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OVARIAN DEVELOPMENT AND REPRODUCTIVE CYCLE OF GARRA GOTYLA GOTYLA

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ABSTRACT
Garra gotyla gotyla is one of the important fish of Jhajjar stream, J&K, India. The present study is aimed to determine the ovarian development and reproductive cycle of this fish. The histological sections of ovaries shows the presence of a large number of oogonia and oocytes in various stages of development, studded along ovigerous lamellae. Based on changes in cytoplasm and its inclusion like vesicles, vitellogenesis, egg membranes and average diameter of oocytes, seven oocyte stages have been recorded viz. Oocyte stage I/ Chromatin nucleolar stage, Oocyte stage II/ Perinucleolar stage, Oocyte stage III/ Early yolk vesicle stage, Oocyte stage IV/ Late yolk vesicle stage, Oocyte stage V/Early yolk stage, Oocyte stage VI/ Late yolk stage and Oocyte stage VII/ Ripe egg stage. Further depending upon the percentage of different developing oocytes and nature of ovarian wall six phases of reproductive cycle has been observed. Immature/ virgin phase (September-October), early maturing phase (November-January), developing phase (February-March), developed phase (April-May), spawning phase (June-July) and spent/ resting phase (August). Gonadosomatic index (GSI) also exhibited remarkable variation during different phases of reproductive cycle and peak was observed during May. Present study therefore suggested that reproductive cycle of G. *gotyla gotyla* follows a cyclic pattern and exhibit remarkable histomorphological changes in ovaries as observed in other teleosts.

INTRODUCTION

Studies on reproductive cycle of fish are of great importance in maintenance of healthy fish population in any water system. Moreover, it has been well established that in most of teleosts the ovaries undergo fairly regular changes. But the variation was observed in different fish species under different environmental conditions. Because gonadal development in fish is affected by environmental conditions *i.e.* temperature, photoperiod, dissolved oxygen, water current etc (Gadekar, 2014). To get a good quality seed, it is desirable to have indepth knowledge on the reproductive biology of fish, so that the exact timing of spawning can be determined. Availability of such information, to a great extent can help a fish farmer to conduct a successful venture induced breeding (Agarwal *et al.*, 2001 and Gogoi *et al.*, 2013).

G. gotyla gotyla is one of the important hill stream fish of J&K, India and in our neighboring country Nepal a major proportion of their population depends upon this for fish protein. Though a lot of work on reproductive cycle of different fishes inhabiting plain as well hilly area of J&K has been done by different workers (Malhotra, 1965; Jyoti, 1972; Gupta, 1980; Singh, 2009 and Vohra, 2011) but there has been no report related to reproductive cycle of *G. gotyla gotyla* so far. Therefore presently an attempt has been made to generate information related to ovarian development and reproductive cycle of *G. gotyla gotyla* from Jhajjar stream, J&K, India.

MATERIALS AND METHODS

Live specimens of *G.gotyla gotyla* were collected monthly from the Jhajjar stream, a tributary of river Tawi for one year. Length and weight of each individual fish was recorded and after that ovaries were carefully removed, excessive moisture was blotted and quickly weighed on an electronic balance. To make permanent slides of ovaries routine methodology was adopted and for that ovaries were firstly fixed in bouin's fixative for 24 hours [freshly prepared from saturated solution of picric acid (75%) to which formalin (20%) and acetic acid (5%) was added at the time of use]. After treatment in bouin's fixative, ovaries were then washed, dehydrated and embedded in paraffin histo wax (54-56°C). 5-7 μm transverse sections of ovaries were cut with the help of microtome and were stained using haematoxylin-Eosin stain. Different phases of gonadal cycle were identified by observing the prepared slides under microscope.

Detailed examination of the histology of the ovaries was attempted by measuring the mean diameter of different stage oocyte by taking the average of the horizontal and vertical diameters of oocyte whereas percentage distribution of different stage oocytes was calculated by studying ovarian microslides at different focal points. Gonadosomatic index (GSI) was calculated by using formula weight of gonads (gms)/ weight of fish (gms) $\times$ 100.

RESULTS AND DISCUSSION

Morphology of Ovaries

The ovaries of *G. gotyla gotyla* are paired (Fig. 1-A and 1-B), sac like structure which lie ventral to kidneys but dorsal to alimentary canal in the body cavity. They are attached to the

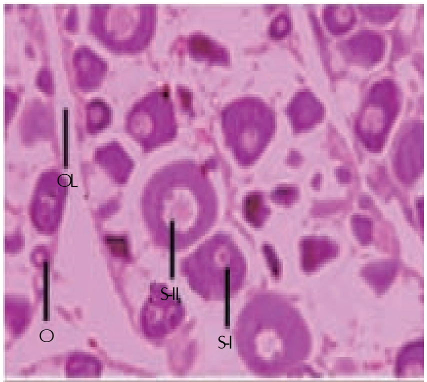

Figure 1: Showing cross section of ovary showing ovierous lamellae (OL) studded with oogonia (O), stage I and Stage II oocytes

Figure 2: showing centripetal invasion of yolk vesicls ii stage III oocyte

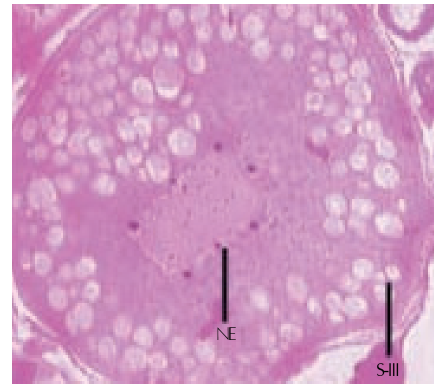

Figure 3: showing nuclear intrusion in stage III oocyte

Figure 4: showing further centripetal invasion of yolk vesicls in stage IV oocyte

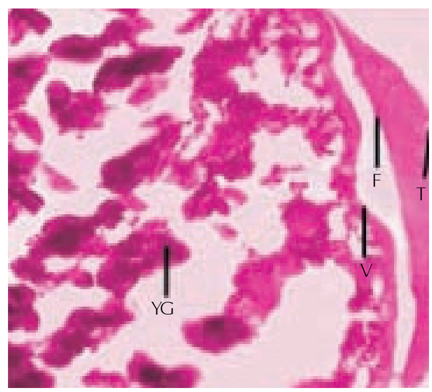

Figure 5: showing Stage V oocyte. Noye the presence ofyolk globules in the ooplasm and three membranes viz. vitelline membrane (VM), follicular membrance (FM) and theca (T)

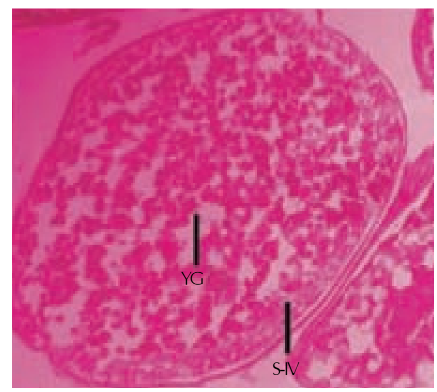

Figure 6: Showing Stage VI oocyte filled with yolk globules

Figure 1A&1B: Showing leaf and right ovary of *G. gotyla gotyla*

body wall by means of a fold of peritoneum, the mesovarium which extends from the dorsal wall to run across the coelom covering the ovaries along their dorsal side as a thin black sheet. The oviduct runs a short distance and after joining the urinary duct, run as an extension of ovarian epithelial covering to open to the exterior through a common urinogenital opening.

The shape, size and colour of ovaries of fish, *G. gotyla gotyla* undergo considerable changes during different stages of maturity. The ovaries usually are transparent and translucent during early stages of maturity but turn opaque and get studded with yolky ova as fish matured (Table 1).

Histology

The wall of ovary is composed of an outer peritoneal membrane overlying tunica albuginea. The innermost layer is the germinal epithelium which projects into the ovocoel in the form of lamellae (Fig. 1). A large number of oogonia and oocytes in various stages of development can be seen studded along ovigerous lamellae (Fig. 1). These oogonia before transforming into an oocyte undergoes various changes pertaining to cytoplasm and its inclusions like vesicles, vitellogenesis and egg membranes. Based on changes an oocyte undergoes during its development into mature ovum, following stages have been identified.

Oocyte stage-I/chromatin nucleolar stage

These are the smallest oocytes observed along the ovigerous lamellae and range in diameter from 0.0231$\pm$.02534mm to 0.0592$\pm$0.01324mm (Table 2 and Fig. 1). They were nearly spherical in form with transparent cytoplasm. The nucleus, which is centrally placed, has a nucleolus (either centric or acentric in position) besides a network of chromatin threads.

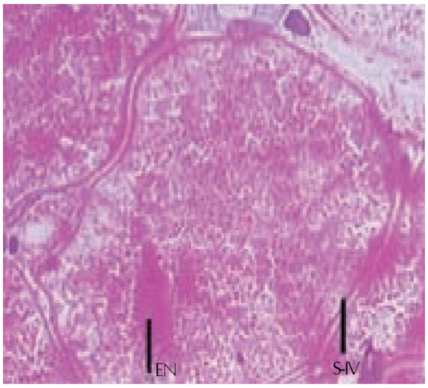

Figure 7: showing stage VII oocyte. Note the presence of ecentric nucleus

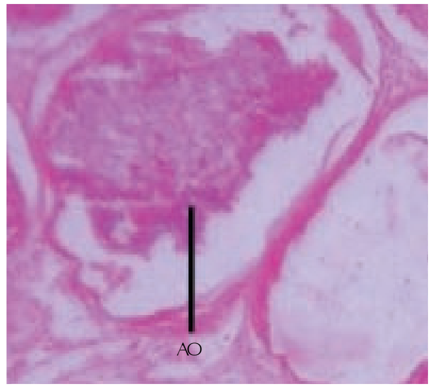

Figure 8: showing atretic ooctye

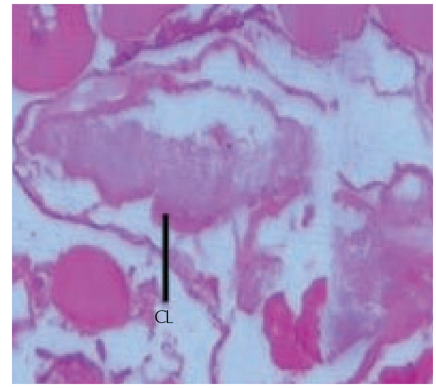

Figure 9: showing corpus luteum

Oocyte stage-II/perinucleolar stage

Oocytes of this stage grow further in size and now vary from 0.0533±0.02310mm to 0.2010±0.01540mm in diameter (Table 2). During this stage nucleus also enlarges proportionally. The nucleoli observe an increase in their number and get distributed adjacent to nuclear membrane (Fig. 1). With the advancement of growth, ooplasm further increases and becomes much greater in volume than nucleus. These oocytes now seem to be surrounded by flattened follicular cells (representing the follicular epithelium).

Oocyte stage-III/early yolk vesicle stage

Oocytes of this stage are characterized mainly by the first appearance of minute vacuoles in the cortical area of cytoplasm, termed as yolk vesicle (Fig. 2). All such oocytes have wavy nuclear membrane in which nucleoli were seen to occupy pockets of waves (Fig. 3). A few oocytes even exhibited the nucleoli piercing through undulating membrane to enter into cytoplasm (Fig. 2 and 3). Follicular layer now is better developed and a thin vitelline membrane internal to follicular epithelium has been noticed in some oocytes of this stage. Diameter of the oocytes of this stage ranged between 0.1150±0.03125mm to 0.3530±0.06425mm (Table 2).

Oocyte stage-IV/late yolk vesicle stage

During this stage, cortical alveoli show random movement/ distribution in the ooplasm that further increases in number and size. The undulation of nuclear membrane becomes clear and more and more nucleoli are seen extruding in ooplasm (Fig. 4). All the oocytes now showed tremendous growth and ranged from 0.2500±0.03520mm to 0.4428±0.02764mm in diameter (Table 2).

Oocyte stage-V/early yolk stage

During this stage yolk granules were seen to be deposited in yolk vesicles. Extra vesicular yolk also has been observed to be deposited in the ooplasm. These extra vesicle granules were observed to form large spherical yolk globules filling almost entire ooplasm. All these oocytes observed an increase in size and now measured from 0.3490±0.01046mm to 0.5210±0.03016mm (in diameter). Nucleus of the oocyte did not observe any increase and rather showed signs of dissolution. An indistinct theca was observed to surround the fully developed follicular epithelium (Fig. 5).

Oocyte stage-VI/late yolk stage

During this stage an extensive deposition of yolk globules is observed which further increases with increase in size of oocyte. Yolk now almost fully occupy whole of ooplasm around the nucleus. Rapid accumulation of yolk globules further results in growth of oocyte. Follicular epithelial layer increase in thickness. The thecal layer surrounding the follicular epithelium becomes more visible and vitelline membrane inner to follicular becomes thicker (Figure 6). Diameter of oocytes of this stage varied from 0.4280±0.02460mm- 0.5910±0.01268mm (Table 2).

Oocyte stage-VII/ripe egg stage

During this stage ovaries were observed to be packed with fully grown yolky oocytes. Nucleus which now becomes indistinct was usually seen to be acentric in position (Figure 7). All these oocytes were observed to be surrounded by well developed egg membranes viz. from inside to outside being represented by vitelline (zona pellucida), follicular epithelium (zona granulosa) and theca. These oocytes ranged in diameter from 0.5710±0.01205mm-0.7500±0.02156mm (Table 2).

Based on the percentage of growing oocytes and nature of ovarian wall on yearly basis, the ovarian cycle has been divided broadly into six phases viz. immature/ virgin phase (September-October), early maturing phase (November-January), developing phase (February-March), developed phase (April-May), spawning phase (June-July) and spent/ resting phase (August) (Tables 1 and 3).

Immature/virgin phase

This phase of reproductive cycle extends from September to October. During this phase, ovaries are thin, translucent, pale and dirty brown in color, with less vascular supply (Table 1). Histologically the ovaries show the presence of ovigerous lamellae (Fig. 1). The ovigerous lamellae are thin and enclose an inner lumen which is filled with oocytes of stage I and stage II. Most of the oocytes (94.8%) are in stage I (chromatin nuclear stage) of development and rest (5.2%) in stage II (perinucleolar stage) (Table 3). GSI was observed to be 2.80±0.16 in the month of September and 0.82±0.24 in the month of October.

Early maturing phase

This phase which extends from November to January, show ovaries to be slightly thicker, opaque and yellowish in color.

Table 1: Shows seasonal changes in degree of maturation, fish length, weight of fish, ovary weight, gonado somatic index, oocyte stage and characteristic features of ovaries

Month	Degree of maturation	Length of fish (cms)	Wt. of fish (gms)	Wt. of ovary (gms)	GSI	Oocyte stage	Characteristic features of ovaries
Nov.	Early maturing	12.5±0.24	27.081±1.20	0.110±0.34	0.40±0.20	I,.II, III	Ovaries become slightly thicker, opaque, yellowish in color, vascularization feeble
Dec.		14.0±0.15	35.576±0.38	1.980±0.14	5.56±0.34	I, II, III	
Jan.		14.4±0.64	38.270±1.06	2.208±0.38	5.76±0.14	I, II, III	
Feb.	Developing	14.6±0.73	41.054±1.46	2.876±0.75	7.0±0.65	IV, V, VI,VII	Ovaries changes into yellow and profuse blood supply.
Mar.		15.4±1.25	48.231±0.87	3.532±0.68	7.53±0.47	IV, V, VI,VII	
Apr.	Developed	15.8±0.38	53.540±0.94	4.312±0.49	8.05±0.54	VI, VII	Ovaries become deep yellow in color, extensive vascularization
May		15.9±0.07	56.046±0.37	5.220±0.38	9.31±0.05	Vi, VII	
Jun.	Spawning	14.5±0.13	42.320±1.46	3.674±0.37	8.68±0.39	VII+ discharged and atretic	Ovaries are yellowish and turgid due to the presence of a large no. of mature ova, vascularization at peak
Jul.		14.1±1.03	38.290±1.59	2.120±0.15	5.53±0.16	VII+ discharged and atretic	
Aug.	Resting	13.5±0.54	33.70±0.58	1.024±0.24	3.03±0.84	Atretic and discharged	Ovaries thin, flaccid, delicate, slender and dull in color, vascularization reduced
Sep.	Immature	13.0±0.65	30.742±0.64	0.862±0.37	2.80±0.16	I, II	Ovaries are thin, transluscent, pale and dirty brown in color with less vascular supply
Oct.		12.9±0.14	28.05±0.44	0.231±0.61	0.82±0.24	I, II	

Table 2: Ova diameter in different maturity stages of oocytes in *Garra gotyla gotyla*.

Maturity Stage	Ova diameter range (mm)
I	0.0231±0.02534-0.0592±0.01324
II	0.0533±0.02310- 0.2010±0.01540
III	0.1150±0.03125-0.3520 ±0.06425
IV	0.2500±0.03520-0.4428±0.02764
V	0.3490±0.01046-0.5210 ±0.03016
VI	0.4280±0.02460-0.5910 ±0.01268
VII	0.5710±0.01205-0.7500 ±0.02156

Vascularization is feeble (Table 1). The ovigerous lamellae are greatly swollen during this phase and laden with oocytes of stage I (64.5%), stage II (28.3%) and rest stage III (7.2%) (Table 3). GSI was noted to be 0.40±0.20, 5.56±0.34 and 5.76±0.14respectively during the month of November, December and January.

Developing phase

This phase extends from February to March. Color of the ovaries during this phase changes to dark yellow. The blood capillaries become inconspicuous because of profuse blood supply (Table). During this phase, the ovaries were observed to be populated by oocytes of stage IV (20.65%), stage V (33.4%) and stage VI (29.45%). Oocytes of stage I and II too are seen but in lesser number (9.1%). Few oocytes of stage VII also make their appearance during this phase (Table 3). The ova are tightly held and the ovary cannot by stripped by applying gentle pressure. An increase in GSI has been recorded during this phase (7.0±0.65 in February and 7.53±0.47 in March).

Developed phase

During this phase which extends from April to May ovaries become deep yellow in color. Vascularization is extensively developed (Table 12). Ovaries now are packed with as high as 85.5% % of stage VII oocytes besides 9.5% stage VI oocytes. Few stage I and II oocytes invariably also can be seen during this phase of ovarian cycle (4.5%) (Table 3). The fish passing through this phase of reproductive cycle have a bulging abdomen (Table 1). Further increment in GSI has been seen in this phase *i.e.* 8.05±0.54 in the month of April and 9.31±0.05 during May.

Spawning phase

This phase begins from early June and last almost till the end of July. During this phase ovaries are yellowish and turgid due to the presence of a large number of mature ova. Ovaries exhibit rich vascularization and called to be in running phase as ova ooze out from the oviduct with slight pressure on the abdomen (Table 1). Histological sections of the ovaries during June and July show oocytes of stage VII (74.5%), 7.2% of stage VI oocytes beside very few stage I and II along with a number of discharged follicles (Table 3). A decrement in the values of GSI has been recorded (8.68±0.39 in June and 5.53±0.16 in July).

Resting/ Spent phase

Ovaries during the month of August appear thin, flaccid, delicate, slender and dull in color. There is decrease in the volume and weight of ovary (Table 1). Vascularization is reduced (Table 1). Histologically, the ovaries show residual

Table 3: Degree of Ovarian maturation and percentile occurrence of different stages of maturity in *Garra gotyla gotyla*.

Stage	Degree of maturation	Months of availability	Percentage of occurrence
I	Immature/Virgin	September-October	Max-I (94.8%) Rest-II (5. 2%)
II	Early maturing	November-January	Max-I (64.5%) & II (28.3%) Rest III (7.2%)
III	Developing	February-March	Max- V (33.4%) & VI (29.45%), 20.65% (IV) and I & II (9.2%) & few VII
IV	Developed/Pre-spawning	April-May	Max-VII (83.5%), Min-VI (9.5%) and few I & II (4.5%)
V	Spawning	June-July	Max-VII (74.5%), VI- (7.2%), few I & II and Discharged and Atretic follicles
VI	Resting adult	August	Atretic and Discharged follicles

oocytes as well as discharged follicles and atretic follicles (Table 1). GSI was found to be 3.03 ± 0.84 during this phase of reproductive cycle (August).

Besides presence of oocytes of different stages viz. stage I, stage II, stage III, stage IV, stage V, stage VI and stage VII, the histological sections of ovaries of *Garra gotyla gotyla* also show the presence of atretic oocytes as well as that of corpus luteum. Atretic oocytes were observed during different phases of reproductive cycle whereas discharges/ corpora lutea were observed only during spawning and post spawning (spent phase) phases of reproductive cycle (Fig. 8 and 9).

DISCUSSION

In females of *G. gotyla gotyla* ovaries are a pair of sac like structure (Figs.1-A and 1-B) that flank an air bladder within their inner margins. Many workers have earlier also reported similar morphology of ovaries in different teleosts like *Salvelinus fontinalis* (Henderson, 1962), *Schizothorax niger* and *Crossochelius latius diplochilus* (Jyoti, 1972), *Channa punctatus* and *Puntius sophore* (Gupta, 1980), *Tor putitora* (Singh, 2009), *Danio devario* (Hina, 2010), *Esomus danricus* and *Rasbora rasbora* (Vohra, 2011) and *Xenentodon cancila* (Subba and Meheta, 2012) but differ from fish like brook lamprey (Okkelberg, 1921), *Botia birdi* (Malhotra, 1965), *Noemachelius kashmiriensis* (Jyoti, 1972) and *Schizothorax niger* (Hajam, 2011) where in the female reproductive system has been observed to consist of a single ovary lying mid ventrally and also from *Trichogaster fasciatus* (Jyoti *et al.*, 1986) whose single ovary is place perpendicular to the genital aperture.

Presently it has been observed that during the different phases of reproductive cycle, the ovaries of fish *G. gotyla gotyla* show considerable change in their shape, size, weight and colour (Table 1). The ovaries were transparent and translucent during early stages of maturity and turned opaque and get studded with ova as they mature. Similar to present findings, Agarwal (1982), Singh (2009), Vohra (2011) and Subba and Meheta (2012) also observed such changes in the ovaries of fish they studied. Histologically, the ovaries of fish *G. gotyla gotyla* under present investigation resemble that of other teleost in being cystovarian type i.e. covered over by an envelope comprising of tunica albugenia and peritoneal epithelium, which at places extends centripetally forming ovigerous lamellae within the ovocoel (Agarwal, 1982; Vohra, 2011 & Gadekar, 2014). Germinal epithelium lying beneath the tunica as well as ovigerous lamellae appears to be the site from where germ cells develop.

Review of literature reveals that mode of origin of new crop of oogonia or germ cells has remained a controversial matter and there are different viewpoints putforth by different workers. Bullough (1939), Mendoza (1943), Dixit (1956), Tromp-Blom (1959), Bara (1960), Jyoti (1972), Malhotra *et al.* (1978), Raina (1999)) postulated that new crop of oogonia originates from the germinal epithelium. On the other hand, according to Wheeler (1924), Yamamoto (1956a, b) and Andrew and Pinto (1957) new crop of oogonia develops from the follicular epithelial cells of spent follicles. Dixit (1956) reported their origin from stromal tissue in *Mystus seenghala*. According to some other authors (Mathews, 1938; Belsare, 1962 and Agarwal, 1982) new crop of oogonia arise from the residual oogonia. In present studies fish *G. gotyla gotyla* is in agreement with those who also have (op.cit) held germinal epithelium and ovigerous lamellae to be the seat of origin of new crop.

An oogonium has a large nucleus and a thin layer of ooplasm. Each oogonium undergoes successive maturation divisions and form new generation of oocytes. Before ripening into a mature ovum, a series of cytonuclear changes take place in the oocyte. Based on these changes, the development of an oocyte has been classified into different stages by various workers (Wood, 1930; Yamamoto, 1956a, b; Srivastava and Rathi, 1970; Jyoti, 1972; Malhotra *et al.*, 1978; Wallace and Selman, 1981; Robb, 1982; Hatikakoty and Biswas, 2004; Agarwal, 2008; Hina, 2010 and Subba and Meheta, 2012).

Presently, based on histological examination and following the pattern of Agarwal (2008) seven developmental stages of oocytes, recognized in the fish *G. gotyla gotyla*. Stage I also known as chromatin nuclear stage have been similarly designated by authors like Yamamoto and Yamazaki (1961), Malhotra *et al.* (1978), Gupta (1980), Wallace and Selman (1981), Agarwal (2008), Singh (2009), Hina (2010) and Vohra (2011). Authors like Chaudhary (1949) and Gupta (1980), however, observed cytoplasmic zonation in oocyte I stage of fishes they studied but presently no such zonation has been observed. Stage II (Perinucleolar stage) shows resemblance to the stage II of Yamamoto and Yamazaki (1961), Malhotra *et al.* (1978), Gupta (1980), Wallace and Selman (1981), Singh (2009), Hina (2010) and Vohra (2011). Many workers (Wheeler, 1924; Chaudhary, 1952; Bara, 1960; Sathyanesan, 1960; Malhotra *et al.*, 1978; Gupta, 1980; Agarwal, 1982 and Singh, 2009) reported the presence of Yolk nucleus of Balbiani in stage II oocytes of various fishes. But presently no such structure has been observed in stage II of *G. gotyla gotyla*.

Phenomenon of nucleolar extrusion which was observed during the Stage III (yolk vesicle stage) has been previously reported by Chaudhary (1951), Jyoti (1972), Gupta (1980), Ali (2003), Agarwal (2008), Singh (2009), Hina (2010) and Vohra (2011). But contrary to it, Nath and Nangia (1931) and Lal (1963) reported the complete absence of nucleolar extrusion in *Rita*, *Ophiocephalus* and *Cirrhinus*. According to Bose and Bose (1964) and Guraya *et al.* (1975) the nucleolar extrusion do not play any direct role in vitellogenesis but others reported that extruded nucleoli play a direct role in vitellogenesis (Chaudhary, 1951; Gupta, 1980 and Singh, 2009) and present author also support the workers who claim their direct role in vitellogenesis.

Kapoor (1977) supporting their role in vitellogenesis held the view that the extruded nucleoli bring some information (probably through messenger RNA) from nucleus into ooplasm. Malhotra (1963, 1965) also supporting their role in vitellogenesis stated that the extruded bodies by the process of disintegration and subsequent action with cytoplasm near the egg periphery bring about the process of yolk formation. Malhotra (1965), Jyoti (1972) and Gupta (1980), however, regarded RNA (acting as messenger) to be the seat of feed back principle, which stimulates the pituitary to release FSH, with which is associated onset of vitellogenesis.

Presently oocytes of stage IV are characterized by appearance of yolk vesicles in peripheral part of ooplasm and their centripetal movement to finally occupy entire ooplasm. During stage V, all the three egg membranes viz. theca, follicular epithelium and vitelline membrane are well developed but get comparatively thicker than the oocyte of previous stage followed by centripetal extension of yolk granules during stage VI and peripheral movement of nucleus during stage VII (Agarwal, 2008 and Subha and Meheta, 2012). Apart from these oocyte stages a large number of corpora lutea were observed during late spawning and spent phase of ovarian cycle. Corpora atretica were however observed during all the different phases of reproductive cycle.

GSI has been used as a model for gonadal development and reproductive efforts in several teleosts (Delahunty and de Vlaming, 1980 and Gadekar, 2014). De Vlaming *et al.* (1972) while discussing the utility of gonadic indices stated it to be a good indicator of reproductive activity of a stock and also mentioned that it can be used as a reliable indicator of spawning period in fishes. The lowest value of GSI (0.40 ± 0.20) was recorded during November and highest (9.31 ± 0.05) during May. After that a decline has been noted in its value. Therefore only one peak is observed for GSI in *G. gotyla gotyla*. Fall in GSI is used as an indicator of spawning period (June and July) and single peak reflects that *G. gotyla gotyla* is a annual breeder.

Thus the overall pattern of oocytes development in *G. gotyla gotyla* is basically the same as in all other teleosts and follows a cyclic pattern. Growth of oocyte which gets initiated in immature phase and extend upto developed phase when they become fully mature and finally start liberating/ oozing during the spawning act. GSI predict that the spawning season extend from June to July.

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