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Original Article

Correlative Cyclical Chances in the Hypothalamo-Neurohypophysial System and Gonads Of A Gar Fish, *Xenentodon cancila* (Ham.)

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ABSTRACT
The hypothalamo-neurohypophysial system (which consists of hypothalamic nuclei, their axonal fibres forming tractus preoptico-hypophyses and the neurohypophysis) works as a morphological as well as a physiological connection between the hypothalamus and the pituitary gland. In fishes, fibres from the neurosecretory hypothalamic nuclei terminate in the neurohypophysis, which remains inter digitized with the adenohypophysis and provides a very close association between the neurosecretory fibres and pars intermedia of the pituitary gland forming a neuro-intermediate lobe. This paper reports that there was periodicity or cyclic changes in the concentration and intensity of neurosecretory material in the cells of nucleus preopticus, nucleus lateralis tuberis, tractus preoptico-hypophseus and neurohypophysis of the fish Xenentodon cancila (Ham.) during different months of a year. It is interpreted that the secretary activity in the hypothalamo-neurohypophysial system follows events of reproductive cycle. But the former is controlled by various extrinsic factors prevailing in the ambient aquatic environment.
KEYWORDS: Hypothalamo-neurohypophysial system, Nucleus Lateralis Tuberis (NPO), neurohypophysis, cyclic, seasonal change and *Xenentodon cancila.*

INTRODUCTION

Neuroendocrine control of reproduction in fishes involves a complex interaction of a variety of neurotransmitters that modulate the stimulatory influence of gonadotropin-releasing hormone (GnRH) on the synthesis and release of the two gonadotropins (FSH and LH). The developing events such as growth, differentiation, sexual maturation and other functional activities are influenced by the endocrine secretion and as such the study of developing endocrine glands carries a special significance in view of their participation in metabolic regulation during the course of development. The physiology of reproduction in teleosts has been a subject, which received wide attention [1-7]. However, the seasonal changes in the neurosecretory hypothalamic nuclei in teleosts in relation to their gonadal cycle have not been much studied.

It is a fact that gonadal hormones have been implicated in one way or the other in reproductive drive. Special behaviours are made possible by the presence or absence of hormones at a particular point. These hormones render the animal sensitive to certain stimuli from the environment. The reproductive and some other behaviors in fishes are associated with hormonal control and regulation. Though, the brain (including Pituitary) and gonads are necessarily involved, but the gonads and gonadal hormones are of much more importance [8-10].

MATERIAL AND METHODS

Living specimens of *Xenentodon cancila*, procured from Lower Lake, Bhopal (Madhya Pradesh, India), were collected in the last week of every month for a period of one year, which were dissected to expose the cranial cavity. The pituitary along with hypothalamus was carefully separated from the cranium and kept in the fixative. A long incision was also made in the abdomen and gonads were taken. The average weights of gonads (testes and ovaries) were calculated separately. Fixation in formaline was done from 4 to 6 hr, while the material was kept in Bouin's fluid for 24 hrs. The material fixed in formaline was washed in running tap water for about 6 hr, and dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Both longitudinal and transverse section of the Pituitary and gonads were cut at 6 µm in thickness for histological preparations & the sections were mounted serially on the slides. Pituitary was stained in Mallory's triple, aldehyde fuchsin (AF) and chrome-alum hemotoxylin-phloxine (CAHP) methods as followed by Bargmann [11], Dawson [12] and Sathyanesan [13] and gonads were stained with Heidenhain's Azan, Ehrlichs acid-alum, haematoxylin-Eosin and Mallory's triple staining method respectively.

RESULTS

Histo-architectural Changes in Hypothalamus

Cytological variations in the nucleus preopticus (NPO) during the reproductive cycle:-

The cytoplasm of nucleus preopticus cell of *Xenentodon cancila* is granular and there is no formation of neurosecretory droplets. Thus the only visible changes in the nucleus preopticus are noticed in the intensity of granular neurosecretory material in the cytoplasm by using different staining method.

In *Xenentodon cancila*, during middle spawning period (June and July), the neurosecretory material is poorly accumulated and less concentrated due to which the vacuoles appear in the cytoplasm. This indicates that the accumulation of neurosecretory material is less than its release. During the late-spawning period (August and September), the NPO cells are fully exhausted with large number of vacuoles, sparsely distributed neurosecretory material and poor staining intensity, due to the higher rate of the release of neurosecretory material (Fig. 1).This condition continues till the post-spawning period, when the neurosecretory material in the cells starts accumulating. During the early post-spawning period (October and November), the cytoplasm of NPO cells shows vacuolization and lesser concentration of neurosecretory granules (Fig.2). The cytoplasm also shows less tinctorial affinity towards CAHP stain. This indicates that the NPO cells are exhausted and in less active phase as far as the production of neurosecretory material is concerned. During late post-spawning period and early pre-spawning period (December to February), the vacuoles disappear from the cytoplasm of the NPO cells and the neurosecretory granules increase quantitatively and become more dense (Fig.3). The neurosecretory material shows greater tinctorial affinity towards CAHP stain. This shows that the production of neurosecretory material by the cell is accelerated During early spawning (May) and late pre-spawning (March) period, the neurosecretory granules are more concentrated (granulation) and uniformly distributed within the cytoplasm and show deep staining intensity (Fig.4). This is due to the accumulation of neurosecretory material in the cytoplasm at a comparatively higher rate.

Cytological variations in the Nucleus Lateralis Tuberis (NLT) during the reproductive cycle

In the present study, the NLT cells of the ventro-lateral group, which are situated posterior to optic chiasma, have been taken into consideration because these cells are larger in size than the ventro-median group of cells and show marked seasonal cytological changes. The course of the distribution of the neurosecretory material in the Nucleus Lateralis Tuberis cells is studied in different phases of reproductive cycle. During the spawning period, the neurosecretory granules in the cytoplasm are sparsely concentrated. This indicates that the elaboration of the neurosecretory material is less than its release. The nucleus is slightly smaller in comparison to that of the pre-spawning period (Fig.5). During the post-spawning period, the cell and the nuclear size are very small and there is a very thin layer of cytoplasm around the nucleus. The cytoplasm contains very little amount of neurosecretory material which indicates the inactive phase of the NLT cells (Fig.6). During pre-spawning period, the nucleus of the NLT cells are larger in size. The neurosecretory granules in the cytoplasm are closely concentrated (Figs.7&8). These changes indicate that the secretion of neurosecretory material is intense during the pre-spawning period (i.e.during gonadal maturation).

Cyclic variations in the tractus preoptico-hypophyseus and neurohypophysis during the reproductive cycle

The intensity of neurosecretory material in the tractus preoptico-hypophyseus exhibits significant variations during different phases of reproductive cycle. During the late spawning and the middle post-spawning period (August to November), the intensity of neurosecretory material in the tractus preoptico-hypophyseus is minimum. This is due to fact that the NPO cells are exhausted and the gonads are fully evacuated. During the late post-spawning period and early pre-spawning period the intensity of the neurosecretory material is less as compared to the spawning period

(Fig.9). From middle spawning period to late pre-spawning period, the intensity of the neurosecretory material is maximum due to higher rate of the release of neurosecretory material from NPO and active maturation of oocytes in the ovary.

The intensity of the neurosecretory material in neurohypophysis also shows noticeable variations during different months of the year. In the month of August, September, October and November, the intensity of the neurosecretory material is maximum and from December onwards, the intensity of the neurosecretory material tends to increase gradually. In the month of March, April, May, June and July, the deposition of the neurosecretory material in neurohypophysis is found to be maximum due to heavy transformation of the neurosecretory material through the tractus pre-optico-hypophyseus.

During the spawning period, the nuclear volume of the cells increases significantly. In the ovaries, most of the oocytes are in advanced stages (i.e. of yolk stage, pre-maturation stage and mature stage) and in the testes, a large number of seminiferous tubules are packed with spermatozoa where as other stages of spermatogenesis are comparatively less when compared with those of the pre-spawning period. The nuclear volume of the nucleus preopticus cells in the spawning period is higher from that of the pre-spawning period which indicates its high degree of metabolic activity during this period which can be related with the process of reproduction and spawning behavior of the fish.

During the post-spawning period, the values of the nuclear volume of the nucleus preopticus cells are very much less indicating a lower metabolic activity which is accompanied by the relative absence of rebuilding process of gonadal tissue after the spawning period. During the pre-spawning period, the nuclear volume of the cells increases steadily and significantly indicating the increased activity of cells. These changes are also reflected in the gonads which show advance in the stages of gametogenesis and the volume of the gonads and the gono-somatic index of the fish also increases gradually during these months.

The Reproductive System and the Reproductive Cycle

In the present work, the reproductive cycle has been assessed on the basis of morphological and histological changes in the gonads so as to correlate it with the seasonal changes in the hypothalamo-neurohypophysis system of the fish.

Morphology and histology of the gonads

The gonads of Xenentodon *cancila* are small paired structures which lie posteriorly in the body cavity ventral to the air bladder. Both halves of the gonads are in close contact with each other. The right half is always larger in size. The gonads open into a common gonoduct posteriorly. The gonoduct and the kidney duct open together in the urinogenital sinus which opens outside behind the anal opening through the urinogenital aperture. The ovary is covered with a thin sheath of peritoneal membrane below which lies the ovarian wall which consists of an outer layer of connective tissue and blood vessels and an inner layer of germinal epithelium. The ovarian wall projects inwards forming ovigerous lamellae containing different stages of developing oocytes. In the present study also ten cytological stages in the development of oocytes have been observed in the ovary which are early and late chromatin-nucleolus stages, early and late peri-nucleolus stages, early and late yolk-vesicle stages, early and late yolk stages, prematuration stage and the mature stage (Figs.10, 13, 15, 16, and 18). Besides these different oogenetic stages, corpora atretica and post-ovulatory follicles are also present in the ovaries. When the immature oocytes fail to attain maturity at any stage of development or when mature oocytes fail to spawn, they result in the formation of corpora atretica. The corpora atretica, thus formed, undergo various stages of resorption until they are completely lost in the ovarian tissue. The post-ovulatory follicle is a mass of follicular cells left behind after the extrusion of mature oocyte from the ovarian follicle. The post-ovulatory follicles undergo gradual degeneration and finally get absorbed in the ovarian stroma.

Table-1: Showing the length, average weight of testes and gono-somatic index of male *Xenentodon Cancila* collected from Lower Lake.

Month	No. of fishes	Length of fish(cm)	Av. Wt. of fish(g)	Av. Wt. of Ovaries(g)	Av. Gonosomatic index(GSI)
Jul.2007	10	10.5-14.4	53.14	0.68	12.8
Aug.	10	11.5-15.0	51.05	0.47	0.92
Sept.	10	11.0-15.5	52.14	0.22	0.42
Oct.	10	10.9-14.5	38.13	0.12	0.31
Nov.	10	10.0-11.0	37.17	0.10	0.27
Dec.	10	10.5-13.5	34.15	0.07	0.20
Jan.,2008	10	9.5-12.5	35.19	0.10	0.28
Feb.	10	11.0-14.5	39.20	0.14	0.36
Mar.	10	11.0-14.0	42.10	0.21	0.50
Apr.	10	11.5-13.7	44.50	0.38	0.85
May.	10	12.0-14.7	47.00	0.50	1.06
June	10	11.4-15.5	48.14	0.60	1.25

Table-2: Showing the length, average weight of ovaries and Gono-somatic index of female *Xenentodon Cancila*, collected from Lower Lake.

Month	No. of fishes	Length of fish(cm)	Av. Wt. of fish(g)	Av. Wt. of Ovaries(g)	Av. Gonosomatic index(GSI)
Jul.2007	10	11.5-15.5	55.64	7.29	13.10
Aug.	10	10.9-14.5	52.12	7.10	13.62
Sept.	10	11.0-15.5	49.57	6.18	12.47
Oct.	10	9.4-12.5	36.16	0.36	0.99
Nov.	10	9.5-10.5	37.14	0.37	1.00
Dec.	10	10.5-13.5	33.14	0.14	0.42
Jan.,2008	10	10.0-12.5	38.18	0.38	0.99
Feb.	10	11.0-15.6	42.10	0.97	2.30
Mar.	10	11.5-14.1	39.64	1.57	3.96
Apr.	10	11.5-15.0	42.50	1.97	4.63
May.	10	12.0-15.5	41.14	2.60	6.31
June	10	12.5-15.0	42.15	3.69	8.75

The testis remains enclosed in a thin peritoneum below which lies a thick layer of epithelial cells, connective tissue fibres and blood vessels. Numerous anastomosing seminiferous tubules extend from the periphery of the testis towards its centre and are separated from each other by intertubular connective tissue. Primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are the six different cytological stages of spermatogenesis (Figs. 11&12).

Seasonal changes in the gonads during the reproductive cycle.

The gonads of *Xenentodon cancila* show well marked changes in their morphological (shape, size and volume) and histological features during different months of the reproductive cycle. On the basis of these changes, the reproductive cycle is divided into spawning, post-spawning and pre-spawning periods. Since the fresh reproductive cycle begins at the end of the spawning period that is why it has been described first in the following account.

Spawning period (May to September)

During this period, the ovaries are large in size, beaded in appearance and orange in colour. In the month of July, the ovarian wall is thin and the ovigerous lamellae lose their identity. The ovary is closely packed with oocytes of yolk stage, prematuration stage and mature stage. In the month of August and September, the ovarian wall is thick, the ovigerous lamellae begin to reappear and the ovary is loosely packed with all the stages of oogenesis. The number of corpora atretica increases during this period while few post-ovulatory follicles are first located in the month of May and their number increases considerably till the end of the spawning period (Fig. 13).

The testes of these months are thick,elongated and creamy-white in colour. The seminiferous tubules filled with spermatozoa extend towards the peripheral region of the testis also. Other stages of spermatogenesis, though few in number, can also be observed in smaller tubules filled with spermatozoa in the peripheral region of the testes (Fig. 14).

(b)Post-spawning period (October to December)

During these months, the ovaries are thin, small in size and smooth in appearance. They are yellowish in colour. The ovarian wall is thick and ovigerous lamellae are distinct containing oocytes of chromatin-nucleolus and peri-nucleolus stages. The corpora atretica are few in number. The post-ovulatory follicles are not present except in the ovary of one of the specimen collected in October where they are few (Fig.15).

The testes are small in size, lamellar in structure and white in colour. All the stages of spermatogenesis are present. The earlier stages are found in the seminiferous tubules of peripheral region of the testes while in the central region the seminiferous tubules are partially filled with spermatozoa. (Fig.16).

Pre-spawning period (January to April)

The ovaries in the pre-spawning period are small and cylindrical in shape. They are smooth in appearance and yellowish in colour. In June, the ovaries of few fishes have a little granular surface. In the ovaries of January, Febuary and March, the ovarian wall is thick. The ovigerous lamellae are distinct and contain oocytes of early and late yolk-vesicle stages alone with the oocytes of the early stages (Fig.17). In the ovaries of April, few oocytes of early and late yolk stages, varying in number from a very few to many, are also present. Few corpora atretica have been observed during this period, while post-ovulatory follicles are absent (Fig. 18).

Morphologically the testes do not show any marked changes from those of the post-spawning period. Process of spermatogenesis is quite active during these months which are evident from the fact that the number of later stages of spermatogenesis is comparatively more. Many seminiferous tubules of the central region of the testes are densely packed with spermatozoa (Figs.19 & 20).

Quantitative study of the reproductive cycle

The quantitative study of reproductive cycle of *Xenentodon cancila* has been made in respect of gono-somatic index during different months of the year. Although this study, regarding the gono-somatic index, is approximate, yet it is quite significant in understanding the gonadal cycle and seasonal changes in the gonads.

The changes in the volume of ovaries and the gonado-somatic index of the female fish

Gonado-somatic index (GSI) of female *Xenentodon cancila* was recorded in every month of the study period. The results are tabulated in (table 1). During the spawning period (May to September),the average values for the ovarian volume and the gono-somatic index show a gradual increase till they attain the peak in the month of July and then on wards these values gradually decrease. It indicates the high metabolic activity of the fish during this period and the maximum spawning of the fish takes place in August and September.

During the post-spawning period (October to December), the average volume of the ovaries and the average gono-somatic index of the female fish decreases gradually which justifies the gradual shrinkage in the ovaries during this period. It indicates that during this period, the metabolic activity of the fish becomes low after spawning.

During the pre-spawning period, the average values for the ovarian volume and the gono-somatic index are observed in an increasing order. It indicates that the ovaries are in active state during this period and the process of oogenesis has been accelerated.

The changes in the volume of testes and the gonado-somatic index of the male fish

Gonado-somatic index (GSI) of male *Xenentodon cancila* was recorded in every month of the study period.The results are tabulated in (Table 2). During the spawning period (May to September),the values for the gono-somatic index of the testes show a further rise as in the case of female fishes, these values reach their maximum indicating that the testes have acquired the state of their functional maturity. A downward trend in these values in August and September indicates active phase of spawning during these months.

During the post-spawning period (October to December), the gono-somatic index of the testes show a trend similar to that in the female fish. A gradual decreasing trend in these values indicates that the testes are in resting stage after the spawning period.

Throughout the pre-spawning period (January to April), the average gono-somatic index of the testes increases gradually which is quite significant particularly in the month of April. It shows an active state of the testes.

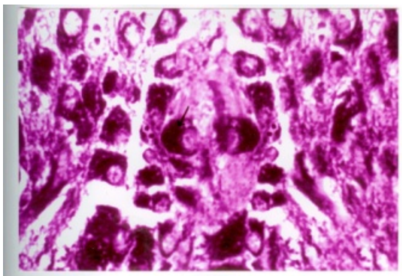
Fig. 1 Showing the cells of nucleus preopticus in the month of August with large number of vacuoles and sparsely distributed neurosecretory material.1000x (arrow)

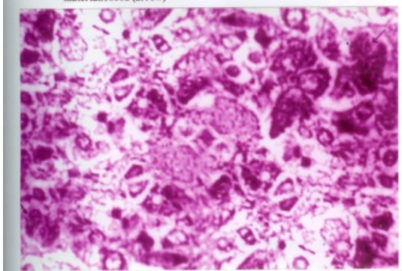
Fig. 2 Showing the cells of nucleus preopticus in the month of October with vacuoles and lesser concentration of neurosecretory material. 1000x (arrow)

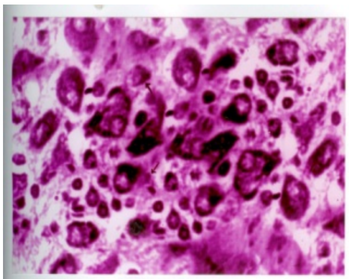
Fig. 3 Showing the cells of nucleus preopticus in the month of February with more concentration of neurosecretory granules.1000x (arrow)

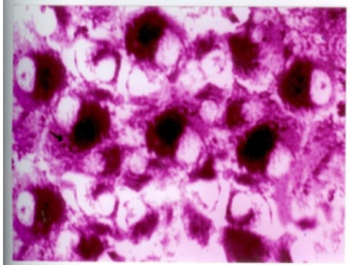
Fig. 4 Showing the cells of nucleus preopticus in the month of May with greater accumulation of neurosecretory material .1000x (arrow)

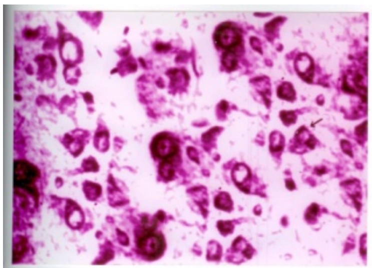
Fig. 5 Showing the cells of nucleus leteralis tuberis in the month of June with lesser concentration of neurosecretory material . 1000x (arrow)

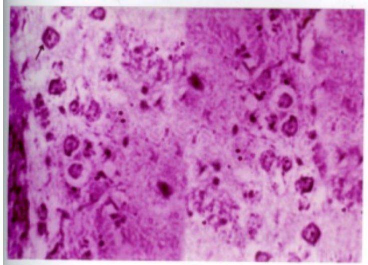
Fig. 6 Showing the cells of nucleus lateralis tuberis in the month of October with a poor accumulation of neurosecrtory material . 1000x (arrow)

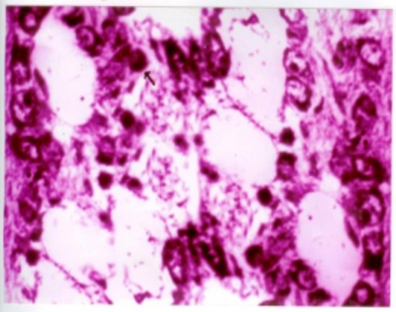
Fig. 7 Showing the cells of nucleus leteralis tuberis in the month of January with more concentration of neurosecretory material. 1000x (arrow)

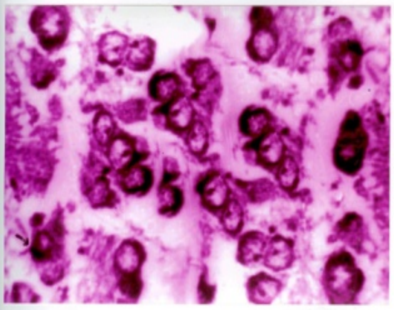
Fig. 8 Showing the cells of nucleus leteralis tuberis in the month of March with greater concentration of neurosecretory material. 1000x (arrow)

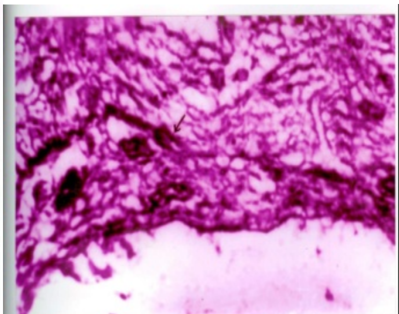
Fig. 9 Showing tractus preoptico-hypophyseus with more intensity of neurosecretory material.1000x (arrow)

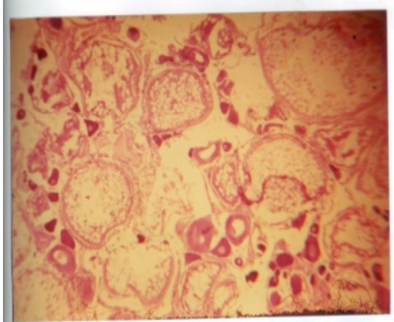
Fig. 10 Showing early yolk stages.28x (arrow)

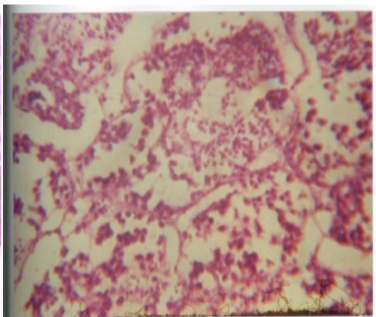
Fig. 11 Showing early spermatogenetic stages.400x (arrow)

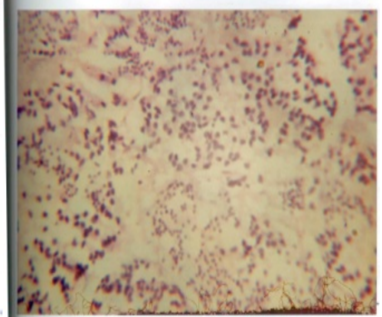
Fig. 12 Showing later spermatogenetic stages.400x (arrow)

DISCUSSION

The cytological activity of NPO cells in *Phoxinus phoxinus* [14] during different months of a year was based on the elaboration of neurosecretory material leading to the formation of microdroplets.

In *Glossogobius giuris* [15], the granulation, degranulation and the intensity of the neurosecretory material were the base of the cytological variations during different periods of the reproductive cycle. In *Alburnus albidus* and *Alosa fallax* [16] and *Coregonus autumnalis migratorius* the cytological variations were based on the quantity of the neurosecretory substance and vacuolization. In *Xenentodon cancila*, during different months of the reproductive cycle, the variations in the NPO cells have been observed on the basis of granulation, degranulation, vacuolization and the intensity of neurosecretory material. During the late spawning and early post-spawning period, the neurosecretory granules are sparsely distributed with large number of vacuoles having less staining intensity. During the late post-spawning and early pre-spawning period, the quantity of neurosecretory granules increases and vacuoles decrease gradually. During the late pre-spawning period and early spawning period vacuoles are found to be absent. The granules are more concentrated and uniformly distributed. During certain months of a reproductive cycle, the appearance of vacuoles in the cytoplasm as mentioned earlier, may be interpreted as a period when the release of the neurosecretory material is more than its elaboration in the cell. Thus in *Xenentodon cancila* the secretory activity of the nucleus preopticus strictly follows the events of reproductive cycle. In *Xenentodon cancila*, the intensity of the neurosecretory material in tractus preoptico hypophysis is the maximum from late pre-spawning to middle of spawning period (March to August) which is in confirmation with the observation of Anant Prakash [17] in *Clarias batrachus* and *Garra gotyla gotyla* Very few authors have observed the cytological variations of NLT during the reproductive cycle of fishes. In *Xenentodon cancila* like *Salvelinus leucomaenis* [18], *Alburnus albidus* and *Alosa fallax* [16], the NLT cells are hypertrophied and show marked vacuolization before the spawning period. In *Salvelinus gontinalis* [19], the Nucleus Lateralis Tuberus cells appear to be in a state of inactivity during the period of gonadal rest and the period of slow growth and development of gonads where they exhibit signs of increased activity during the period of renewed activity of gonads and the period of rapid growth and development of gonads. The secretion of NLT which is intense before the spawning and of NPO during the spawning [16,20-21] is also observed in *Xenentodon cancila*. After spawning period, a reasonable decrease in the cell size and nuclear size is observed in *Xenentodon cancila* which is similar to the findings of Billenstein [19] in *Salvelinus fontinalis* and Pavlovic and Pantic [16] in *Alburnus albidus* and *Alosa fallax*. In the present fish, NLT is more active before the spawning and NPO during the spawning, both are inactive after spawning. Thus, these observations indicate that both the NLT and NPO are associated with the reproductive activity of the teleost fish, but their independent role in reproduction is yet to be investigated.

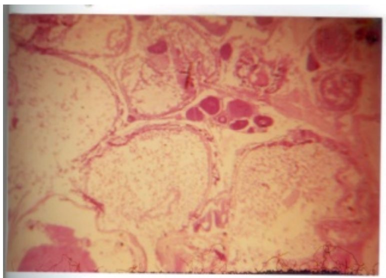

Fig 13 Showing oocytes of early and late yolk stages.28x (arrow)

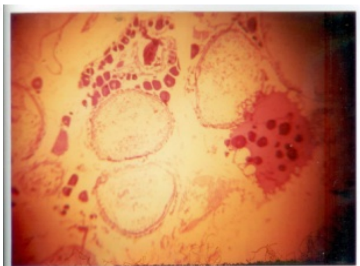

Fig 15 Showing oocytes of chromatin-nucleolus and peri-nucleolus stages.28x (arrow)

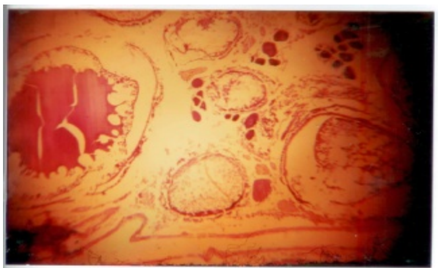

Fig 17 Showing oocytes of early and late yolk vesicle stages.28x (arrow)

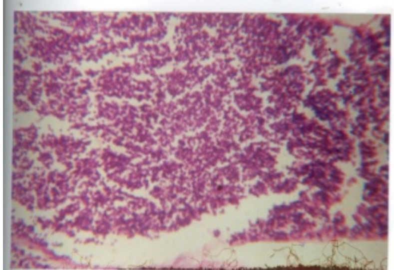

Fig 14 Showing seminiferous tubules closely filled with spermatozoa.400x (arrow)

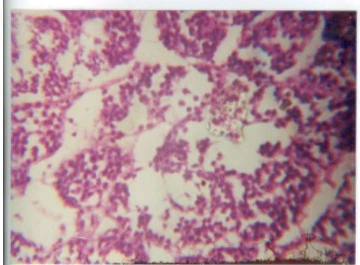

Fig 16 Showing seminiferous tubules partially filled with spermatozoa.400x (arrow)

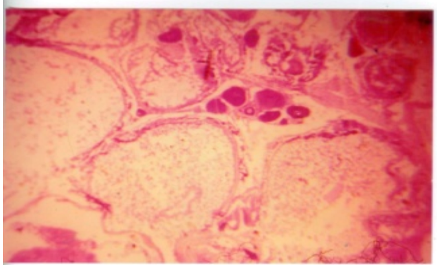

Fig 18 Showing oocytes of early and late yolk stages.28x (arrow)

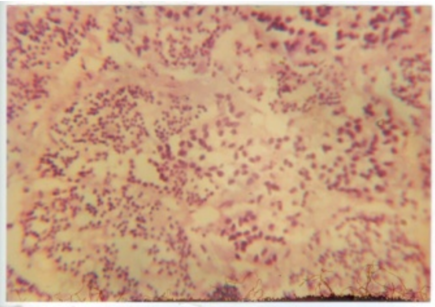

Fig 19 Showing different stages of spermatogenesis.400x (arrow)

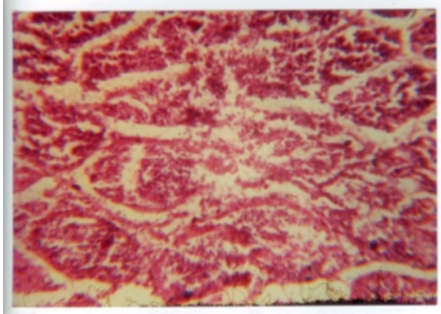

Fig 20 Showing most of the seminiferous tubules filled with spermatozoa.400x (arrow)

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