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DETERMINATION OF PROTEIN IN THE GERM LINE CELLS OF ZIG-ZAG EEL *Mastacembelus armatus*, LACEPEDE 1800 (ACTINOPTERYGII: MASTACEMBELIDAE) FEMALE IN THE DIFFERENT REPRODUCTIVE PHASES: A HISTOCHEMICAL APPROACH

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AUTHOR'S CONTRIBUTION
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ABSTRACT

Mastacembelus armatus, is an indigenous fish species of southern Asia, which also resides in Indian subcontinent. This fish species is facing an alarming declining in their number in the last decade. Due to its moderate cost, it is mainly taken by the lower income group of people of the society. The proteins play very important role in the oocytes specially in external fertilization. Maternally inherited proteins play pivotal role in pattern formation as well as in the growth and development of the embryo. Microscopic ovarian features with respect to accumulation of protein of *Mastacembelus armatus* were analyzed. Samples were fixed in Carnoy's fixative, mounted in 54° - 56° paraffin and cut into 8 µm-thick slices which were stained with Mercury – Bromphenol Blue (MBPB) method. After staining the thin sections with the thickness of approximately 8 µm were examined. Ovary of *Mastacembelus armatus* is extremely dynamic organ in which follicles underwent asynchronous development. The oocytes of ovary were observed in various phases. Accumulation of protein in the different stages of oocytes in zig-zageel with the advancement of spawning season shows a positive correlation.

Keywords: *Mastacembelus armatus*; ovary; oocytes; protein.

1. INTRODUCTION

The chemical composition of the different regions of the fish oocytes such as theca, granulosa, zona radiata, cortical alveoli and cytoplasm during the reproductive cycle of fish and the mobilization of macromolecules and cations from the liver to gonads specially in the ovary have been studied by several authors [1] Selman and Wallace [2], Sarasquete et al. [3], Munoz-Cueto et al. [4], Corriero et al. [5]. Beams and Kessel [6], Guraya [7], Wallace and Selman [8] observed the presence of lipid granules in the yolk nuclei of the teleost ovary. Sardul and Garaya [9] reported the steroid synthesizing cellular sites of ovarian tissue include theca interna of developing follicles, the granulosa luteal cells of postovulatory corpora lutea as evidenced by the sudanophilic lipids in the cytoplasm. Khoo [1] emphasized that yolk vesicles of oocytes are composed of glycogen and complex acidic mucopolysaccharides while very little or no lipids are present. In contrast yolk granules contain neutral fats and phospholipids only. He further stated that protein content of yolk granules are larger than yolk vesicles and the zona radiate contains

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phospholipids. Craik and Harvey [10]; Cussac and Maggese [11]; detected the presence of lipids in follicular epithelium of oocytes. Munoz et al. [12] reported small lipid droplets on the perinuclear ooplasm just before the formation of cortical alveoli in *Trigla lyra*. In the first vitellogenic stage the lipid droplets greatly increased in the central area of the cytoplasm while in second vitellogenic stage lipid droplets occupied in the perinuclear area. Several researchers advocated that the accumulation of PAS-positive carbohydrates generally occurred in the yolk vesicles during secondary growth of the oocytes [1]; Nagahama, [13]; Selman and Wallace [2]. Selman and Wallace [2] confirmed the presence of acidic glycoconjugate substances in the yolk vesicles of *Fundulus heteroclitus*. Mandich et al. [14] histochemically detected proteins, lipids and carbohydrates during oocyte development in dusky grouper *Epinephelus marginatus*. They reported the appearance of lipid vesicles in the middle ooplasm of the oocytes, at the beginning of the secondary growth phase, and afterwards PAS and Alcian blue positive small carbohydrate granules appeared before the occurrence of the first yolk granules. Ortiz-Delgado et al. [15] histochemically established the distribution of carbohydrate, proteins, lipids, calcium, iron, vitellogenin, zona radiata protein during different phases of oogenesis. During the initial vitellogenic phase, the oocytes showed cortical alveoli with neutral lipids exclusively while the yolk granules were composed of glycoproteins, Calcium, Iron and proteins rich Lysine, Arginine, Tyrosine, Tryptophan and Cysteine. The cortical alveoli contained carboxylated and neutral glycoconjugates, the follicular envelope contained protein and calcium and the zona radiata was mainly proteinaceous in nature along with calcium and neutral glycoproteins.

2. METHODOLOGY

Adult male (average length 15.5 cm) and mean body weight (62 g) and female (average length 17.6 cm) and mean body weight (62.5 g) of *M. armatus* were procured fortnightly throughout the year from particular pond of Asansol near Chapui village. The size of the pond is 50 m x 40 m x 10 m, in order to avoid ecological variations than can affect development of hypothalamus, pituitary and gonads. The fishes were collected during the second week of every month from January 2018 to December 2018. To study the gonadosomatic index, fishes were sacrificed and ovaries of the fishes were dissected-out minutely, after giving an incision along the mid-ventral line. The ovary was cut into small pieces and then the tissues were fixed in Carnoy's fixative.

After proper fixation in Carnoy's fixative the pieces of and ovary were then thoroughly washed in 70% as well as absolute alcohol overnight and then dehydrated in acetone, cleared in xylene and embedded in paraffin wax (54°C - 56°C melting point) in vaccum embedding medium for one hour. Serial paraffin sections were cut at 8 μm thickness using Leica RM 2125 RT microtome and were subjected to histochemical test for protein by Mercury – Bromphenol Blue (MBPB) method [16]. Deparaffinized sections were brought to distilled water and immersed in a freshly prepared bromphenol blue solution for two hours at room temperature. After rinsing in 0.5% acetic acid for five minutes, the sections were transferred directly to the tertiary butyl alcohol for overnight. Then the slides were cleared in xylene and mounted permanently in DPX. Slides were examined under microscope.

3. RESULTS AND DISCUSSION

The chromatin materials of nuclei of oogonia and primary oocytes are positive to protein histochemical test due to proteinaceous nature as very less protein is accumulated in the cytoplasm thus oogonia and primary oocyte's cytoplasm give a negative result during the resting phase of reproductive cycle (Fig. 1). In the growth phase the cytoplasm of early and late perinucleolar oocytes furnish comparatively intense reaction (Fig. 2). The ooplasm around yolk vesicles show positive result for protein of stage V oocytes has been noticed during maturation phase (Fig. 4). In the spawning phase the yolk granules of mature follicles have been found to be more reactive. The germinal layers are also positive to protein reaction (Figs. 3 and 5). The granulosa cells of granulosa layer exhibit intense protein reaction during spawning phase (Fig. 5).

The intensity of reaction of protein molecules vary in different oocytes during oogenetic cycle. During the course of present investigation it has been noted that the ooplasm is rich in proteinaceous substances. The yolk globules of stage V oocytes contain high amount of protein. This is in conformity with the findings of Cussac and Maggese [11] in *Rhamdia sapo* and Srivastava and Srivastava [17] in *C. striatus*. It has been reported that in lower vertebrates the principal events responsible for the enormous growth of the oocytes involve sequestration and packing of a hepatically derived plasma precursor or vitellogenin which takes part in the formation of yolk protein. This was supported by number of researchers (de Vlaming et al. [18]; Shackley and King [19]; Idler et al. [20]. Therefore, it is assumed that the phosphoprotein together with lipoprotein, form the principal nutritive reserve of the growing and mature oocytes of

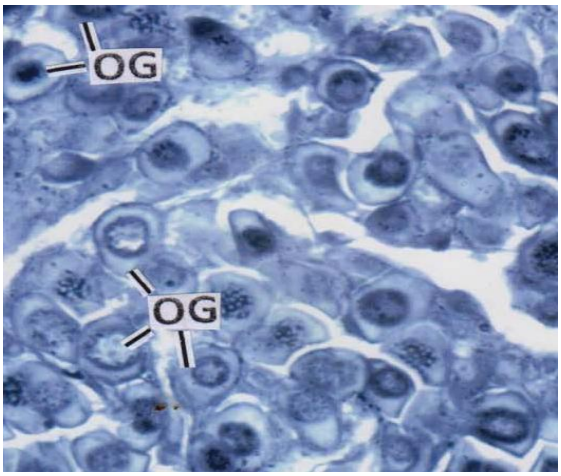

Fig. 1. Intense protein reaction in the nuclei of oogonia (OG) and weak reaction in cytoplasm during resting phase
(MBPB) x 600

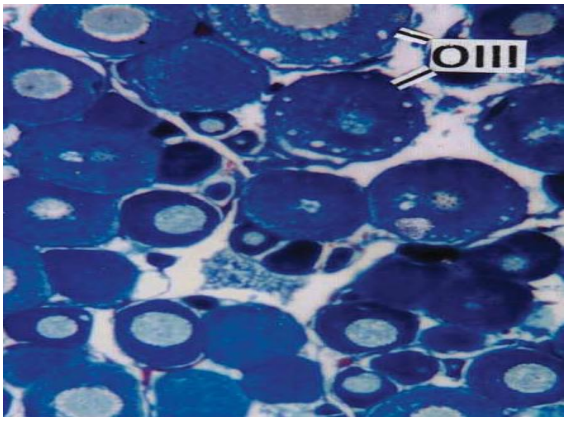

Fig. 2. Intense protein reaction in the cytoplasm and nucleoli of oocyte II (O II) and oocyte III (OIII) during growth phase
(MBPB) x 400

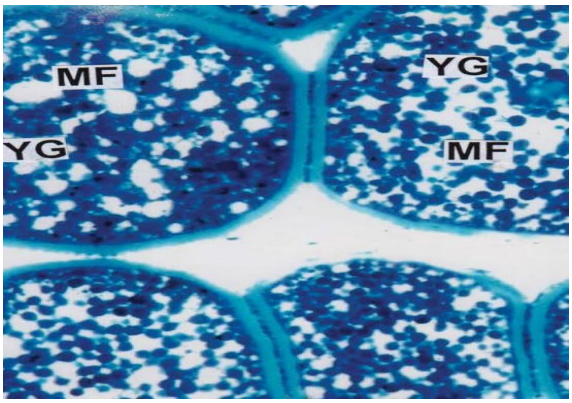

Fig. 3. Maximum protein reaction in the yolk granules (YG) of mature follicles (MF) but moderate reaction in the germinal layers during spawning phase
(MBPB) x 400

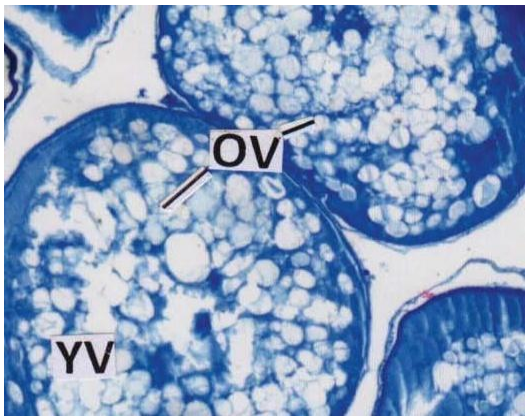

Fig. 4. Intense protein reaction in the cortical dense ooplasm of oocyte V (O V) and yolk vesicles (YV) during maturation phase
(MBPB) x 400

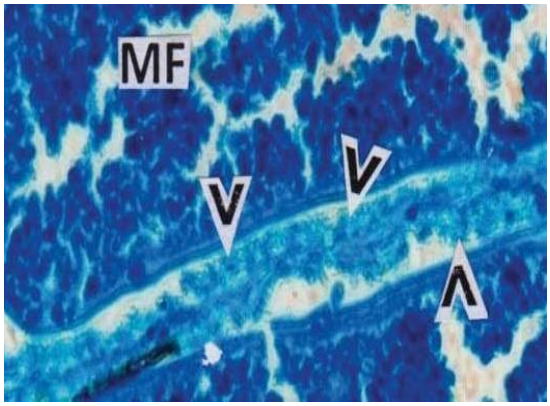

Fig. 5. Intense reaction of protein in the granulosa cells (arrow heads) of zona granulosa and yolk granules (YG) of mature follicles (MF) during spawning phase
(MBPB) x 400

oviparous vertebrates [21]. The zona ganulosa and zona radiata contain glycoprotein in stage IV and stage V oocytes as were reported by Gutierrez et al. [22] in *Solea senegalensis* during final process of oogenesis. The localization of protein containing cysteine in the middle layer i.e. zona granulosa in *Mystus tengara* also reported by Guraya et al. [23]. Ortiz – Delgado et al. [15] have detected general protein along with protein rich in amino acids such as arginine, cysteine and tryptophan in the zona radiata in *Xiphias gladius*. They also concluded that the zona radiata externa is rich in neutral glycoproteins whereas zona radiata interna contain proteins rich in disulphide groups.

4. CONCLUSION

The oocytes growth is more or less similar in the teleosts. In most of the teleosts, the progress of oogenesis might be in four, five, six and eight stages. The oocytes development in the *Mastacembelus armatus* was manifested in a series of changes, which their division into six stages. During the oocytes development, the oocyte enlarged due to hydration and preteolysis of the yolk protein. During the present investigation the accumulation of protein in the early oocytes proceeds rapidly from the growth phase onwards and this situation continues till the maturation phase where maximum deposition of protein has been recorded. Therefore, it is possible that the protein demand for the advanced oocytes is largely met with this accumulated protein which is being synthesized in the course of oogenesis. Results of the present study hopefully would contribute knowledge to the research on the process of the oogenesis of the *Mastacembelus armatus*.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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