PA Histomorphological and Histometric study of the co-relation between the development of the Zona Radiata and the Zona Granulosa during oogenesis in the Gangetic mystus - *Mystus cavasius* (Hamilton, 1822)

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(Received: 26/10/14) (Accepted: 22/11/14)

**ABSTRACT**

The growth and development of the zona granulosa (ZG) and the zona radiata (ZR) layers from the different oocytic stages of the Gangetic catfish, *Mystus cavasius* (Hamilton, 1822) has been studied. The ZR layer appeared earlier during the late perinucleolus stage whereas the ZG layer was detected for the first time in the yolk vesicle stage. The two layers were found to increase in thickness with the growing oocyte cell diameter and a strong positive correlation was predicted by Pearson correlation coefficients for both the layers. The maximum height of the ZR (6.8 ± 0.64 µm) and ZG (10.8 ± 0.27 µm) was recorded from the mature oocytes which exhibited maximum yolk incorporation with eccentric nuclei. The ZG provided with prominent syncytial nuclei have shown more significant growth than the ZR throughout the study indicating its active role in the synthetic process while the ZR was known for its permeable and porous nature. Some inter cellular as well as intra cellular connections were also detected in between the Zona layers proving that the ZR and ZG layers play some vital role in the oocyte development process and hence in regulating the reproductive cycle of the female fish under study.

**Keywords:** Histology, Histometry, Zona granulosa, Zona radiata, Oocyte, *Mystus cavasius*

**INTRODUCTION**

*Mystus cavasius* (Hamilton, 1822) or Gangetic tangra is a commercially important fresh water catfish distributed throughout the Indian sub continent, Myanmar, Thailand, Malaysia, East Indies, Syria and West Africa [1]. Though its conservation status is under the least concerned category in India, it was declared as a vulnerable species in Bangladesh [2]. This catfish being an important food fish is exploited largely and hence a sound knowledge of its oocytic growth and configuration is essential to know the reproductive mechanism.

In addition to the oolemma, the two vital layers occurring in the oocytes of the teleost fishes are the zona radiata (ZR) and the zona granulosa (ZG). As reported by Rizo *et al.* (2003) [3], in between the oolemma and the follicular layer lies the ZR, which is porous in nature and hence regulates the movement of important substances from the follicular layer into the oocyte. Several groups of workers have contributed to the study of the ZR layer in the teleost fishes [4-11]. The inner follicular layer is actually the ZG layer which performs several functions for the growing oocytes including the process of vitellogenesis [6]. Hence, it is quite obvious that during the developmental process of *Mystus cavasius*, the oocytes will definitely undergo some morphohistological as well as morphometric changes whose knowledge may help to increase the productivity of the fish.

So, in the present research an attempt has been made to correlate development of the zona granulosa and zona radiata layers in different oocytic stages during the course of oogenesis in *Mystus cavasius*.

**MATERIALS AND METHODS**

**Specimen collection and data analysis**

Adult female *Mystus cavasius* (Hamilton, 1822) were collected during the third week of every month throughout the year from a particular stocking pond located in Bishnupur (23.08° N, 87.32° E) subdivision of Bankura district, West
Bengal, India, in order to avoid ecological variations in different water bodies that can affect the ovarian development. The fishes were collected at an interval of about 30 days in order to obtain all the oocytic stages during the different reproductive phases- growth, maturation, spawning and post spawning. About 130 female fishes with mean body mass 48 ± 0.37 g were collected round the year and every month ovaries from 10 fishes were dissected out for the measurement of oocytic cell diameters and histological and histometric observations of the ZR and ZG required for our experiment.

**Histological methods**

The ovaries were dissected out, cut into small pieces and then fixed in aqueous Bouin’s fluid [12] for 18 hours. The fixed tissues were then dehydrated through up graded alcohol, embedded in paraffin (melting point-56º C-58º C) and sectioned at 4µm thickness.

The ovarian sections were stained with the Mallory’s triple (MT) and haematoxylin-eosin (H&E) stains [12]. The stained sections were mounted permanently with DPX and examined under compound light binocular microscope.

**Histometric measurements**

From the histological preparation of the ovaries, the mean heights of the ZR and ZG were measured at 10 different locations for each sample. The diameters of the various oocytic cells were measured with the help of reticulo-micrometer and ocular micrometer at four points within each cell at 90º from one another.

**Statistical analysis**

All data are reported as the mean ± Standard Error of Mean (SEM). The correlation between the oocyte cell diameters and the heights of two layers (ZR and ZG) were determined using the Pearson correlation coefficient.

**RESULTS AND DISCUSSION**

In the histological sections of the ovary in *M. cavasius*, six different stages (excluding the atretic follicles and the discharged follicles) (Table I) of oocytes are identified on the basis of their morphohistological characteristics and staining intensity. The height of both the ZR and ZG layers are found to increase with the increasing diameter of the oocytes as shown in figure 1 (discharged follicles are not shown) and showed strong positive correlation having the value of Pearson correlation coefficients of 0.937 and 0.976 respectively.

The ZR and the ZG layers are not visible and hence could not be identified during the early developmental stages i.e., in the oogonial and the early perinucleolus stage of the oocyte (fig. 2) under the light compound microscope. However, the ZR layer is observed for the first time during the late perinucleolus stage (III) (fig. 3) whereas the ZG layer could be identified for the first time during the yolk vesicle stage (Figs. 3- 5).

In between the oolemma and the ZG layer of the oocytes appears a banded and striated acellular layer known as the ZR layer (Figs. 3-10). Striations have been reported in the ZR by several workers during early and late vitellogenesis. [13-15]. The perforations or pores of the ZR layer were identified by Shabanipore et al. (2004) [16] as the dotted structures of the inner surface of the membrane. Shabanipour et al. (2004) [16] reported that during the vitellogenic stages like the yolk deposition stage and the mature oocytic stage bud like structures occur in the form of undulations in the ZR layer. (Fig.8). Larger number of undulations corresponds to larger number of pores which is again an indication of greater porosity and hence greater permeability of the ZR for the yolky materials and other factors, enzymes etc, the ZG and from the cell exterior into the oocyte. Koç (2010) [12] reported that the ZR was located in between the oocytes and the follicular cells and thickened at the vitellogenic stage.

Unlike the ZR layer the ZG layer is a cellular layer (figs. 3-10) composed of distinct nucleated cells whose diameter changes with the progression of the oocytic stages finally reaching its peak value in the mature oocytic stage (Table-1). It is interesting to note that the maximum incorporation of yolk materials takes place in the mature oocytes from across the ZR and the ZR also exhibits maximum height during this stage (figs.7-10). Selman and Wallace (1989) [17], reported that in teleost fish, a rapid thickening process is observed in the zona radiata during maturation and ovulation stages. It was also supported by Guraya (1986) [18] that the thickening tendency of the ZR in teleost during oogenesis is caused by the increase in the ovule diameter and it lessens after ovulation.

As reflected from figure. 11, the height of the ZG increases greatly when the follicles undergo hypertrophic atresia and this is due to the fact that the yolk materials of the atretic follicles are engulfed by the phagocytic action of the ZG nuclei. In the mature oocytes that are exhibiting eccentric nuclei i.e., in the late mature oocytes, and in the discharged follicles the ZR layer decreases in height and appears as loosely oriented structures enveloping the oolemma. In some of the mature oocytes a perivitelline space is observed in between the oolemma and the ZG layer.

(figs. 7 and 8), this character can be attributed to the fact that a little shrinkage of the yolky cytoplasm occurs in the oocytic cells after hydration of the yolky materials that appear inside the oocyte. Cruz-Höfling et al., (1993) [6], found similar structure in the teleost, *Crenicichla johanna*. The ZG layer also becomes irregular in appearance (fig. 8) and slightly decreases in height although the nuclei inside it can still be identified. This is due to the fact that during the late maturation phase the rate of yolk incorporation into the oocyte declines sharply and almost ceases. Some intercellular thread like connections or desmosome like structures are noticed in between the ZG layers of adjacent oocytes (figs. 9 and 10). Similar structures are also found within the cell in between the ZG and the ZR interna. (fig. 10).

### Table I Different oocytic stages showing their average diameters and respective heights of their zona radiata (ZR) and zona granulosa (ZG)

<table>
<thead>
<tr>
<th>Terminology and stage</th>
<th>Cellular features</th>
<th>Average cell diameter (µm)</th>
<th>Height of ZR±SEM (µm)</th>
<th>Height of ZG±SEM (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oogonia (I)</td>
<td>a) Occur mostly in clusters or nests attached to the germinal epithelium. b) Scanty chromophobic cytoplasm with a large centrally placed nuclei.</td>
<td>7.5 ± 0.24</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Early perinucleolus stage (II)</td>
<td>a) Ooplasm basophilic with a thin layer of follicular epithelium. b) Large centrally placed nucleus with an average diameter of 16 ± 0.98 µm. c) 5-10 visible nucleoli and some fragmented chromatin observed in each nucleus.</td>
<td>28 ± 1.08</td>
<td>00</td>
<td>00</td>
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<tr>
<td>Late perinucleolus stage (III)</td>
<td>a) Ooplasm basophilic and stains lighter. b) Nucleus centrally located with an average diameter of 67± 0.69 µm. c) 12-30 visible nucleoli and dispersed chromatin material. d) Appearance of zona radiate (ZR) for the first time.</td>
<td>86.5 ± 0.86</td>
<td>1.2 ± 0.04</td>
<td>00</td>
</tr>
<tr>
<td>Yolk vesicle stage (IV)</td>
<td>a) Small colourless vesicles appear in the family stained cytoplasm. b) Large irregular shaped nucleus with nucleoli in its periphery. c) Syncitial zona granulosa (ZG) is clearly visible along with ZR.</td>
<td>210 ± 1.46</td>
<td>3.8 ± 0.12</td>
<td>7.8 ± 0.06</td>
</tr>
<tr>
<td>Yolk deposition stage (V)</td>
<td>a) Yolk granules are deposited in yolk vesicles. b) Ooplasm changes from basophilic to acidophilic in staining nature. c) ZR and ZG both increase in thickness.</td>
<td>392 ± 1.23</td>
<td>4.2 ± 0.32</td>
<td>8.2 ± 0.14</td>
</tr>
<tr>
<td>Mature oocyte (VI)</td>
<td>a) Yolk laden oocytes with eccentrically placed nuclei. b) Intensely stained voluminous cytoplasm with granular appearance. c) Fibrillar inter-cellular connections are frequent d) ZR and ZG are thickened to its maximum possible limits.</td>
<td>518 ± 1.87</td>
<td>6.8 ± 0.64</td>
<td>10.8 ± 0.27</td>
</tr>
<tr>
<td>Atretic follicle (Hypertrophid)</td>
<td>a) Nuclei of the ZG invade the yolky cytoplasm. b) The yolk materials are phagocytised by the nuclei of ZG. c) The ZG attains almost the double height of that in stage VI with apical nuclei</td>
<td>No definite size</td>
<td>1.5 ± 0.01</td>
<td>18.6 ± 0.18</td>
</tr>
<tr>
<td>Discharged follicle</td>
<td>a) Discharged state of matured follicle. b) Theca and follicular cells are visible with irregular outlines.</td>
<td>No definite size</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>
Figure 1: Variation in the heights of the Zona Granulosa (ZG) and the Zona Radiata (ZR) layers with diameters of the oocytes during different stages of oogenesis.

Figure 2: Optical micrograph of the histological section of the ovary of *Mystus cavasius* showing an early perinucleolus oocyte (EP) without any visible zona layer, only the oolemma are visible (arrow heads) as the outer layer and the nucleus (Nu) is provided with distinct nucleolus (orange dots). (MT)
Figure 3: Late perinucleolus oocyte (LP) with a visible zona radiata (ZR) layer (white arrow). The distinct zona granulosa (ZG) layer with syncytial nuclei (black arrow) is visible above the ZR layer (white arrow) in the oocyte of Yolk vesicle (YV) stage. (H&E)

Figure 4: YV stage oocyte and a yolk deposition (YD) stage oocyte with a large nucleus. Note the presence of distinct ZG and ZR layers in both the oocytes. (MT)

Figure 5: YV stage oocyte with densely stained ZR and ZG layers. Note the presence of an adjacent YD stage with ZG and ZR and an early perinucleolus oocyte (EP) without any zona layers. (H&E).

Figure 6: Two YD stage oocytes located side by side bearing ZG layers with distinct nuclei and band like ZR layers. (H&E).
Figure 7: Mature oocytes (Mo) showing ZG and ZR layers. The occurrence of a gap (thin black arrow) (perivitelline space) between the ZR and the oolemma can be noticed. (MT).

Figure 8: MO bearing ZG and ZR layers. A gap (thin arrow) (perivitelline space) between the ZG and the ooplasm can be seen and the ZR at places appears undulating. (H&E).

Figure 9: A triad of mature oocytes (Mo) showing inter-cellular fibrillar connections (arrow head). The nucleated ZG layers and the band like ZR layers are also visible. (H&E).

Figure 10: Two adjacent Mo showing intracellular as well as intercellular connections (arrow head). The intercellular connections can be noticed between the ZG layer and the ZR layer within the Mo. (H&E).
Figure 11: A MO undergoing atresia showing increased height of ZG. The ZG is showing columnar cells with apically placed nuclei. Some yolk granules (black stars) can also be noticed in the ZG layer. Beside the Mo, an early YV stage can be seen with normal ZG layer. (MT).

CONCLUSION

It is clear from our study that the ZR and the ZG are the two important structures of the oocytes that varies in thickness during the various stages of the oocytic development and bears some positive co-relations with the increasing oocytic size. The structures show great variation in height in the breeding and non-breeding seasons indicating their direct involvement in the process of vitellogenesis. Hence, a control over the transporting or communicating system of the ZR layer synthetic activity of the syncytial ZG cells would definitely prove to be helpful for the researchers in regulating the reproductive behaviour of the female *M. cavasius* through target specific action on these zona layers.

Acknowledgements

The author is grateful to the University Grants Commission, for financing this research work. The author is also thankful to, the department of Zoology, Ramananda College, Bishnupur, Bankura, for providing the laboratory facilities required for this work.

CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this paper after the follicle is discharged as indicated by the decreasing diameters of the discharged oocyte.

REFERENCE


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