ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 458-463

www.fisheriesjournal.com
Received: 22-11-2015
Accepted: 24-12-2015

P Chakrabarti
Fisheries Research Laboratory,
Department of Zoology,
The University of Burdwan,
Burdwan 713104, West Bengal,
India.

M Banerjee
Fisheries Research Laboratory,
Department of Zoology,
The University of Burdwan,
Burdwan 713104, West Bengal,
India.

Correspondence
P Chakrabarti
Fisheries Research Laboratory,
Department of Zoology,
The University of Burdwan,
Burdwan 713104, West Bengal,
India.

Histocytological changes in the corpuscles of stannius in relation to ovarian development of freshwater featherback *Notopterus notopterus* (Pallas) during growth, maturation and spawning phases

P Chakrabarti, M Banerjee

Abstract
The corpuscles of Stannius (CS) of important food fish *Notopterus notopterus* were studied in relation to ovarian maturation. Cytological status of CS were correlated with the ovarian development during growth, maturation and spawning phases. The cytoarchitecture of CS in *N. notopterus* showed two principle types of secretory cells (Type I and Type II) arranged along the connective tissue septa. Different germ line cells were recognized on the basis of size and histoarchitectural morphology of cells. The Type-II cells varied according to the proliferation of different stages of oocytes in harmony with the various reproductive phases. It was found that at the end of growth phase and onset of maturation phase the diameter along with cytoplasmic granules of Type-I cells increased considerably than the Type-II cells, which was correlated with the occurrence of cortical alveolus and yolk granule stages in the ovary. During the end of maturation and spawning phases the diameter of Type I cells further increased along with chromophobic cytoplasm and hypertrophied nuclei were arranged in clusters encircling the blood vessels. No significant changes were noticed in the Type II cells. These momentous changes correlated with the dynamic cytological activities like vitellogenesis and occurrence of mature oocytes in the ovary. Thus the cytological changes of the CS during growth, maturation and spawning phases accomplished with the ovarian maturation in *N. notopterus.*

Keywords: corpuscles of Stannius, Ovarian development, Growth, Maturation, Spawning, *Notopterus notopterus.*

1. Introduction
Corpuscles of Stannius are clearly distinguished from the remaining kidney tissue as small, oval, cream or white colour bodies, whose disposition and number vary between species [1-3]. Several studies has been conducted on histoarchitechture, nature and mode of secretion of Corpuscles of Stannius in a number of teleosts [4-7]. Alim and Ehsan [8] have reported histology, location and functions of the corpuscles of Stannius in freshwater teleost, *Rasbora daniconius.* The oogenesis is a very active process in the ovaries, in which the oogonia passes through various phases of development that are very similar in different fish species. The ovarian cycle in majority of freshwater teleosts whish are seasonal breeders undergo remarkable changes during various periods of the season [9-14]. The growth phase includes upto the cortical alveoli stage while germinal vesicle breakdown, coalescence of yolk globules are the major features in the process of maturation of ovary [15, 16]. Loretz [17] had stated that the cells of Corpuscles of Stannius in fish possess extracellular calcium sensing receptors and help in calcium uptake from gill epithelium. Ahmed and Swarup [18] have reported that seasonal changes in the structure and behavior of corpuscles of Stannius in relation to ovaian cycle and corresponding changes in serum calcium level of *Mystus vittatus.*
The aim of the present study is to identify and localize the different cell types of corpuscles of Stannius and the event of oogenesis during different reproductive phases in *Notopterus notopterus.*

2. Materials and Methods
2.1 Gonadosomatic index (GSI)
Ten adult females of *N. notopterus* (average length 26 to 28 cm and average body weight 100 to 120 gm) were collected from local freshwater body during the second week of every month

from January to August, 2015 to calculate the GSI from the following formula:

$$GSI = \frac{\text{Weight of the ovary}}{\text{Weight of the fish}} \times 100$$

2.2. Histological methods

For histological studies after decapitation of the fish, the spherical, nodular structure of corpuscles of Stannius lying on or embedded in the anterio-posterior part of the kidney were fixed in Bouin's fixative. The pieces of ovarian tissues were also dissected out and were fixed in Bouin's fixative for 16 hours. The corpuscles of Stannius and ovarian tissues were placed in 70% ethanol and subsequently dehydrated with increasing ethanol concentration followed by acetone and cleared in benzene. Tissues were then embedded in paraffin (melting point 56- 58 ^{0}C). The tissues were serially sectioned at 4 µm thickness. Deparaffinized sections were brought to water and stained with Delafield's haematoxylene - eosin (H & E), Mallory's triple stain (MT), Iron alum haematoxylene, Periodic acid- schiff's stain [19] and Aldehyde fuchsin stain [20]. Sections were dehydrated through ascending series of ethanol, cleared in xylene and mounted permanently with DPX. The diameter of the Corpuscles of Stannius and various oogenetic stages of the fish were measured with the help of reticulo - micrometer and ocular micrometer respectively.

3. Results

3.1. Gonadosomatic index (GSI)

In present study it has been observed that the values of GSI in *N. notopterus* follow a regular changes during growth, maturation and spawning phases. During the growth phase i.e. in January and February the GSI value ranges from 0.967±0.013 to 1.08±.04. However, from March onwards when the ovary enter into the maturation phase GSI aligns between 1.753±0.625 to 5.51±0.17 and in May GSI increases sharply to 13.35±1.452. In June the ovary constitutes with full mature follicles and the GSI is maximum i.e. 18.571±1.01 and in July and August the GSI value shows a declining trend and is noticed to be 15.712±1.468 and 8.388±2.71 respectively.

3.2. Histological characteristics of corpuscles of Stannius and ovary

In the present investigation the corpuscles of Stannius (CS) in *N. notopterus* are superficially embedded on either side of the posterior kidney. Histologically each CS is invested by connective tissue capsule adjacent to renal tubules and rich in vascularization which results in a better access of gland cells to the blood circulation (Fig. 1). The cytoarchitecture of the CS shows two principle types of secretory cells (Type I and Type II). The Type I cells are round and homogeneously stained with eosin and aniline blue with oval or rounded nucleus (Figs. 1, 4, 9). Type II cells are oval in shape and having chromophobic cytoplasm (Figs. 4, 11). Sometimes Type I cells are arranged in the form of cords lined by single lager of cells (Figs. 5, 9).

On the basis of nucleocytoplasmic ratio and the mean oocyte diameter for each developmental stage, the sequence of oocyte maturation in *N. notopterus* has been divided into five distinct developmental stages viz. oogonia (Stage I), perinucleolus stage (Stage II), yolk vesicle or early maturation oocytes (Stage III), yolk granules or late maturation oocytes (Stage IV), mature follicles (Stage V) and atretic follicles. Oogonia are more or less spherical or oval in shape with their diameter ranging from 10 to 20 µm. They are provided with a large distinct nucleus (Fig. 2). Perinuclear oocytes are found to be almost oval with diameters ranging from 80 to 200 µm. The centrally placed nucleus provided with fragmented nucleoli with chromatin materials (Fig. 2). Yolk vesicle or early maturing oocytes is characterized by the appearance of vesicles in the cytoplasm (Fig. 3). Yolk granules or late maturing oocytes increase in size and the diameter ranges from 700 - 850 µm. Yolk vesicle or early maturing oocytes is characterized by the appearance of vesicles in the cytoplasm (Fig. 3). Yolk granules or late maturing oocytes increase in size and the diameter ranges from 700 - 800 µm (Figs. 3, 7). The thickness of theca, zona pellucida and zona radiata increase gradually. Yolk granules remain tightly packed with each other (Fig. 7). Mature follicles are more or less spherical in shape and attains an average diameter of 1,100 µm. Yolk granules occupies the entire oocyte and the germinal vesicle is formed to be eccentric in position (Figs. 8, 12). The developing oocytes that fail to attain maturity and undergo resorption are called atretic oocytes. These are characterized by irregular shape, disintegrated nuclei and liquefied yolk granules (Fig. 8).

3.3. Sequential changes in corpuscles of Stannius (CS) and ovary during different reproductive phases

The activities of the Type-I and Type-II cells of CS and frequency of various oogenetic cells, as revealed by the histological studies are found to undergo changes in harmony with growth, maturation and spawning phases.

3.3.1. Growth phase (January to February)

In this phase the Type I cells are round or oval in shape, arranged in clusters or scattered adjacent to the blood vessels and between renal tubules (Fig. 1). The cellular diameter of Type I cells ranging from 4 to 5 µm and Type II cells from 2 to 3 µm. At the end of growth phase the increment of the Type-I cells including their chromophobic cytoplasm is clearly detected in comparison to Type-II cells.

Dominant cell types in this period are the early perinucleolar oocytes. However, the percentages of late perinucleolar oocytes increased during the end of this period which shows cortical alveoli (Fig. 3). A sizeable blood vessel inscribing a near circular path is associated in between the oocytes.

3.3.2. Maturation phase (March to May)

During this phase the number of Type-I cells increase in number and are arranged in clusters encircling the blood vessels (Fig. 4). The diameter of Type I cells increase to 6 to 8 µm and undergo hypertrophy during this phase (Fig. 4). The type I cells show intense reaction with AF and PAS respectively (Figs. 5, 6). No significant changes have been observed in Type II cells. They have a prominent nucleus and chromophobic cytoplasm and the diameter ranging from 3 to 4 µm (Figs. 4, 5).

The highest oogenetic activity has been found to occur during this phase and the majority of the oocyte enter into its secondary growth phase when cytoplasmic materials increase and vitellogenesis started and as a result, primary oocytes are converted into yolk vesicular oocytes (Fig. 7). From April to May a sharp increase in number of yolk filled mature follicles is well marked (Fig. 8).

3.3.3. Spawning phase (June to August)

In the spawning phase the Type I cells undergo momentous changes having hypertrophic nuclei and depleted cytoplasm (Figs. 9, 10). In the early spawning phase, the Type I cells contain chromophobic cytoplasm and acentric hypertrophic nuclei and they are arranged in cords and / or remain scattered in between the blood vessels (Figs. 9, 10, 11). The diameter of Type I cells increase to 9 to 10 µm. However, some of the Type-II cells are found to increase in diameter which has been recorded to 4 to 5 µm (Fig. 10).

The predominant cell types in this period are the mature follicles of stage V along with a few resting primary oocytes in between (Figs. 8, 12). The mature follicles are provided with eccentric nucleus (Fig. 12).

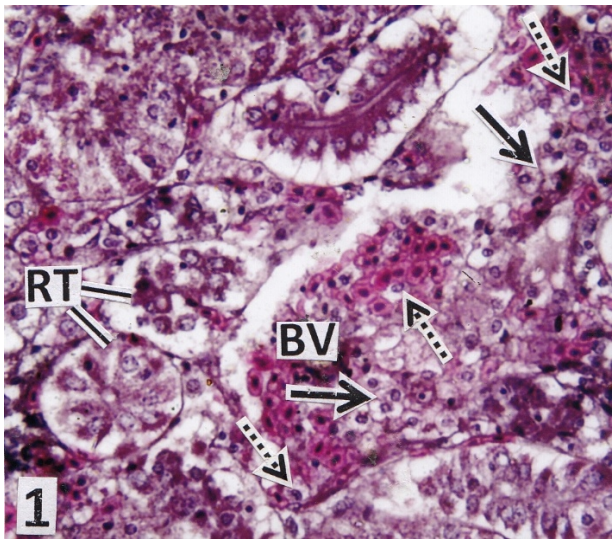

Fig 1: Showing disposition of Type I cells (solid arrows) and Type II cells (broken arrow) adjacent to blood vessels (BV) during growth phase. Note renal tubules (RT) in between Type I and Type II cells; (H & E) × 400X.

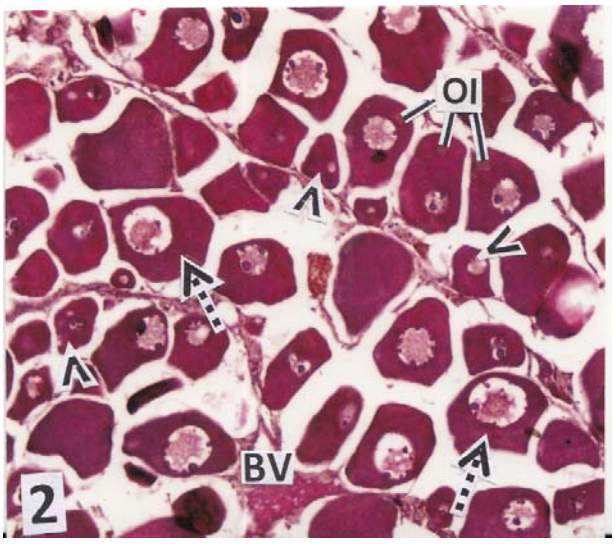

Fig 2: Showing oogonia (arrow heads), oocyte I (OI) and oocyte II (broken arrows) during growth phase. Note the presence of BV in between; (H & E) × 150X.

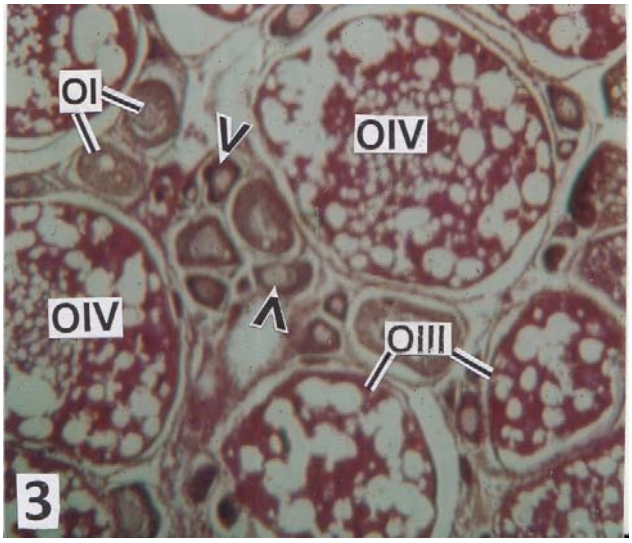

Fig 3: Showing cortical alveoli in oocyte III (OIII) stage and yolk vesicles in oocyte IV (OIV) stage during end of growth phase. Note oogonia (arrow heads) and oocyte I (OI) in between; (H & E) × 400X.

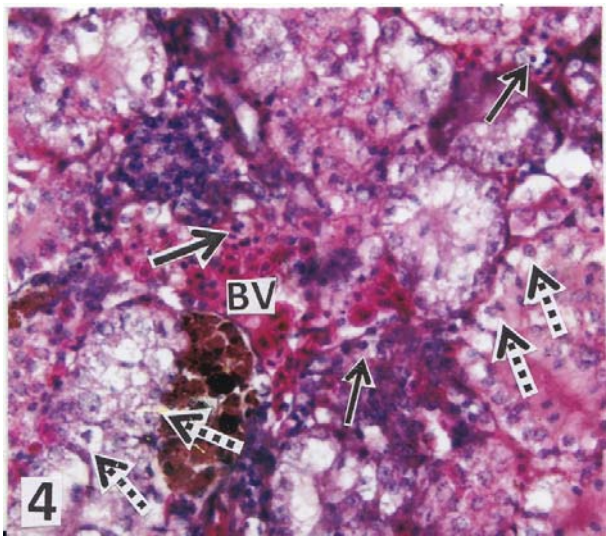

Fig 4: Showing enlargement of Type I cells (broken arrows) with chromophobic cytoplasm and Type II cells (solid arrows) having faintly stained cytoplasm in between BV during maturation phase. (H & E) × 400X.

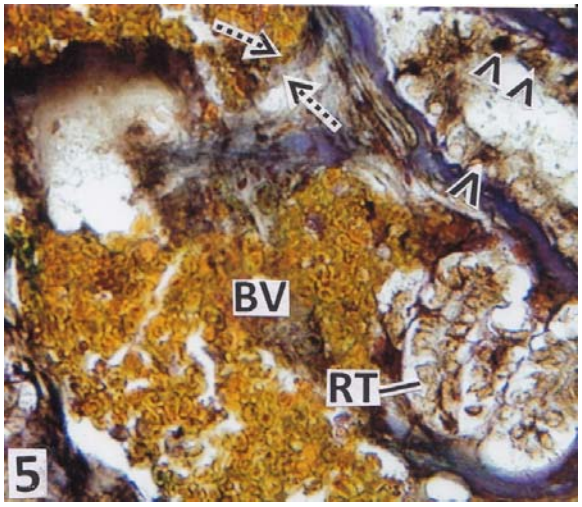

Fig 5: Showing intense AF reaction in Type I cells (arrow heads) while faint reaction in Type II cells (broken arrows) in maturation phase. Note positive reaction in BV and RT; (AF) × 400X.

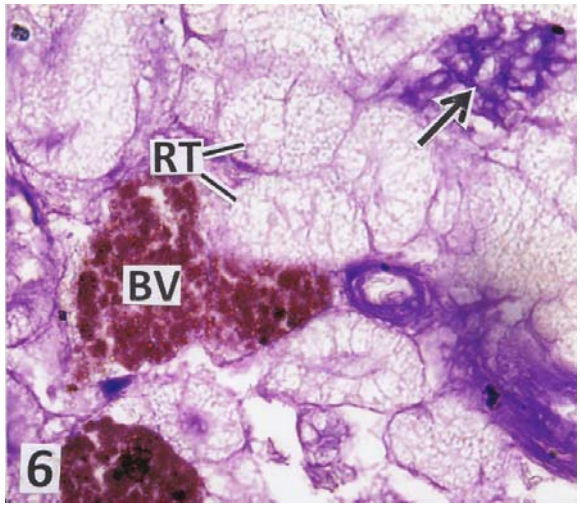

Fig 6: Showing intense PAS reaction in Type I cells (solid arrow) and BV and faint reaction in RT during end of maturation phase. (PAS) × 400X.

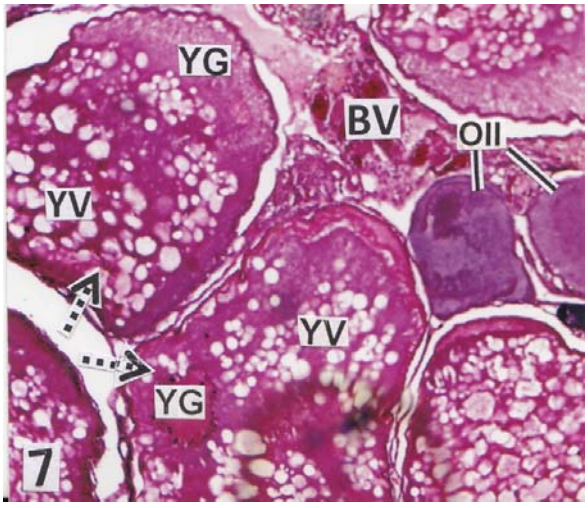

Fig 7: Showing yolk vesicles (YV) and yolk granules (YG) in mature oocytes (broken arrows) during maturation phase. Note the presence of oocyte II (OII) and BV in between; (H & E) × 400X.

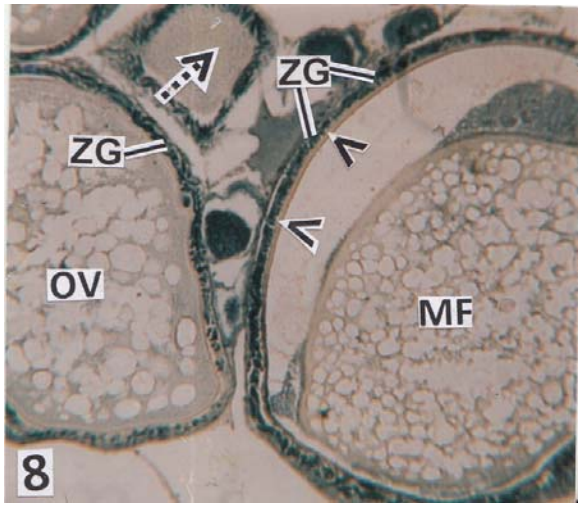

Fig 8: Showing mature follicles (MF) and oocyte V (OV) with prominent zona granulosa (ZG) and zona radiata (arrow heads) during maturation phase. Note atretic oocyte (broken arrow) in between MF; (IA) × 400X.

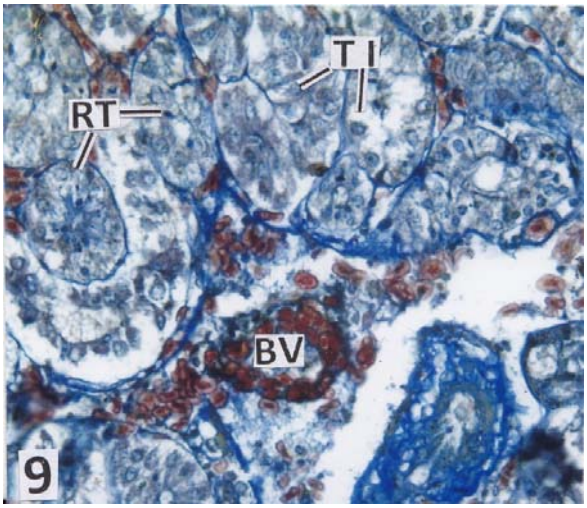

Fig 9: Showing hypertrophy of the Type I cells (TI) adjacent to blood cells and BV and RT during spawning phase. (MT) × 400X.

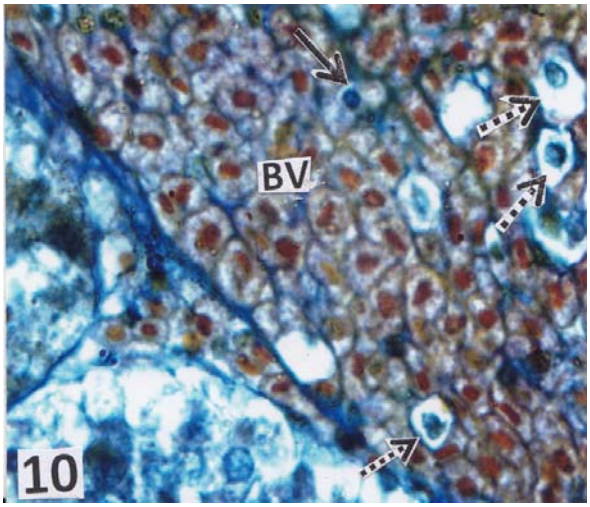

Fig 10: Higher magnification of hypertrophic Type I cells (broken arrows) and slight increment of Type II cells (solid arrow) in between blood cells of BV during spawning phase. (MT) × 1000X.

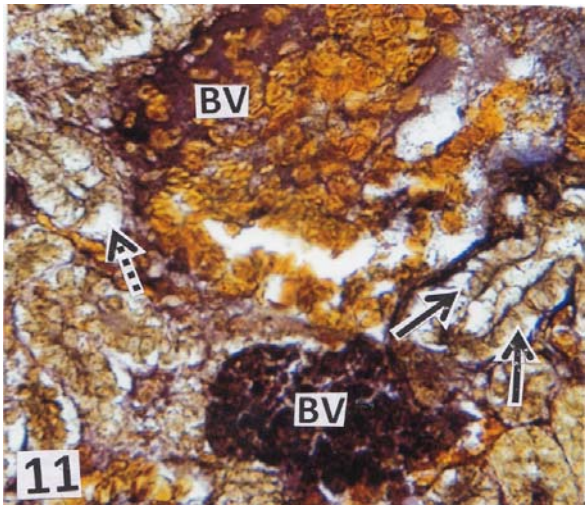

Fig 11: Intense AF reaction in Type I cells (solid arrows) and comparatively feeble stain in Type II cell (broken arrow) adjacent to BV during spawning phase. (AF) × 400X.

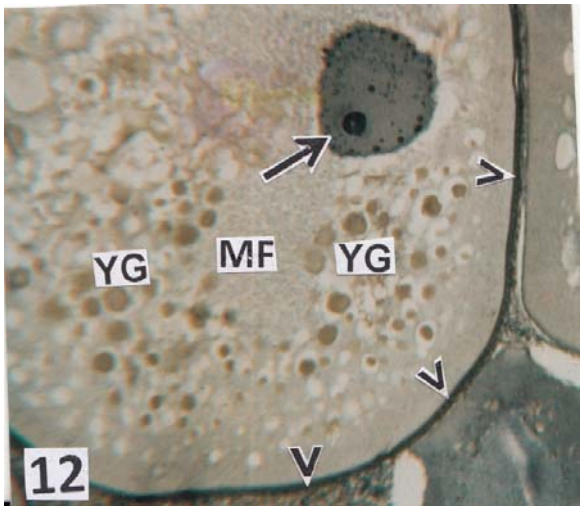

Fig 12: Showing mature follicle (MF) having dense yolk granules (YG) and eccentric germinal vesicle (solid arrow) during spawning phase. Note prominent ZG (arrow heads); (IA) × 600X.

4. Discussion

In the present investigation a pair of corpuscles of Stannius are present in *N. notopterus* and are located in the anterior part of the posterior kidney. However, Belsare [21] has reported that in *N. notopterus* only one Corpuscles of Stannius is present. In *N. notopterus* rounded or oval structure of Type I and Type II cells are usually arranged in cords, lobules or scattered and remain closely associated with the blood vessels and renal tubules. The cytoarchitecture of CS of catfish *Heteropneustes fossilis* has been investigated by Subhedar and Rao [22] based on the arrangement of cells and reported that at least four principle architectural patterns have been distinguished.

Bedjargi and Kulkarni [23] have observed four types of CS in *N. notopterus*. However, several studies confirmed that the CS of teleostean fishes contain two distinct secretory cell types, Type I and Type II cells [24, 25]. Aida *et al* [26] has reported that in *Onchorhynchus kisutch* CS contains two structurally different cells namely Type I and Type II. They further noticed that Type I cells were numerically predominant and possessed large abundant electron dense secretory granules, prominent RER and Golgi bodies. Ahmed *et al* [27] have stated that Type I cells were the source of stanniocalcin while the nature and function of Type II cells was not clear. In the present investigation it has been found that oogonia passes through a number of maturation stages before it becomes a ripe ovum. The formation of yolk globules in the oocyte begins in the periphery of the developing ooplasm, later the wave of yolk deposition moves towards the centre of the ovum. Bist and Joshi [28], Kapoor [29] have observed similar pattern of yolk deposition in *Schizothorax richardsonii* and *Puntius ticto.* In *N. notopterus* the mature ova are enveloped by zona radiata, zona granulosa and outermost theca. In the late developing oocytes it may be assume that these radial striations are relatively active in the transport of essential substances from granulosa layer to the oocyte cytoplasm for building up the protoplasm of the oocyte. Similar observation has also been made by Bromage and Cumaranatunga [30] and Shabanipour and Heidari [31] in the mature ovary of rainbow trout and *Liza aurata.*

In *N. notopterus* the development of ovary can broadly be divided into two phases. In the previtellogenic phase growth is slow and comparatively few cytoplasmic changes have been found. During growth phase the accumulation of cytoplasmic granules in the Type I cells has been started which is clearly reflected in tinctorial reactions. The vitellogenic phase is characterized by rapid growth and deposition of large amount of yolk in the cytoplasm. In the present investigation the significant increase in the number of vitellogenic oocytes is noticed during maturation phase. This is in conformity with the findings of Tyler and Dunn [32]. During maturation and spawning phases in *N. notopterus* Type I cells of CS begins to be hypertrophied including the increase in nuclear size and are found to be closely associated with the blood vessels indicating a relationship of releasing their contents in the blood circulation for the purpose of gonadal development. Ahmed and Swarup [18] have reported that the seasonal changes in the structure and behavior of CS in relation to ovarian cycle and corresponding changes in the serum calcium level of *Mystus vittatus*. Verma and Alim [7] have also noticed the simultaneous increase in the serum calcium and calcitonin level with the advancement of the reproductive cycle reaching the peak during pre- spawning and spawning phases. Urasa and Wendelaar Bonga [33] emphasized that in *Oreochromis mossambicus* the CS are enlarged in female fish, because of an increase in size and number of the Type I cells. Concurrently total plasma calcium increase markedly until spawning which is mainly accounted for calcium bound to vitellogenins.

5. Acknowledgements

The authors are grateful to the Department of Science and Technology, New Delhi for providing necessary instruments for this research work.

6. References

1. Hirano I, Hasegawe S, Yamauchi H, Orimo H. Further studies on the absence of hypocalcamic effect of eel calcitonic in the eel, Anguilla japonica. General and Comparative Endocrinology. 1989; 43:42-50.
2. Marra LE, Youson JH, Wendelaar Bonga SF, Hanssen RJGM, Wagner GF. Immunoreactivity of the corpuscles of Satannius of the garpike, Lepisosteus osseus to antisera against salmon and trout stanniocalcins. Cell and Tissue Research. 1994; 277:511-518.
3. Wagner GF. The molecular biology of the corpuscles of

Stannius and regulation of stanniocalcin gene expression. In Hochachka PW, Mommsen TP (eds.). Fish Physiology, Academic Press, Amterdam, 1994, 273-306.
4. Bhattacharya TK, Butler DB. Fine structure of the corpuscles of Stannius in the toad fish. Jounal of Morphology. 1978; 155:271-286.
5. Alim A. Studies on the corpuscles of Stannius of a hillstream teleost Crossocheilus latius. Indian Zoologist. 1986; 10:15-17.
6. Alim A. Studies on the corpuscles of Stannius of a teleost, Garra mullya. Zoologische Angizen. 1988; 221:314-318.
7. Verma SK, Alim A. Differences in the activity of prolactin cells in male and female freshwater teleost Mastacembelus armatus during gonadal cycle. Asian Pacific Jounal of Reproduction. 2014; 3:231-237.
8. Alim A, Ehsan S. Histology of corpuscles of Stannius of a teleost Esomus danrica. Biology Bulletin of India. 1988; 3:196-197.
9. Srivastava SJ. Seasonal histological changes in the ovary of a freshwater large murrel, Channa marulius. Zoologische Jahrbiicher. 1980; 104:492-499.
10. Mukherjee J, Bhattacharya S, Nath P. Annual changes in ovary and vitellogenin content of liver, serum and ovary of murrel, Channa punctatus. Journal of Experimental Biology. 1989; 27:764-769.
11. Manna PR, Bhattacharya S. Gonadotropin binding to the ovary of an Indian major carp, Catla catla of different stages of reproductive cycle. Journal of Bioscience. 1993; 18:361-372.
12. Aroskiaraj A, Haniffa M, Seetharaman S, Singh S. Cyclic changes in gonadal maturation and histological observations of threatened freshwater catfish Narikeliru Mystus montanus. Acta Ichthyilogica *et* Piscatoria. 2004; 34:253-266.
13. Singh A, Singh IJ, Ram RN, Kushwaha B. Ovarian development in Labeo dyocheilus during active reproductive phase under captive and wild condition. Journal of Environmental Biology. 2008; 29:169-174.
14. Yin JX, Racey P, Li J, Zhang YG. The ovarian cycle of the fish Leptobotia elongate endemic to China. Pakistan Jounal of Zoology. 2012; 44:997-1005.
15. Nagahama Y. The functional morphology of teleost gonads. In Fish Physiology, ed. Hoar WS, Randall DJ, Donaldson EM. New York; Academic press. 1983, 233-275.
16. Yueh W, Chang C. Morphological changes and competence of maturing oocytes in the Protandrous black porgy, *Acanthopargus schlegeli*. Zoological Studies. 2000; 39: 114-122.
17. Loretz CA. Extracellular calcium- sensing receptors in fishes. Comparative Biochemistry and Physiology Part A 2009; 149: 225-245.
18. Ahmed N, Swarup K. Seasonal changes in the functional morphology of corpuscles of Stannius body in relation to the reproductive cycle and changes in serum calcium level of a freshwater female catfish, Mystus vittatus. Proceedings of National Academy Science, India, 1990; 58:359-363.
19. Mc Manus JRA. Histological and histochemical uses of periodic acid. Stain Technology. 1948; 23:99-108.
20. Halmi NS. Differentiation of two types of basophils in the adenohypophysis of rat and the mouse. Stain technology. 1952; 27:61-64.
21. Belsare DK. Comparative anatomy and histology of the corpuscles of Stannius in teleost. Zeitschrift fur Mikmskopisch- Anatomische Forschung (Leipzig) 1973; 87:445-456.
22. Subhedar N, Rao Z. On the cytoarchitechture of corpuscles of Stannius of catfish, Heteropneustes fossilis. Mikroskopie Anatomische Forschung, Leipzig. 1976; 90:737-748.
23. Bedjargi PRC, Kulkarni RS. Light and electron microscopic structure of corpuscles of Stannius of the freshwater fish, Notopterus notopterus. International Journal of Applied and National Science. 2014; 3:45-54.
24. Wendelaar Bonga SE, Graven JAA. A second cell type in Stannius bodies of two euryhaline teleost species. Cell and Tissue Research. 1975; 159:287-290.
25. Wendelaar Bonga SE, Grevan JAA, Veenhius M. The relationship between the ionic campaign ion of the environment and the secretory activity of the endocrine cell types of Stannius corpuscles in the teleost, Gasterosteus aculeatus. Cell and Tissue Research. 1976; 175:297-312.
26. Aida K, Nishioka RS, Bern HA. Degranulation of the Stannius corpuscles of coho salmon (Onchorhynchus kisutch) in response to ionic changes in vitro. General and Comparative Endocrinology. 1980; 41:305-313.
27. Ahmed Firoz M, Alim A, Sen NS, Lakra G, Mishra KR, Raza B *et al*. Electron microscopic studies of the corpuscles of Stannius of an air breathing teleost (Heteropneustes fossilis). Jouanal of Biosciences. 2002; 27:509-513.
28. Bist JS, Joshi ML. Seasonal histological changes in the ovaries of a mountain steam teleost Schizothorax richardsonii. Acta Anatomica. 1975; 93:512-525.
29. Kapoor CP. Nucleolar extrusion in the oocytes of Puntius ticto. Zoologische Beitrage. 1977; 23:221-226.
30. Bromage N, Cumaranatunga R. Egg production in the rainbow trout. Tn Recent advances in Aquaculture, ed. Muir JF, Roberts RJ. London; Croom Helm, 1988, 63-138.
31. Shabanipour N, Heidari B. A histological study of the zona radiata during late oocyte developmental stages in the Caspian sea magilid, Liza aurata. Brazilian Journal of Morphological Sciences. 2004; 21:191-195.
32. Tyler AV, Dunn RS. Ratio growth and measures of somatic and organic conditions in relation to the frequency in winter flounder Pseudopleuronecter americanus with hypothesis regarding population homeostasis. Journal of the Fisheries Research Board of Canada. 1976; 38:63-75.
33. Urasa FM, Wendelaar Bonga SE. Stannius corpuscles and plasma calcium levels during the reproductive cycle in the cichlid teleost fish, Oreochromis mossambicus. Cell and Tissue Research. 1985; 241:219-227.