Fate of unspawned oocytes in the ovary of Labeo rohita (Ham.) during inter spawning period through multiple breeding

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ABSTRACT
Labeo rohita was induced bred repeatedly three times during May to August. GSI (ovary) was 18.2 \(\pm\) 0.547 in non-bred fish which was reduced to 6.983 \(\pm\) 1.004 soon after spawning and further reduced to 4.226 \(\pm\) 0.603 during 1st week. GSI again slowly increased up to 17.178 \(\pm\) 0.497 at the end of 6 week of initial spawning. Histology of spent ovary exhibited 70-80% empty follicle and 20-30% of unspawned oocytes of different stages viz. previtellogenic oocytes (PO), vitellogenic oocytes (VO), non-spawned loose mature oocytes (NLMO) and non-spawned overmatured, oocytes (NOMO). The empty follicles started disintegrating within 1st week of the spawning. Some of the maturing oocytes gradually become overmatured whereas NLMO and NOMO were entered to the atretic process and were absorbed to the system. Most of the non-spawned previtellogenic eggs and early vitellogenic eggs were used to develop in the ovary as growing oocytes and entered the maturation phase towards 6th and 7th week.

Note
Indian major carps viz. Catla catla, Labeo rohita and Cirrhinus mrigala were considered as seasonal breeders. Spawning of mature oocytes, degeneration of matured oocytes when they fail to spawn, recruitment of new oocytes, and their maturation are the cyclic events of the year. All the mature oocytes do not get scope to be ovulated out during spawning. The non-ovulated mature eggs are degenerated in the ovary. Degeneration process of the ovarian oocytes and their resorption into the sytem is widely known as follicular atresia. In teleostean species, occurrence of atretic oocyte is a common phenomenon of prespawning, spawning and postspawning ovaries (Guraya, et al 1975; Guraya, 1986, 1993). Bhowmic et al. (1977), Chondar (1986) and Gupta et al (1995) could bred IMC species twice, thrice and four times respectively during same season. The present communication is based on the histological changes of unspawned oocyte that occur during inter spawning period of two successive induced breeding in Labeo rohita through multiple breeding.

Labeo rohita could be induced bred during May. Three spent females were sacrificed on the day of breeding (0 day spent) for histological and GSI study of the ovary. Thirty spent brood were tagged with dye marking (Khan et al., 1988) and reared in pond for further study on spent brood till subsequent breeding in the same season. These spent brood have been sampled at weekly intervals during the interspawning
period of 1-2nd spawning for histological as well as GSI study. Thirteen females out of the above spent brood could be induced bred for the second time during June. Again the spent brood were reared. The spent brood of 2nd breeding were also sampled at weekly interval between 2-3rd breeding (Table 1). Six female could be bred for third time during August. Ovary samples were processed for histoanatomical study. The histoanatomical fate of EF, NLMO, NOMO and other primary oocytes were categorically studied. GSI (ovary) of non bred fish, at termination of induced breeding experiment, after one week, three weeks and six weeks of 1st and 2nd breeding were recorded.

Gonosomatic index (ovary) of non-bred individual was recorded as 18.2 ± 0.547. GSI of induced-bred spent was immediately reduced to 6.983±1.004. The so called spent female were expelling out the non-viable spawned eggs upto at least 12 hours after termination of the induced breeding experiment, hence GSI was further reduced to 4.226±0.603 within first week of induced breeding and then increased gradually upto 17.178±0.497 within next five week. Ovary, soon after the breeding operation retained 20-30% unspawned nonvitellogenic and vitellogenic oocytes of different growing stages (Fig.1) along with 70-80% of empty follicles (Fig 1 and 3). Those vitellogenic eggs which were in the process of ovulation (oocyte with ruptured follicular epithelium), but could not be ovulated (Fig. 1&2) were named as non ovulated loose matured oocytes (NLMO). Generally these oocytes were always counted less than 10% of total unspawned oocytes. In a partially bred fish these percentage were found considerably high. 3-5% of non spawned oocytes in the spent ovary were found as over matured (initial stage of atresia) oocytes (Fig.1) and named as non-spawned over matured oocytes (NOMO). Rest of the unspawned oocytes were under the category of previtelligenic and vitellogenic primary oocytes (PO). NLMO, NOMO and PO underwent atresia during interspawning development of ovary in the course of multiple breeding. The atretic process of these three categories were initiated since the first day of spawning and completed within two weeks before the subsequent spawning. Unhealthy spent brood with prolonged recovery period from spawning stress showed mostly previtelligenic and vitellogenic ovular atresia in enmasse which was started with ooplasmic vacuolization (Fig.6). The vacuoles were fused together as a space in the ooplasm and then gradually invade the cytoplasmic and nuclear content. Atresia of NLMO started with a perioocytic space between follicular epithelium and oocytes (Fig.2). Then the oocyte shrink and become atretic till it was absorbed in to the system (Fig.4.). NOMO showed characteristics such as undulating outer surface, pearl spot at

**Table 1**: Sampling schedule of brood during the interspawning period for GSI and histology study

<table>
<thead>
<tr>
<th>Inter spawning period</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st &amp; 2nd spawning</td>
<td>3*</td>
<td>2</td>
<td>3*</td>
<td>2</td>
<td>2</td>
<td>3*</td>
<td>*Sampled for GSI</td>
</tr>
<tr>
<td>2nd &amp; 3rd spawning</td>
<td>1</td>
<td>1</td>
<td>3*</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>*2 dermal infection</td>
</tr>
</tbody>
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Fig. 1. Ovary, soon after spawning, showing non-spawned loose matured oocytes (NLMO), non-spawned over matured oocytes (NOMO), previtellogenic oocyte (PO) and empty follicles (EF). Haematoxilin & Eosin. X 75

Fig. 2. NLMO is in the focus. Note the space between the oocyte and follicular layer (arrow). Bromophenol blue. X 150

Fig. 3. Inset of Fig. 1 showing empty follicle after few hours of spawning. Haematoxilin & Eosin. X 400

Fig. 4. Spent ovary after 2nd week. Note NLMO and shrunken oocyte with thick follicular layer. Sudan black-B. X 150

Fig. 5. Oocyte with 'cell pearl' in NOMO after two week of spawning. Sudan black-B X 200
Fig. 6. Spent ovary of 6 week of spawning. Note the previtellogenic oocyte atresia (arrow). Haematoxilin & Eosin. X 150.

Fig. 7. NOMO after 2nd week of spawning. Note the liquifaction of yolk (LY) and wrinkled zona. Haematoxilin & Eosin. X 150

Fig. 8. Atretic follicle after three weeks. Note the yolk colloid (CY) and folded chorion (arrow). Haematoxilin & Eosin. X 150

Fig. 9. Atretic oocytes after four weeks. Note the left out chorion layer without any yolk material. Haematoxilin & Eosin. X 150

Fig. 10. Growth of PO (arrow) and newly recruited oocyte (arrow heads) after three weeks of spawning. Note the remnants of absorbed empty follicles adjacent to these growing oocytes. Bromophenol blue X 100
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One end of oocyte as the initial point (Fig. 5) or disorganization and liquefaction of yolky materials (Fig. 7). NLMO atresia took less time than NOMO atresia to get absorbed to the system. In both the cases, chorion was retained till the end of the absorption (Fig. 8 and 9). The empty follicles, which were recognized as the site of evacuated oocytes underwent hyperplasia and were disintegrated within a week of induced breeding. Mostly the unspawned previtellogenic and early vitellogenic oocytes along with the newly recruited oogonia were found as growing oocytes during inter spawning period and reached the final stage of maturation within six to seven week of the preceding spawning (Fig. 10). The above histological observation and GSI trend of 1st interspawning period were repeated during the 2nd interspawning period.

Oocyte atresia in teleostean ovaries are generally described as a degenerative process in annual ovarian cycle and broadly categorized as previtellogenic and vitellogenic types. Presently oocytes atresia of induced bred *Labeo rohita* has been described within a period 60 days after each spawning through multiple breeding. The PO atresia, which had been described by the earlier workers as uncommon, was found en masse in the spent ovary specially of the fish which persist the dermal infection in the present study. This might be due to low intake of food during post spawning sickness or due to the microbial infection. Srivastav (1979) recorded different types of yolk nuclear atresia, which coincides the present observation of PO atresia, and he described it as poor nourishment of oocytes. Guraya, (1986) reported such type of atresia as due to bacterial infections. Atresia of NOMO and NLMO were also recorded during the routine observation in the inter spawning ovarian development through multiple breeding. Guraya (1993) described that 'after breeding is over the residual eggs are generally eliminated by undergoing atresia'. He also added that 'the atretic yolky eggs seen during the post spawning period are possibly those eggs which actually developed atresia during the prespawning period but failed to ovulate and thus persist in the post spawning ovary'. Presence of over matured eggs in the zero day spent ovary confirms the above statement. Experimental evidences suggested that the follicular atresia of the ovary are also caused either due to lack of proper gonadotropin stimulation or due to inadequate availability of hormones including steroids (Guraya, 1973). As the brood were induced bred by the administration of gonadotropin or gonadotropin releasing hormone, the lack of gonadotropin as a factor for the formation of atresia might not hold good here. Of course the statement could not be fully interpreted in the present study as selected oocytes only undergo atresia and rest of the oocytes developed progressively for subsequent maturity. Bieniarz et al. (1979) while studying the changes in the ovary of adult carp also described that small oocytes developed simultaneously with large atretic oocytes. In the present study NLMO showed shrinkage and distortion of the follicle, whereas zona pellucida was found in folded shape. NOMO showed disorganization, fusion and liquefaction of yolk granules as ooplasmic events and wrinkled zona pellucida. Multiplication of follicular epithelium made this layer thick and in some places it appeared as 'cell-pearl' in the peripheral ooplasm. During the process of reabsorption of yolky ooplasm, retention of chorion was observed till the end of atresia in the
present study as observed by Srivastava (1979). Reduction of GSI during first week of spawning was obviously due to expelling of some more ovulated eggs from the eggs from the ovocoel and degeneration of the empty follicles. Rise of GSI during second week onward was definitely due to growth of considerably large number of oocytees towards maturation.

Acknowledgements
The authors are grateful to Dr. S. Ayyappan, and Dr. R.K. Jana, former Directors, and Dr. N. Sarangi Director, C.I.F.A. for th facilities. Critical suggestions of Dr. P.C. Thomas, Scientist, Emeritus (ICAR) to improve the manuscript is duly acknowledged.

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