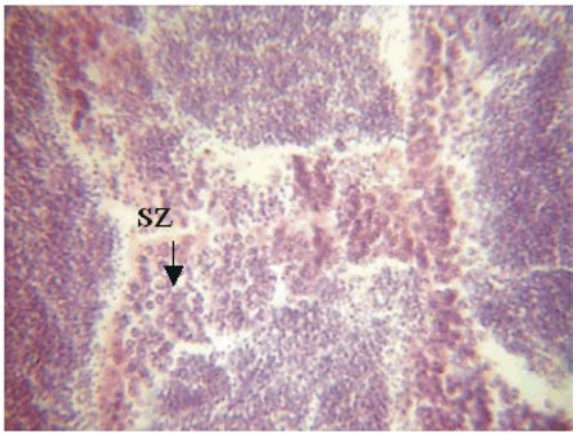


Fig. 8: Photomicrograph of section of testis showing Spermatozoa (SZ) filled lobules in matured testis (1000X)

Extensions of Sertoli cells envelop germ cells forming spermatocysts. These cells had triangular or round nucleus with chromatin in a dispersed pattern or forming small clots. When early spermatogonia were the only germ cells present next to residual sperm being progressively removed by Sertoli cells via phagocytosis.

DISCUSSION

The testes in trout are paired organs that show seasonal variations in shape, volume and weight, depending on the degree of maturation. The annual cyclic changes occur mainly due to spermatogenic activity and maturation of germ cells. Prior to spawn, the testes undergo preparatory stages during the remaining part of the season, which includes various degrees of histological and cytological changes in relation to spermatogenetic activity. In the present study the testes of trout exhibited phenomenal variations both in volume as well as size during different seasons. It has also been observed that the GSI values vary greatly during the different months of the year. It remains very low throughout during the spent phase when spermatogenetic activities almost ceases. However, because of the proliferation of the spermatogonial cells and subsequent formation of spermatocytes in the testis, gradual increase of GSI has been noticed. The highest GSI value was found in maturation and spawning phases due to the active proliferation of the later stages of the spermatogenetic cells causing the relative increase of the testes weight. Similar changes of the GSI values in relation to spermatogenetic activity in the testes of different teleosts like *Channa gachua, Etroplus suratensis, Puntius javanicus* and *Liza parsia* [19-22]. It has been observed that the fish spawn only after gaining the highest GSI. Therefore the GSI is closely related with the maturity and spermiation of the fish. Pattern of changes in GSI observed in male gonad corresponds to GSI fluctuation in female gonads of rainbow trout [23]. The testis of rainbow trout during its seasonal testicular cycle exhibit a period of growth, maturation, depletion and rest. Similar set of changes have been reported in other teleosts by Umeda and Hesangawa [24]. In present study, dormant nests of spermatogonia occur in the resting periods. Similar types of nests were reported in other teleosts by Htun-Han [25], Sinha and Mandal [26] and El-Boray [27]. In the present study it was observed that spermatids were finally metamorphosed into spermatozoa during the maturation phase. During immaturation phase cells like primary spermatogonia (PSG), secondary spermatogonia (SSG) and spermatocytes (SC) were present. In maturation phase, the testes were predominated by spermatocytes, spermatids and spermatozoa. Similar results were also observed in Mahseer where in spermatogenic activity starts from preparatory phase and continues up to spawning or maturation phase. During spawning season it was found that the seminiferous tubules were filled with spermatozoa [28]. The testes of *Gasterosteus aculeatu*s remain mature throughout the year but their functional maturity is attained only in the breeding season (April-May) [29]. In present study it become evident that the testes in rainbow trout reveal structural and functional

cyclicity from the immature phase to regression phase its spermatogenic stages are similar to that of seasonally breeding fish *Labeo rohita.*

REFERENCES

1. Nelson, J.S., 2006. Fishes of the World, 4th ed, pp: 601. John Wiley and Sons, New York.
2. Nagahama, Y., 1983. The functional morphology of teleost gonads. In: Hoar, W.S. and Randall, D.J. (Eds): Fish Physiology, Vol.9. Part A, pp. 433. Part B, pp: 477. Academic Press, New York.
3. Pudney, J., 1995. Spermatogenesis in non-mammalian vertebrates. Microsc. Res. Tech., 32: 459-497.
4. Pudney, J., 1996. Comparative cytology of the Leydig cell. In: Payne, A. H., Hardy, M. P. and Russell, L. D. (Eds): The Leydig Cell, pp: 98-142. Cache River, Vienna, Ill.
5. Nakatani, K., A.A. Agostinho, G. Baumgartner, A. Bialetzki, P.V. Sanches, M.C. Makrakis and C.S. Pavanelli, 2001. Ovos e Larvas de Peixes de A´ guaDoce: Desenvolvimento e Manual de Identificacao, pp: 378. Eduem, Maringa´.
6. Bromage, N., M. Porter and C. Randall, 2001. The environmental regulation of maturation in farmed finfish with special references to the role of photoperiod and melatonin. Aquaculture, 197: 63-69.
7. Alvarenga, E.R., N. Bazzoli, G.B. Santos and E. Rizzo, 2006. Reproductive biology and feeding of *Curimatella lepidura* (Eigenmann & Eigenmann) (Pisces, Curimatidae) in Juramento reservoir, Minas Gerais, Brazil. Revista Brasileira de Zoologia, 23: 314-322.
8. Singh, R., S.K. Chaturvedi and Abhinav, 2010. Effect of photoperiod and temperature on testicular regression in *Channa punctatus*. J. Env. Biol., 31: 307-310.
9. Turner, C.L., 1919. Seasonal changes in the spermary of the perch. J. Morph., xxxii, 681-711.
10. Van Oordt, G.J., 1925. The relation between the development of the secondary sexual characters and structure of the testes in the teleost *Xiphophorus helleri*. Brit. J. Expt. Biol., iii, 43-49
11. Craig-Bennet, A., 1931. The reproductive cycle of three spined stickleback *Gacterosteus aculeatus*. Phil. Trans. Roy. Soc. London. ccxix, 197-279.
12. Nair, P.V., 1965. Studies on the male reproductive system of some siluroid fishes. Ind. J. Zool., pp: 1-35.
13. Grier, H.J. and R.G. Taylor, 1998. Testicular maturation and regression in the common snook. J. Fish Biol., 53: 521-542.
14. Taylor, R.G., H.J. Grier and J.A. Whittington, 1998. Spawning rhythms of common snook in Florida. J. Fish Biol., 53: 502-520.
15. Grier, H.J., 2002. The germinal epithelium: its dual role in establishing male reproductive classes and understanding the basis for indeterminate egg production in female fishes. Proceedings of the Fifty-third Annual Gulf and Caribbean Fisheries Institute, Biloxi, Mississippi November 2000 (Creswell, L. R., ed.), pp. 537–552. Fort Pierce, FL: Alabama Sea Grant Consortium.
16. Agarwal, N.K., 1996. Testicular morphology, histology, histochemistry and seasonal cyclicity. Fish Reproduction, APH Publishing Corporation, New Delhi, pp: 16-33.
17. Thorpe, J.E., C. Talbot, M.S. Miles and D.S. Keay, 1990. Control of maturation in cultured Atlantic salmon, *Salmo salarin* pumped seawater tanks, by restricting food intake. Aquaculture, 86: 315-326.
18. Jobling, S., S. Coey, J.G. Whitmore, D.E. Kime, K.J.W. Vanlook and B.G. Mcallister, 2002. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. Biol. Reprod, 67: 515-524.
19. Sanwal, R. and S.S. Khanna, 1972. Seasonal changes in the testes of a freshwater fish *Channa gachua*. Acta Anat., 83: 1-6.
20. Jayaprakash, V. and N.B. Nair, 1981. Maturation and spawning in the pearl spot, *Etroplus suratensis*. Proc. Indian Natn. Sci. Acad., 47: 828-836.
21. Chakrabarti, P. and A.K. Gupta, 1994. A histological study of the testes during growth, maturation and spawning phase in the exotic fish *Puntius javanicus* (Blkr.) Ad. Bios., 13: 11-12.
22. Rheman, S., M.L. Islam, M.M.R. Shah, S. Mondal and M. Alam, 2002. Observation on the fecundity and gonadosomatic index (GSI) of the grey mullet *Liza parsia* (Ham.). J. Biosci., 2: 690-693.
23. Sharma, R.K. and R.A. Bhat, 2014. Histoarchitectural variations during oocyte growth in Rainbow trout (*Oncorhynchus mykiss*). Int. J. Fish. Aquat. Stud., 2(2): 177-183
24. Umeda, S. and I. Hesangawa, 1984. Annual cyclic changes in the testicular activity of the yellowtail cultured in Komame Bay, Japan. Report USA Mar. Biol. Inst. Kochi Univ., 6: 41-47.

25. Htun-Han, M., 1978. The reproductive biology of the dab, *Limanda limanda* (Ham.) in the North Sea: Seasonal changes in the testes. J. Fish Biol., 13: 361-368
26. Sinha, G.M. and S.K. Mandal, 1982. Detection and localization of phosphatases, DNA, glycogen, mucopolysaccharides and bound lipids in testis of a teleost fish, *Anabas testudineus* (Bloch) during the annual cyclical changes by histochemical methods. Mikroskopie, 39: 1-13.
27. El-Boray, K.F., 2001. Histological changes in the testes of the fish *Gerres oyena* (Forsskål, 1775) during the reproductive cycle in Suez bay, red sea, Egypt. Egypt. Aquat. Biol. Fish, 5: 83-93.
28. Rai, B.P., 1965. Seasonal changes in the testis of the Mahseer, Barbus tor (*Tor tor*). Acta. Anat., 62: 461-475.
29. Swarup, H., 1958. The reproductive cycle and development of the gonads in *Gasterosteus aculeatus* (L.). Proc. Zool. Soc. Bengal., 11: 47-61.