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Histological Changes In Pituitary Gonadotrophic Hormone Cells In Relation To Seasonal Changes In Ovaries Of The Fish *Orieochromis Mossambicus*

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Abstract: An attempt was made to study the correlation of pituitary gonadotrophs (GTH) with the simultaneous changes in the ovaries throughout the year. The pituitary and ovary of the same fishes were prepared for the histological studies. According to gonadosomatic indices (GSI) and histological position ovarian development was categorized in three phases,1.recrudesence (Dec-April) 2. Spawning (May-July) and Quiescent (Sep-Nov). The pituitary GTH-cells during recrudescent period were small in size and granulated. Degranulation was noticed at the time of spawning when ovary filled with mature follicles. Chromophobic condition was observed at the time of quiescent period.

Keywords: Gonadotrophic hormones, fish, ovaries, pituitary, histopathology, seasonal changes.

1. INTRODUCTION

Tilapia, *Orieochromis mossambicus* is an exotic fish which belongs to family Cichlidae. It was introduced in Pakistan from Malaya in 1951(Naik, 1973).These fish are also widely distributed in fresh waters of Africa and Middle East. The present paper deals with the data on the structure of ovaries on the basis of seasonal variation during reproductive cycle.

Considerable information is available on the breeding activity of various fishes through out the year (Chubb and Potter, 1984 Cyrus and Balber, 1984: Jalali and Haider, 1985: Shaikh and Jalali, 1991).

It is well established that the pituitary gland are dispensable for the maturation of the gonads in all vertebrates. The physiology of reproduction in fishes has been extensively reviewed by several investigators (Dodd, 1955 Hoar, 1955 Pickford and Aatz, 1962).

Patterns of oocyte development in teleost are also described. Marza (1938) was the first to describe the synchronous and group-synchronous pattern of oocyte development. Most teleostean species manifest annual rhythm of breeding which in many cases is synchronized or controlled by environmental factors (de Vlaming 1975). In large number of species the gonadotrophs exhibit hypertrophy vacuolization granulations, numerical increase and other singns of intense activity in correlation with maturation of the gonads (Sundaraj, 1959 Sathyanesan, 1963 Rai, 1965 Oztan, 1966).

During investigation an attempt is made to study the annual reproductive cycle and correlative changes in the gonadotropins in relation to gonadal activity throughout the year.

The productivity of female fish is the integral part of aquaculture programmed with regard to the development and enhance of fishery. This fish was selected for the study because: i) No information is available regarding its annual reproductive cycle. ii) As the fish *Orieochromis mossambicus* is the larvivorous fish and mainly fed upon the eggs and the larvae of the mosquito. The result of reproductive history can help to enhance its production artificially.

Present studies were carried out in order to understand actual timings of the development of the oocytes synchronizing pituitary gonadotrophs.

2. MATERIALS AND METHODS

Specimens of female fish, *Orieochromis mossambicus,* were collected with the help of cast net twice a month for a period of one year from the waters around Hyderabad city. The fish was transported to the laboratory in live condition. The fish were kept outside the water in order to allow them to die. The dead fish were cleaned and dried with filter paper and weighed on the Harvard trip balance.

Histological examination

In order to dissect out the gonads and the pituitary an incision from the base of the pelvic fins extending up to the operculum the alimentary canal was given to expose the gonads, which were then carefully taken out and cleaned of adhering fats/tissues. The gonads were weighed on electronic balance to the nearest mg. to access the variations in their gonad somatic index.

$$GSI = \frac{\text{Total gonadal weight}}{\text{Total body weight}} \times 100$$

After weighing of gonads the pieces of each gonad were immediately fixed in the bouin's fluid for histology. After 24 hours the pieces of tissue were washed and dehydrated in ascending grades (30%-100%) of alcohol cleared in Benzene and then embedded in paraffin wax at a temp 62C for 1-2 hours depending upon the size of the tissue; Sections of the gonads were cut at 6um and stained in Haemotoxylin and Eosin. Stained sections were photomicrograph. At the same time pituitary gland of each fish was isolated carefully and was fixed in Bouin's fluid and processed for section cutting. The sections of pituitary glands were stained in periodic acid Schiff's reagent (PAS).

Statically Analysis:

The data collected for investigating the seasonality in the reproductive cycle was statically analyzed by taking mean (M) and Standard error (SE).

3. RESULTS

Gonadosomatic index (GSI)

The GSI (1.38±0.25) of the fish started increasing in late November immediately shoot up to 2.45±0.21 in late December. This is maintained till April and reached the maximum (4.75±0.13) in the month of May when maturation was completed. Abrupt decrease (1.73±0.12) was noticed as the breeding was over. The GSI again increased in the month of August i.e. 3.28±0.16 and dropped in the quiescent period (September- early Nov.).As shown in the following **(Table-1 and Fig.1)**.

Present studies were carried out in order to understand actual timings of the development of the oocytes synchronizing pituitary gonadotrophs.

Table 1: Seasonal variation in the mean body weight, ovary weight, gonadosomatic index (gsi) in female fish. *Oriochromis mossambicus* (Peters)

S.No.	Date	No. of Animals	Body Weight (gms)	Ovaries Weight (mgs)	GSI	Water Temp ℃
01	January	7	20.80±2.62	450±12.0	2.16±0.24	12℃±1.0
02	February	8	23.70±1.87	490±8.50	2.06±0.33	13℃±1.2
03	March	7	12.99±2.14	221±9.12	1.70±0.32	18℃±2.2
04	March	9	11.26±1.36	259±9.80	2.30±0.75	20℃±2.6
05	April	8	30.82±2.67	595±8.50	1.93±0.22	25℃±3.6
06	May	8	16.32±2.20	776±13.18	4.75±0.13	30℃±4.6
07	June	6	30.05±2.24	530±12.82	1.73±0.12	35℃±5.6
08	July	7	16.05±2.20	362±12.90	2.25±0.21	37℃±6.0
09	July	8	19.02±2.24	320±7.80	1.66±0.19	32℃±5.0
10	August	10	18.16±1.22	595±6.32	3.28±0.16	26℃±3.8
11	September	7	21.60±2.62	185±2.30	0.39±0.14	23℃±3.2
12	October	8	18.07±1.50	155±2.30	0.85±0.22	21℃±2.8
13	November	8	22.30±2.56	300±7.23	0.10±0.17	20℃±2.6
14	November	8	18.60±2.66	258±8.40	1.38±0.25	18℃±2.2
15	December	8	27.13±3.98	374±7.52	1.37±0.33	16℃±1.8
16	December	8	16.05±2.94	394±8.29	2.45±0.21	15℃±1.6

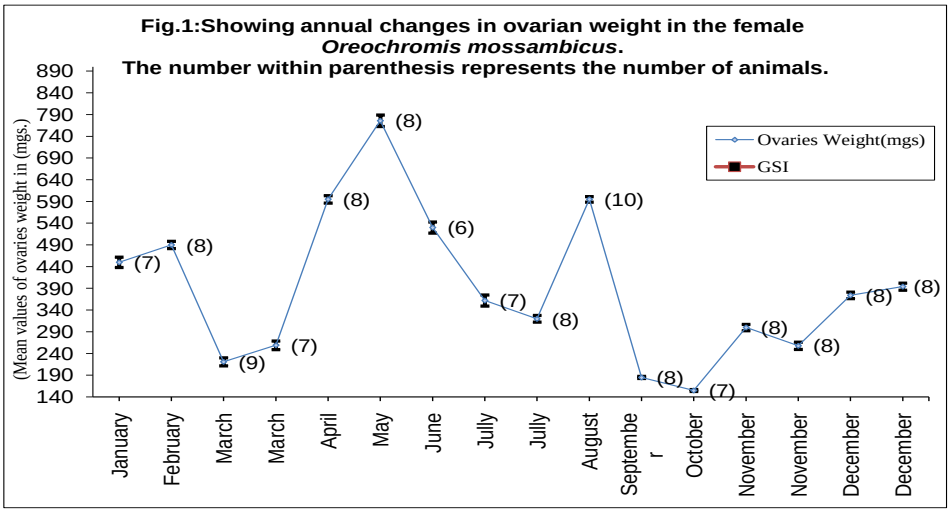


Fig. 1. showing annual changes in ovarian weight in the female oreochromis mossambicus
The number within parenthesis represents the number ofanimals.

Histological observation of ovaries
Recrudescent period: (Dec-April)

The recrudescence starts from December and continue till April. During early recrudescence the ovary possess germ cells, oogonia and oocytes in stage I, II and stage III also nucleoli appears at the inner periphery of the nuclear membrane indicates the initiation of the oocyte development. **(Fig. 1a)**.This condition of the gonad continues till early January. Appearance of stage III cells with formation of granulose and theca cells starts in the month of January and continued till February during this many follicles are also seen. In these follicles the layer of theca cells and the zona radiate are well developed. The changes in the cytoplasm and nuclear membranes also appeared. The cytoplasm shows vacoulation towards their periphery and heavily filled with the yolk granules. The nuclear membrane is uneven and nucleoli are migrated towards periphery **(Fig.2a and 3a)**. The follicles at this stage are matured and ready to spawn.

Breeding period: (May-July)

The breeding period according to the studies starts in May. The ovary is mainly filled with the stage v follicles. Other premature and maturing stages are also seen. The matured follicles are large in size and deeply eosinophilic **(Fig.4a).** The nucleus possesses evenly distributed chromatin material with several nucleoli around. The nuclear membrane at this stage is not clearly visible **(Fig.6a)**. Zona radiate become striated and clearly bifurcated in zona radiate externa and zona radiata interna**.(Fig.4a and 5a)**. At this stage the ovary attained a maximum weight (776±13.18mg).Shown in **(Table 1)**.

Quiescent or Inactive period: (Sept-Nov)

The breeding period continues up to the August as the ovary is filled with the maturing active follicles. This starts regressing in the month of September and continues till November. During this phase the ovary remains inactive. No any activity is noted in the ovarian contents. However, many atretic follicles are seen **(Fig.7a and 8a).** The ovarian weight during this phase reduced to its lowest level (85±2.30mg).

Pituitary Gonadotrophs
Seasonal changes:

During recrudescent period when the gonads are preparing the granulated GTH-cells are also increasing in number but the size of the cells is small **(Fig.1b).** At the end of the recrudescent period (April) the numbers of granulated GTH-cells compared to the early recrudescent period (Dec) are increased **(Fig.2b)**. During breeding season when the gonads have become are matured, the GTH- cells are large but less granulated in early May **(Fig.3b and 4b)**. Complete degranulation of GTH-cells noted in the Pituitary of late May **(Fig.5b and 6b)**.The **(Figure 7b)** shows that the spawning almost completed and reduction in the number but not in size of GHT-cells was observed in the quiescent period when the gonads were regressing. Degranulation

which was noted in the breeding season persisted throughout it quiescent phase **(Fig. 8b)**. GTH cells of the anterior pituitary gland are responsible to produce the gonadotrophs that act upon the follicles to produce ovarian hormones. These ovarian hormones Estrogen and Androgen promote the development of oocytes. Oocyte progression reflects the granulation in the GTH cells. The cyclical changes in GTH cells takes place accordingly.

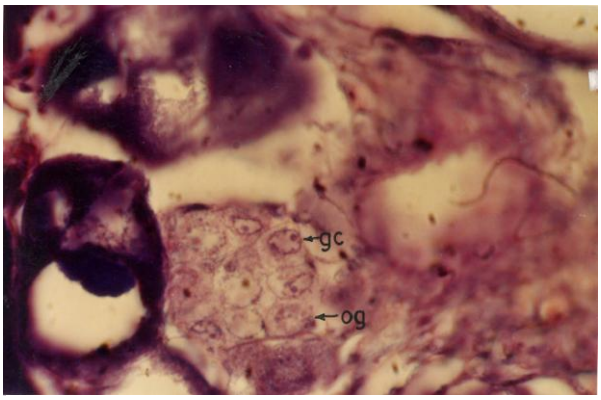


Fig. 1a, Section of the ovary taken from the fish in Dec indicates recrudescence by the presence of oogonia(og) in cyst.

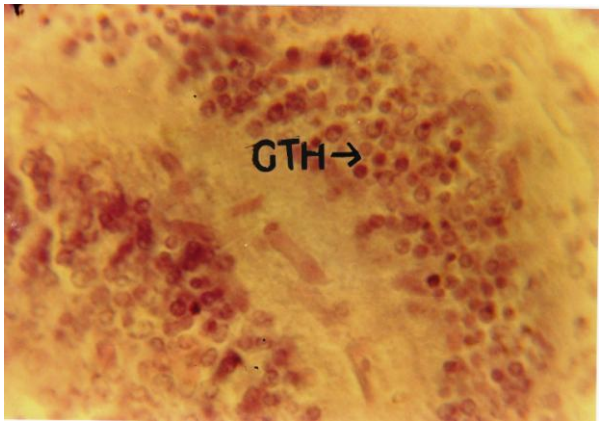


Fig. 1b, Section of the pituitary gland of the same the fish it shows small sized gonadotrophs (GTH- cells)

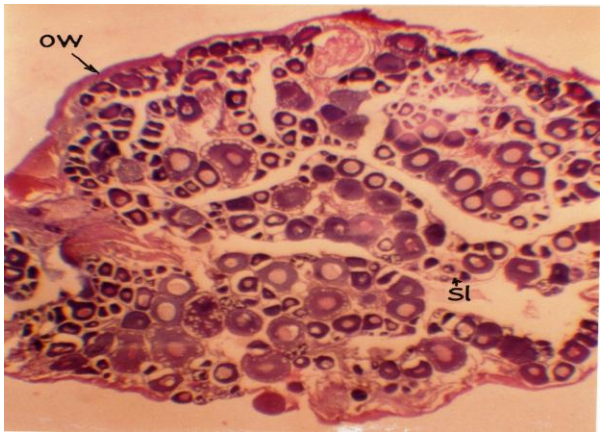


Fig. 2a, Section of the ovary of the fish collected in January it shows the cysts containing many variable sizes.

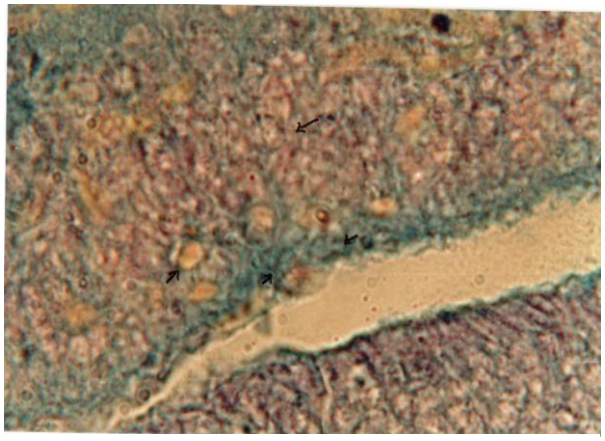


Fig. 2b, Section of the pituitary gland of same fish shows proliferation of GTH-cells with the increase in size and granulation.

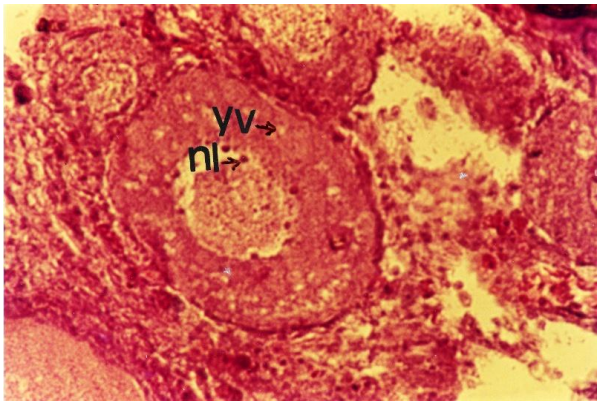


Fig. 3a, Section of the ovary of the fish collected in February it shows the maturing follicles. Yolk vesicles (yv) at the periphery of cytoplasm. Nucleoli (nl) are arranged at nuclear membrane . X 40.

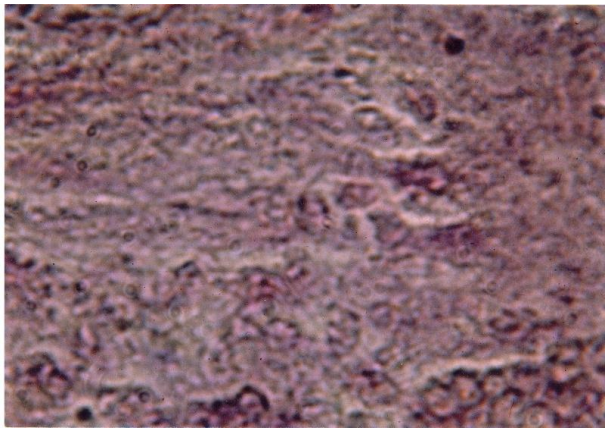


Fig. 3b, Section of the pituitary of the same fish it shows the accumulation of the cytoplasmic granules. X 100.

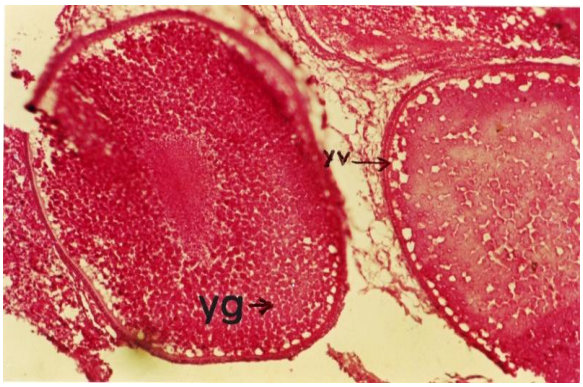


Fig.4a, Section of the ovary fish collected in the month of march that showing opaque oocytes with yolk granules(yg) and gravid follicles. X 40.

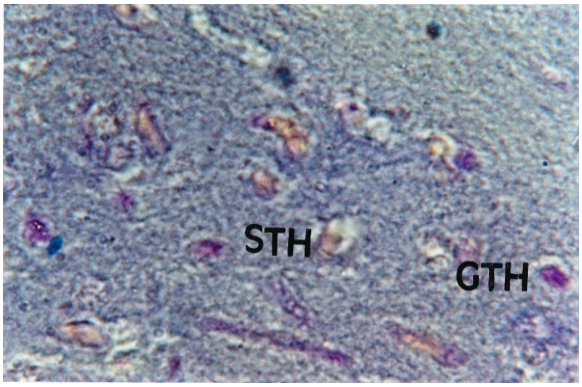


Fig. 4b, Section of the pituitary gland of same fish it shows the large sized gonadotrophs (GTH-cells) heavily granulated. X 100.

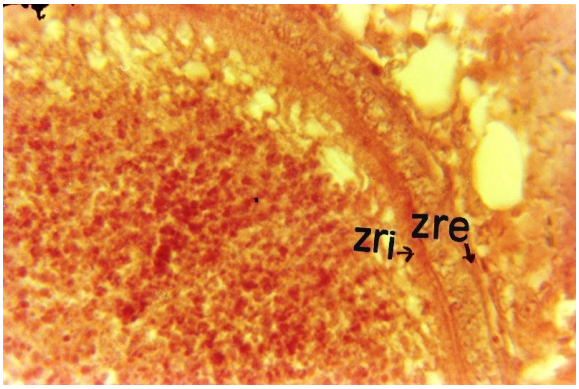


Fig. 5a, Section of the ovary of the fish collected in the month of July shows disintegration of zona radiata externa (zre) and interna (zri) X 400.

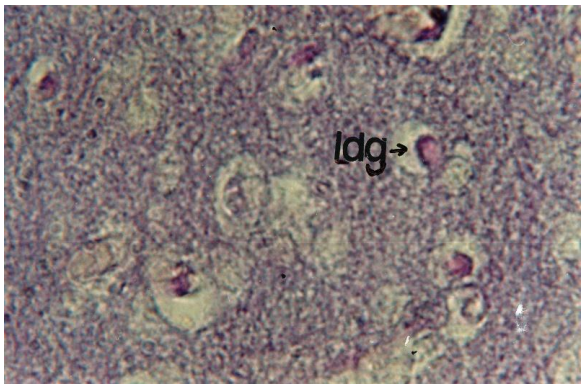


Fig. 5b, Section of the pituitary gland of same fish it shows very large sized chromophlic GTH-cells. Note that few active granulated GTH-cells are still present X 100.

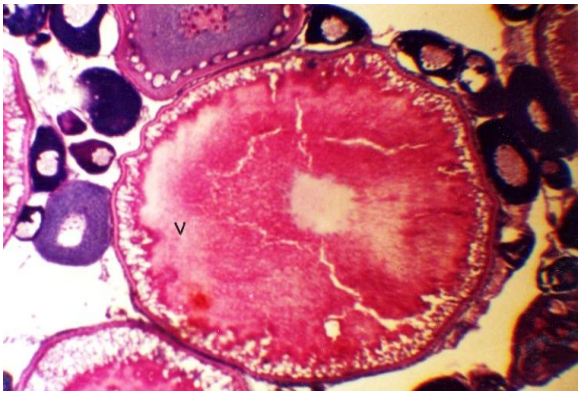


Fig. 6a, Section of the ovary of fish collected in April/May shows the matured follicle. Here the nuclear membrane is uneven & ready to migrate to periphery for ovulation X 40.

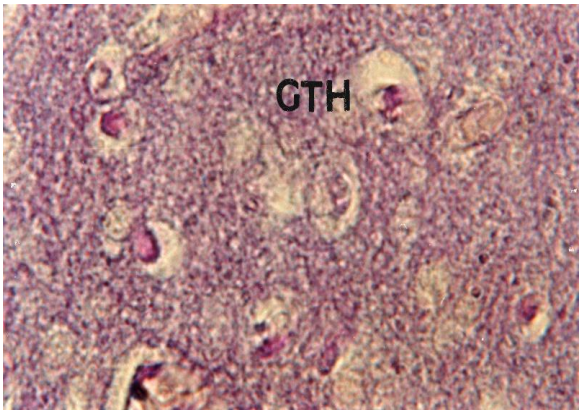


Fig. 6b, Section of the pituitary gland of same fish the gonadotrophs(GTH-cells) are even larger containing magneta red granules X 100.

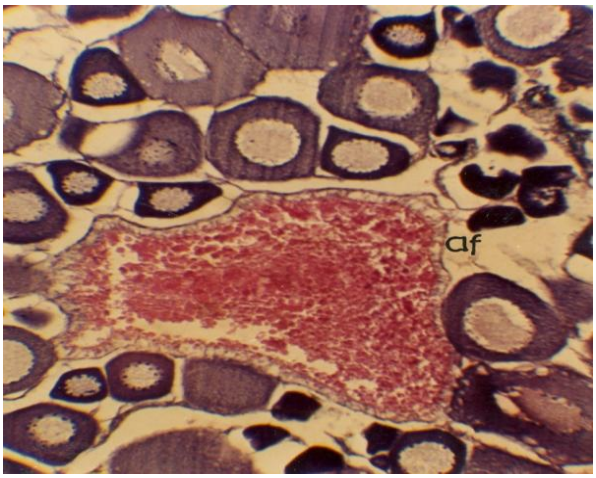


Fig. 7a, Section of the ovary of the fish collected in the month of June shows the appearance of atretic follicles(af) and also the presence of active oocytes of variable size X 40.

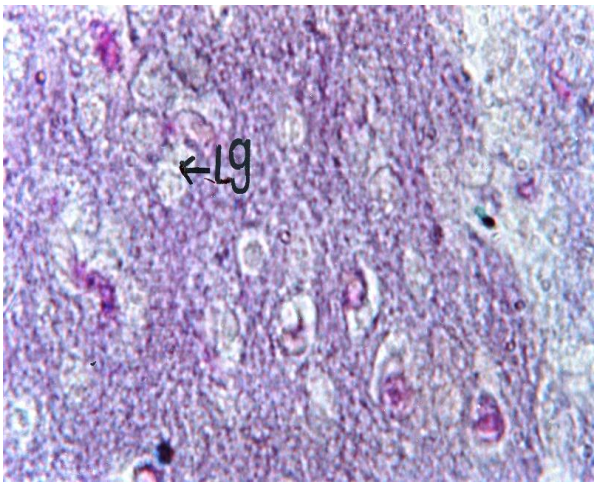


Fig. 7b, Section of the pituitary gland of the same fish when spawning was over. The granulation is partially disappeared from the GTH-cells X 100.

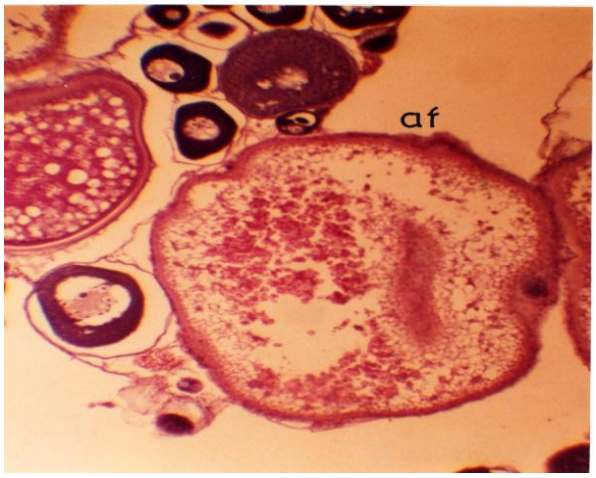


Fig. 8a, Section of the ovary of the fish collected in the month of August shows atretic follicles (af) including few active oocytes X 40.

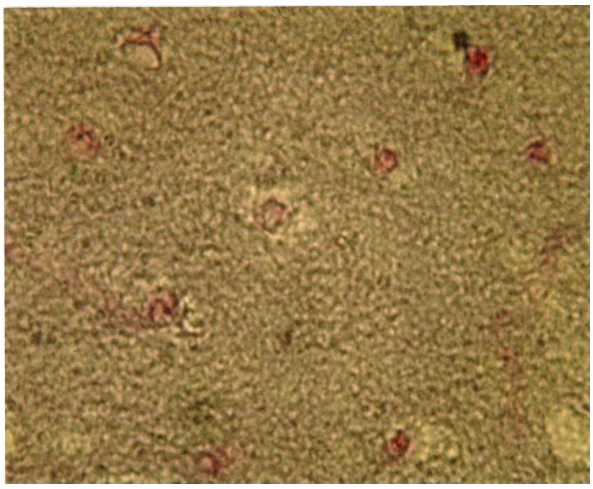


Fig. 8b, Section of the pituitary gland of the same fish shows the disintegrated GTH-cells X 100.

4. DISCUSSION

The study of seasonal changes in the ovary and their relationship with gonadotrophs in the fish *Orieochromis mossambicus* reveals that the gonadal development is group synchronous. Both serial and single spawning patterns have been reported among the teleosts, *Chrusophrys auratus (*Crossland, 1977).

At least two populations or clutches can be distinguished in the ovary of the *Orieochromis mossambicus*.This pattern of gonadal development is also reported by Wallace and Selmon, 1979. Maximum gonadal weight was recorded during the main breeding season (May). However the rise in the gonadal weight was also recorded in August. This rise in gonadal weight reveals that the temperature has a role to synchronize the gonadal development. This type of temperature dependent, gonadal development already reported by many workers (de Valming, 1972, 1975 Gillet and Billarad, 1978; Shaikh and Hafeez, 1993; Scott, 1979). In the many Clupids and Gerres species breeding periods is not restricted to the particular period (Ellis, 1971 Vender Puye, 1973 Otobo, 1978 Cyrus and Blabber,1984). Whereas, in Barillius Vagra Ham. (Jalali and Haider, 1985), the breeding period extends from March to May and Illisha africana (Marcus and Kusemiju, 1984) breed during May to December. The gonadotrophs exhibited a gradual increase in size and number. These were very active and more in number when ovaries were in prespawning (April-May). During the resting phase (Sept-October) of the

ovaries, the gonadotrophs are numerically reduced. These finding are in agreement with those of Kerr, 1948; Scruggs, 1957; Beach, 1959; Sundaraj, 1960; Skoll, 1961; Robertson and Wexter, 1962; Sathyanesan, 1963; Bar and Hobsan, 1964; Lagios, 1965).The role of gonadotrophs in the ovarian activity was assessed by the changes in the GSI which seems to be more reliable criterion (Pickford and Atz, 1957). The GSI in *Orieochromis mossambicus* was minimum during resting phase of the ovaries. It progressively increased during the preparatory and prespawning phase and reached to its peak at the spawning phase. The maximal GSI in *Orieochromis mossambicus* coincided with the heightened activity of gonadotrophs. Such information was also been reported by Ahsan, 1966).

It is concluded that changes in gonadotrophs in relation to ovarian cycle in the fish *Orieochromis mossambicus* shows almost the same as in fishes reported elsewhere.

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