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The Studies of Cholesterol Variation in Relation to the Breeding Phases of Female Tilapia, *Oreochromis mossambicus* (Peters)

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Abstract

The reproductive cycle of African multiple spawner Tilapia, *Oreochromis mossambicus,* is divided into preparatory, prespawning, spawning and postspawning phases, after evaluating the gonadosomatic index and percentage dominance of particular types of oocytes. In the preparatory phase GSI is 0.21887+0.021 and ovary is dominated by stage I and stage II oocytes which constitute 36.873% and 32.992% of the total oocytes respectively. Some developing oocytes of stage III (14.467%), stage IV (5.895%) and stage V (9.715%) are also observed. In the spawning phase, the ovaries are very much elongated, occupying about 2/3rd of the body cavity. They are turgid and yellow in colour holding large number of translucent eggs. Ovarian wall becomes very thin. The GSI increases to 3.24932. The ovary is dominated by stage VI follicles; which are 45.731% of the total oocyte count. Positive staining of cholesterol is observed in the ovary, as maturation proceeds. Intensity of staining increases in the ooplasm successively from stage I to stage VI oocytes. Cholesterol in nervous, endocrine and somatic tissues with respect to the ovarian cycle is ascertained by their histochemical demonstration and biochemical estimations. Brain and pituitary has highest cholesterol in prespawning phase, lowest in spawning phase. On the contrary, ovary has cholesterol is highest in the prespawning and lowest in the spawning phase. Liver and muscles show highest cholesterol in the preparatory and lowest in the spawning phase.

Keywords: *Tilapia,* cholesterol, breeding phases, *Oreochromis mossambicus*

1. Introduction

Tilapia, *Oreochromis mossambicus*, is a continuous breeder in tropical climate where it breeds every 2 months (Chang Kong Tom, 1962) or six to eleven times a year with an interval of 25 to 44 days in between consecutive spawning (Chimitz, 1955). Cholesterol, as a main structural component of animal cell membranes and a precursor for biosynthesis of vitamin D3, prostaglandins, steroids and bile acids, is an essential nutrient for eukaryotic animals **(**Steffens, 1989; Fast and Boyd, 1992; Sheen et al., 1994). Cholesterol in the steroidogenic tissue has been shown to be associated with reproduction and its fluctuation in relation to maturity has been reported in the fish, *Etroplus suratensis* (Diwan and Krishnan, 1986; Premjith et al., 1992), suggesting cholesterol being the precursor for the synthesis of steroid hormone influencing the maturation phenomenon. Cholesterol is closely linked to fish stress through the

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hypothalamic-pituitary-interrenal axis (HPI axis) (Mormède et al., 2007). The metabolic aspects of oogenesis have been partly assessed and changes in several aspects of carbohydrate and lipid metabolism, in gonad, liver and muscles of several species are found (Washburn et al., 1990; Kjeshbu et al., 1991; Olin et al., 1999; Nair and Mathew, 2000; Shamsan and Ansari, 2010; Mathana et al., 2012; Jyrwa and Bhuyan, 2016). Chang et al. (2018) studied the growth and stress axis responses to dietary cholesterol in nile tilapia (*oreochromis niloticus*) in brackish water.

All these studies however have mainly focused upon the gonads but studies on the possible biochemical interactions in teleosts along the hypothalamic-pituitary-gonadal axis are not reported. Therefore, in the present work, brain (telencephalon, diencephalon including hypothalamus), pituitary and ovary are selected to study the possible correlation of cholesterol in these tissues. Liver and muscles are selected to find out the correlation between somatic and reproductive components.

2. Materials and Methods

The mature females of *Oreochromis mossambicus*, for two years were collected from natural water bodies of the Futala Lake, near Botanical Garden, Nagpur, India.

All fishes were transported alive to the laboratory in open bucket and maintained in 3×2×1.5 feet rectangular tanks (10 fish per tank) which were supplied with non-circulatory fresh water maintained at 28±2 °C with water exchanged at the rate of 15 liters/day. The fish were weighed, killed by decapitation and brain, pituitary, ovaries, liver and muscles were dissected out in Ringer's solution. Telencephalon and diencephalon portions of brain were separated as described by Senthilkumaran and Joy (1993) and Pepels et al. (2002). Weight of ovaries was noted and gonadosomatic index was calculated using the formula,

$$GSI = \frac{\text{Weight of ovaries}}{\text{Weight of Fish}} \times 100$$

Different stages of oocytes were identified as specified by Tacon et al. (1996) and Degani et al. (1997) and gonadal state was ascertained. Individuals were grouped on the basis of identical state of oocytes, their percentage dominance was calculated and the gonadal cycle was reported in four phases.

Ovary of one side in each fish was processed for histology to assess the gonadal state and ovary of the other side alongwith selected tissues were processed for histochemical and biochemical studies. For histology, Heidenhein's Iron-Haematoxylin (Kiernan, 1999) method was used. Schultz-Smith Method (Weber et al., 1956) was adapted for histochemical demonstration of cholesterol. Cholesterol were estimated by Ferric Chloride ($FeCl_3$) (Zlatkis et al., 1953) method (Table 1).

3. Results and Discussion

In *O. mossambicus,* maturation with positive staining of cholesterol is observed in the ovary. Intensity of staining increases in the ooplasm successively from stage I to stage VI

Table 1: Variations in GSI Values of Female

Phases	Female
Postspawning	0.52665 + 0.018
Preparatory	0.21887+0.021 NS
Prespawning	0.88778 + 0.067 p<0.01
Spawning	3.24932+0.205 p<0.01

Value Present mean+SE of observations; NS: Non Significant

oocytes (Figures 1 to 4). In *O. mossambicus* intense staining of these membranes for cholesterol can be the indication of their involvement in vitellogenesis. Granulose has the responsibility of deposition of yolk in developing ovum and of its removal in ova which degrade before ovulation that is granulosa has

Cholesterol histochemistry

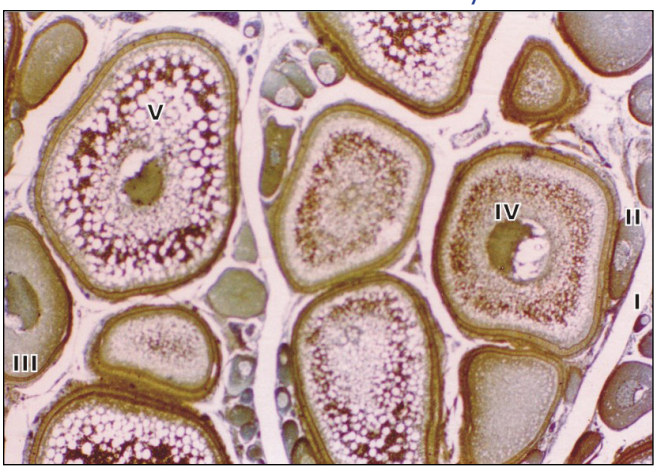

Figure 1: T S of ovaru during preparatory phase showing darkly stained ooplasm of stage I oocytes, nucleoplasm in stage III, IV, V are modetately stained, theca shows faint staining and radiata is weakly stained. X25

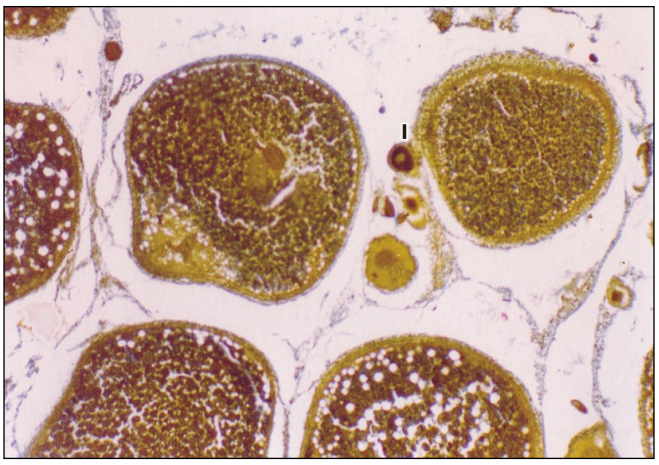

Figure 2: T S of ovary during pre spawning phase showing moderately stained theca and granulosa, radiata is weakly stained, yolk granules are darkly stained and nucleoplasm is faily well stained, nucleoli are darkly stained in stage I ooplasm intensely stained. X25

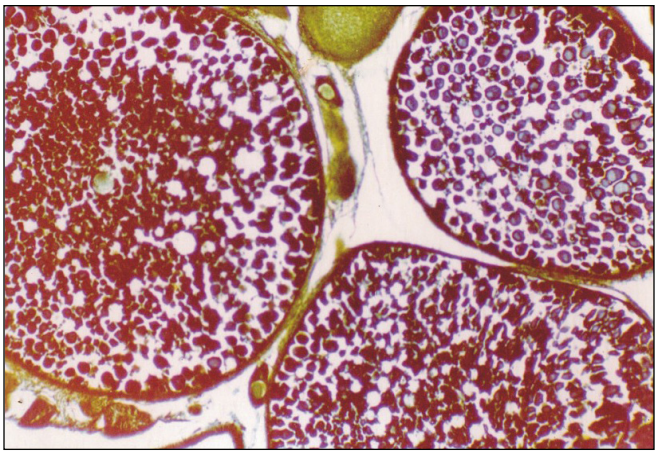

Figure 3: T S of ovary during spawning phase showing oocytes moderately stained, wall of the oocytes in intensely stained. X25

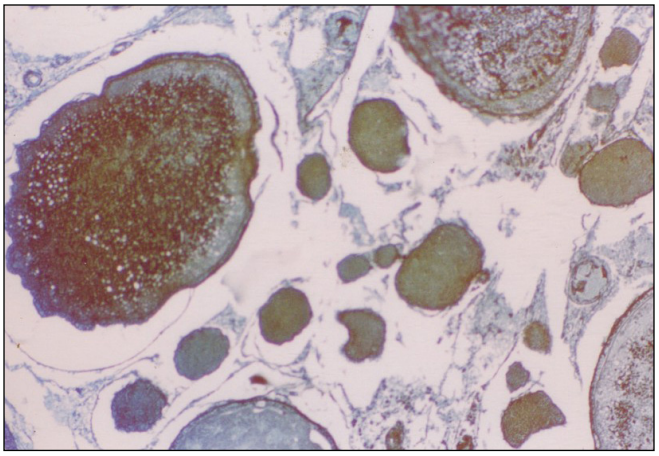

Figure 4: T S of ovary during postspawning phsase showing darkly stained wall, oocyte granules intensity stained. X25

both nutritive and phagocytic functions (Hoar, 1969).

Cholesterol content is highest in the ovary of this fish during prespawning period and the increase is statistically significant (p<0.01). Changes in the cholesterol level of gonads are an indirect measure of steroid biosynthesis and are considered as a marker of ovarian activity (Saksena and Saxena, 1999). Prespawning phase shows the dominance of stage III, IV and V types of the oocytes in *O. mossambicus*, indicating active proliferation of oocytes (Table 2). High level of ovarian cholesterol has been observed during prespawning and spawning phases of *Schizothorax richardsoni* and *Glyptothorax pectinopterus* (Singh and Nauriyal, 1990), *Channa orientalis* (Sharma and Saksena, 1991), *Clarias batrachus* (Saksena and Agarwal, 1991) and *Heteropneustes fossilis* (Singh et al., 1993) owing to greater demand of precursor material for enhanced requirement of steroids during these phases. Cholesterol content in *O. mossambicus* is lowest in liver, muscles and ovary (p>0.01) during spawning phase. This reduction of free cholesterol in the ovary is in response to gonadotropins (Mukerjee and Bhattacharya, 1982). In female plaice *Pleuronectus platessa* (Dawson and Grimm, 1980) and in Arctic capelin, *Mellotus villosus* (Henderson et al., 1984), marked depletion of body lipid and/or glycogen occurs during gonadal development. Changes in the cholesterol level of gonads are an indirect measure of steroid biosynthesis and are considered as a marker of ovarian activity in *Channa orientalis* (Saksena and Saxena, 1999) and *Notopterus notopterus* (Shankar and Kulkarni, 2007). From somatic tissues, these are diverted to gonads for maturation. Siddique (1966) recorded maximum ovarian cholesterol in the *Ophiocephalus punctatus* at the end of maturing phase, while in *H. fossilis,* Singh and Singh (1989) observed declined cholesterol level in ovaries during preparatory phase and increased level during spawning phase. In *Anabus testudineus,* ovarian cholesterol is low during prespawning and high during postspawning. In *O. mossambicus* ovarian cholesterol is lowest in spawning indicating its utilization as a precursor for steroid synthesis during this phase.

In telencephalon, in prespawning phase there is significant increase (p<0.01) for cholesterol. Brain cholesterol in *O. mossambicus* is reported to be 16.10±2.2 by Reddy et al., (1981), but phasic studies are not reported. In our study, cholesterol content is highest in prespawning which is 3.71±0.050 and 2.731±0.036 in telencephalon and diencephalon respectively. Interplay of number of factors like synthesis, hydrolysis and nature of fatty acid pool available for cholesterol esterification (Swell and Law, 1968) controls the turnover of tissue and serum cholesterol esters, which may

Table 2: Percentage of developing oocytes in the ovary at different reproductive phases in Tilapia, *O. mossambicus*

Phases	Stage I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-VI
Postspawning	28.773+0.905	27.089+1.603	23.523+1.162	14.596+0.574	6.017 + 0.754	0.00+0.000
Preparatory	36.873+4.366 p<0.01	32.992 + .872 p<0.01	14.467+0.782 NS	5.895 + 0.997 NS	9.715 +0.183 p<0.01	0.0+0.000
Prespawning	6.249+0.609 NS	10.340+0.126 NS	21.641+1.603 p<0.01	26.768+1.473 p<0.01	29.215+0.456 p<0.01	5.785+0.346 p<0.01
Spawning	7.019+0.412 NS	11.508+0.685 NS	13.053+ .438 NS	9.875 +0.648 NS	11.874+0.519 p<0.01	45.731+2.553 p<0.01

Values are mean+SE of Observations; NS: Non significant

result in such high amount in prespawning phase because in this phase all the types of oocytes are recruited and an array of biochemical reactions take place.

Pituitary gland seems to be essential for cholesterol utilization, as its surgical removal results in hypercholesterolemia (Friedman et al., 1970). It also influences the secretion of the other endocrine glands in *H. fossilis* (Jaiswal and Belsare, 1976). In *O. mossambicus*, cholesterol content in the pituitary during spawning phase is the lowest (0.578±0.017). Lowest content during this phase might be due to its diversion to other tissues especially to gonads which use cholesterol as a precursor for synthesis of sex steroids during this phase. Cholesterol plays a role in protein synthesis, as well, even at the level of DNA action (Sabine, 1977). Cholesterol and its esters are responsible in large measures for maintaining the structural integrity of various protein-nucleic acid complexes (Bandyopadhyay and Deutscher, 1973; Manzoli et al., 1974).

Variation in both environmental as well as intrinsic (hormonal) factors affect the cellular constituent of liver, muscles and gonad which show seasonal fluctuations (Plack et al., 1971; Emmersen and Emmersen, 1976; Medda and Mahapatra, 1980; Okuzawa et al., 1986; Quinito et al., 1989). A seasonal co-relation exists between liver metabolic activity and egg maturation in *Garra mullaya* also (Khan and Mehrotra, 1991). In *O. mossambicus* lowest cholesterol is noticed in liver and muscles in the spawning phase. Lovern and Wood (1937), Hickling (1947) and Milroy (1998), revealed that during maturation, fats which are stored in various organs are transferred to maturing gonads. In *Pseudosliaena aneus* and *Johnius carutta* (Appa Rao, 1967), there is abundance in fat during ovarian stages in I to IV indicating and there is gradual reduction during stages V to VII indicating its utilization first by the ovaries and later by the body during spawning and postspawning periods. Cholesterol is not only a precursor for the synthesis of sexual hormones but is an important component in the cell membrane which is formed in high rates during gonadal development (Saksena and Saxena, 1999). Thus variations in its content during different ovarian stages are observed in *O. mossambicus* and a definite correlation in nervous, endocrine, somatic and reproductive components is established in this fish (Table 3).

Table 3: Variations in cholesterol content of telencephalon, diencephalon, pituitary, ovary, liver and muscle extracts of Tilapia, *O. mossambicus* (mg g^{-1} wet weight of tissue)

Phases	Telencephalon	Diencephalon	Pituitary	Ovary	Liver	Muscle
Postspawning	1.137±0.032	1.784±0.072	0.844±0.031	4.804±0.047	5.531±0.069	1.050±0.036
Preparatory	2.460±0.078	2.695±0.042	0.978±0.043	5.245±0.075	10.618±0.085	2.320±0.056
	$p<0.01$	$p<0.01$	$p<0.05$	NS	$p<0.01$	$p<0.01$
Prespawning	3.710±0.050	2.731±0.036	1.118±0.036	6.215±0.029	9.357±0.154	1.362±0.098
	$p<0.01$	$p<0.01$	$p<0.05$	$p<0.01$	$p<0.01$	$p<0.01$
Spawning	0.798±0.035	0.645±0.024	0.578±0.017	3.000±0.142	5.162±0.126	0.550±0.025
	NS	NS	NS	NS	NS	NS

Values are mean+SE of Observations; NS: Non significant

4. Conclusion

GSI values lowest in the preparatory phase and highest in the spawning phase. In the preparatory phase, ovary is dominated by stage I and II oocytes, in the spawning phase VI and V oocytes is dominated. Brain and pituitary has highest cholesterol in prespawning, lowest in spawning phase. The ovary has highest cholesterol content in prespawning, and lowest in the postspawning phase. On the contrary, liver and muscles show highest cholesterol in the preparatory and lowest in the spawning phase.

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