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STUDIES ON THE SEASONAL HISTOMORPHOLOGICAL CHANGES IN THE OVARY OF INDIAN MAJOR CARP, *LABEO ROHITA* (HAM.)

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ABSTRACT

Seasonal changes with reference to histomorphological changes in the oocytes of a fresh water Indian major carp *Labeo rohita* have been described. The gonadosomatic index indicated that, the spawning seasons of *Labeo rohita* was in July and August. The different stages of oogenetic development were examined microscopically. The study revealed a close correspondence among gonadosomatic index, ova diameter and water temperature. It was concluded that five stages of oocytes developments of *Labeo rohita* under study were identified.

INTRODUCTION

The fresh water Indian major carp is a prized food fish of India but it is facing tough competition in Indian water against the exotic fishes. A thorough study of gonad morphology, anatomy and histology is required for proper management of the fishery (Mahmoud, 2009). Knowledge on reproductive biology of fish is essential for evaluating the commercial potentialities of its stock, life history, culture practice and management of its fishery (Doha and Hye, 1970). Moreover the histological studies of the gonads form an initial stage in the attempt to make a fish breed and thus boost the production of the desired species (Malhotra, 1970).Temperature is a major environmental factor affecting the reproductive cycle and spawning fishes (Lam, 1983). Gonadosomatic index (GSI) is used as an important criterion for expression of gonadal development and reproductive effort in fishes (Saxena, 1987).

Labeo rohita do not breed in ordinary perennial tanks, attempts to induce carp breeding by hypophysation have been made. Moreover, the only carp that has been studied in detail with reference to gonadal cycle is *Cirrhinus mrigala* (Lehri, 1968) but other major carps do not seem to have received adequate attention. Therefore, the present study has been undertaken to gain insight into the seasonal changes undergone by the ovaries in *Labeo rohita*. The objective of the present study is to describe the phases of gonadal development and determine the spawning season of Indian major carp, *Labeo rohita*.

MATERIALS AND METHODS

Monthly collections of the fishes were made for one complete year. Length and weight of each individual and ovaries were recorded and gonadosomatic index was calculated by formula: weight of ovary $\times$100/ weight of body. Fixation of ovary was done in Bouin's fluid for 24 hours. Sections were cut ranging from 6-10μ and stained by Delafield's haematoxylin, counterstained by eosin. The diameters of the oocytes were measured by the oculometer standardized against a stage micrometer on random sampling basis.

RESULTS

Histological changes in Ovary

Ovaries of *Labeo rohita* are paired lying in the posterior half of abdominal cavity ventral to the air bladder. The ovaries are covered by an outer peritoneum membrane and an inner ovarian wall. The ovarian wall is distinguished into an outer tunica albuginea and inner germinal epithelium (Fig. 1). The innermost germinal epithelium projects inside the ovarian lumen forming finger shaped ovigerous lamellae (Fig. 2). Each lamella holds ova at different stages of development. All the oocytes in the ovary do not mature at one time.

On the basis of cell and nuclear structure, staining intensity of the cytoplasm and yolk formation, five stages of oocytes are identified. These are immature (type-I and type-II), maturing (type-III), matured (type-IV) and atretic follicles (type-V).

Immature oocytes

They are small in size with large nuclei. They are separated from the germinal epithelium and are known as oogonia. The immature oocytes (type-I and type-II) remain unstained, but later they are stained deeply with haematoxylin. The nucleus

Table 1: Average Oocyte diameter and Gonadosomatic index of the fish during its reproductive phases

Phases	Month	Oocyte diameter in μm	Oocyte diameter mean	Gonadosomatic index	Gonadosomatic mean
Resting(Control)	November	67.82 ± 1.42	70.83±2.65μm	0.69± 0.10	0.74±0.12
	December	66.22 ± 1.91		0.60± 0.12	
	January	82.27 ± 1.90		0.95± 0.16	
Preparatory	February	99.89 ± 0.47	111.9±7.09μm	1.26± 0.05	1.89±0.24 p<0.01
	March	124.0 ± 3.44		2.56± 0.63	
Prespawning	April	313.60 ± 4.73	502.60±44.95μm	3.43± 0.89	10.42±0.79 p<0.01
	May	545.00 ± 2.46		8.83 ±1.66	
	June	649.10 ± 2.69		19.00 ±1.02	
Spawning	July	665.29 ± 5.38	583.80±62.13μm	21.98± 1.80	16.49±1.70 p<0.01
	August	506.00 ± 3.81		11.01 ±1.60	
Postspawning	September	99.29 ± 3.11	94.19±2.84μm	1.30 ± 0.36	3.08±0.34 p<0.01
	October	89.08 ± 2.14		0.87±0.09	

Table 2: Percentage of immature, maturing, matured and atretic oocytes during different phases of the reproductive cycle in *Labeo rohita*

Phases	Immature	Maturing	Matured	Atretic
Resting (Control)	98.44±0.44	1.55 ±0.40	0.00±0.00	0.00±0.00
Preparatory	75.58±0.05NS	24.41±0.05 p<0.01	0.00±0.00	0.00±0.00
Prespawning	22.75±0.16NS	33.14±0.14 p<0.01	43.83±0.44P<0.01	0.00±0.00
Spawning	14.58±0.12NS	0.00±0.00	83.83±0.50P<0.01	1.58±0.09 p<0.05
Postspawning	67.50±0.52NS	7.58±0.02 p<0.01	0.00±0.00	24.01±0.07 p<0.01

Values represent mean ± SE of observation based on data on 48 fishes; NS-Not significant

is large with many prominent nucleoli (Fig. 3). The number of nucleoli increases and are arranged along the inner margin of the nuclear membrane. This stage is the perinucleolar stage and the oocytes are of type-II (Fig. 4). The diameter of immature oocytes ranges from 30 to 330 μm.

Maturing oocytes

They are larger in size with prominent nuclei. During maturation, yolk deposition takes place. At a perinucleolar stage, the yolk nucleus is located close to the nuclear membrane (Fig. 5). However, yolk nucleus migrates to the periphery of the ooplasm during further maturation (Fig. 6).

The maturing oocytes (type-III) are marked by the appearance of yolk vesicles in the peripheral zone of the ooplasm is yolk vesicle sage (Fig. 7). Afterwards they increase in number and size (Fig. 8). The diameter of oocytes at these stage ranges from 90 μm to 630 μm.

Mature oocytes

Mature oocytes (type-IV) are characterized by heavy deposition of yolk and gradual disappearance of nuclear membrane (Fig. 9). The ripe eggs are spherical and full of yolk globules. They are coated with two layers- an outer zona granulosa and an inner zona radiata (Fig. 10). The diameter of mature oocytes ranges from 320 μm to 790 μm.

Atretic oocytes

The ova which fail to attain maturity or fail to spawn undergo resorption and are called as atretic follicles (type-V) (Fig. 11). Atresia occurs frequently in mature oocytes, than in the maturing ones. The process of resorption is more prominent during postspawning period.

In *Labeo rohita* during the formation of atresia, firstly the nucleus disappears. Zona granulose undergo hypertrophy and shows cellular appearance. Zona radiata ruptures at many places which is visible in the form of number of pores (Fig. 12). During atresia, the cytoplasm of the eggs first takes a lumpy and granular appearance and the nucleus shows signs of disintegration. Oval or round vacuoles containing granules are found along the periphery of the oocytes (Fig. 13) and gradually they occupy the whole of the oocytes. They become reduced in size and finally disintegrate.

Seasonal changes in the Ovary

The annual cycle of *Labeo rohita* has been divided into following five phases (Table 1 and Table2)

Resting phase (November to January)

GSI is 0.74 ± 0.12. Ovary is dominated by immature or type-I and II oocytes which constitute 98.44% ± 0.40% of the total oocyte count. The average diameter of these oocytes is 70.83 ± 2.65 μm. These are type-I immature oocytes. Some type-II oocytes are also seen which are larger in size but less in number. In this phase mature oocytes are not observed. However, few maturing oocytes (type-III) are observed and constitute about 1.55 ± 0.40% of the total oocytes. Immature oocytes are seen towards the periphery whereas large maturing oocytes are located towards the centre (Fig. 14).

Preparatory phase: (February to March)

The ovaries increase in weight and size. GSI in preparatory phase is 1.89 ± 0.24. Histologically, ovaries show type-II immature oocytes and type-III maturing oocytes which constitute 75.58 ± 0.05% and 24.41 ± 0.05% respectively. These are at perinucleolar stage. Few maturing oocytes (24.41 ± 0.05%) are also present. Yolk vesicles appear along the periphery in the ooplasm but in this phase matured and degenerating follicles are not observed (Fig. 15).

Prespawning phase (April to June)

GSI suddenly shoots up to 10.42 ± 0.79. In this phase, all

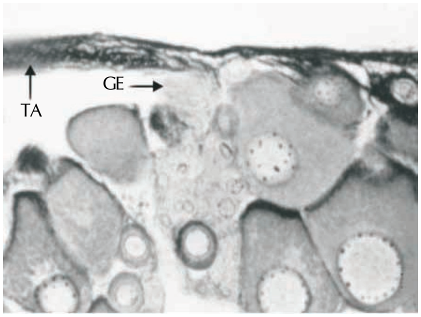

Figure 1: Photomicrograph of section of the ovary showing outer layer of Tunica albuginea (TA) and inner layer of Germinal epithelium (GE) X200.

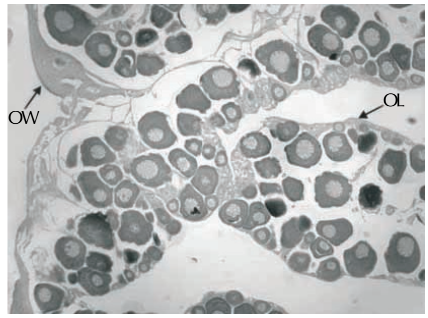

Figure 2: Photomicrograph of section of the ovary showing ovarian wall (OW) along with finger like projection of ovigarious lamelle (OL) X200

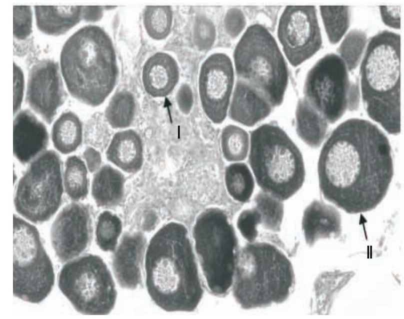

Figure 3: Photomicrograph of section of the ovary showing immature oocytes (type I and II). Type-II oocyte with prominent nucleoli X200

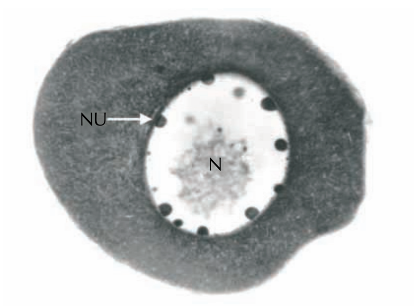

Figure 4: Photomicrograph of section of the immature oocyte (type II) at perinucleolar stage X200

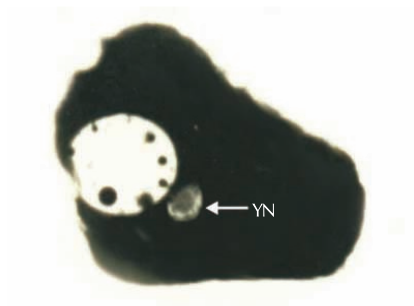

Figure 5: Photomicrograph of section of an oocyte showing the yolk nucleus (YN) close to the nuclear membrane X200

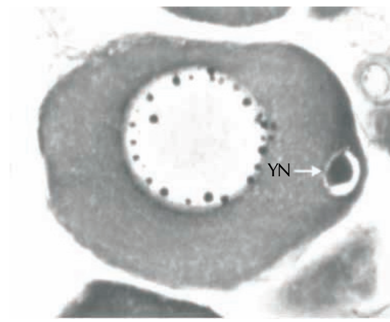

Figure 6: Photomicrograph of section of an oocyte showing the yolk nucleus (YN) towards the periphery X200

types of oocytes are observed except the atretic follicles. Maturing oocytes (type-III) have yolk vesicles near the periphery. Histologically, immature (type-I and II), type-II and type-III oocytes are observed which constitute about 22.75 $\pm$ 0.16%, 33.41 $\pm$ 0.14% and 43.48 $\pm$ 0.44% respectively. The average oocyte diameter of type-III is 502.60 $\pm$ 44.95 μm. There is reduction in the interfollicular space because oocytes increase in size due to yolk formation. Some type-I and type-II oocytes are also located in the peripheral area of the ovary (Fig. 16).

Spawning phase (July to August)

Ovaries are large, fill the entire peritoneal cavity and contain fully matured oocytes ladden with yolk. GSI becomes 16.49 $\pm$ 1.70. During this phase the ovaries are predominated by mature oocytes (type-IV). Eggs are present in the oviduct and fish spawns number of times during this period. The ova can be extruded by applying a pressure on the abdomen. Histologically, type-IV, type-V and some immature (type-I and type-II) oocytes are observed which constitute 83.83 $\pm$ 0.50%, 1.58 $\pm$ 0.09% and 14.58 $\pm$ 0.12% respectively. The average diameter of type-IV oocytes is 583.80 $\pm$ 62.13 μm. However, few oocytes at perinucleolar and yolk vesicle stage are present in the peripheral area of the ovary. Type-IV oocytes are characterized by the presence of yolk in the form of granules in the ooplasm. Some atretic follicles (type-V) are also visible in this phase (Fig. 17).

Postspawning phase: (September to October)

A sharp decline in the GSI is obtained in this phase which is 3.08 $\pm$ 0.34. Histologically, ovary shows atretic or discharged follicles (type-V), immature and some maturing oocytes. The oocytes are surrounded by follicular cells. The granulosa cells are responsible for deposition of yolk in developing ovum and also for its removal in ova which undergoes degeneration and become atretic follicles. Vitelline membrane is wavy, looses contact with granulosa cells and broken at some places. Yolk shows liquification and has fine granular appearance. Vitelline membrane is collapsed at certain places and the cells form small ball. As the yolk is consumed, the follicular cells collapse, shrink and disappear. These cells digest the yolk by phagocytosis. The percentage of immature, maturing and atretic follicles is 67.50 $\pm$ 0.52%, 7.58 $\pm$ 0.02% and 24.01 $\pm$ 0.07% respectively (Fig. 18).

DISCUSSION

The ovary of *Labeo rohita* is of cystovarian type because the lumen of ovary is continuous with oviduct as in *Clarias batrachus* (Lehri, 1968). Yamamoto (1956) has stated that the new oocytes are produced by the follicular epithelial cells, while Tromp- Blom (1959) and Khanna and Pant (1967) suggest origin of oocytes from the germinal epithelium. In *Labeo rohita* oocytes is adult developed from the germinal epithelium of ovigerous lamellae. During resting phase, the ovary is dominated by the immature oocytes. These are smaller in diameter (70.83 $\pm$ 2.65 μm) with darkly stained ooplasm and large nuclei are known as type I oocytes. GSI (0.74 $\pm$ 0.12) is lowest during this period.

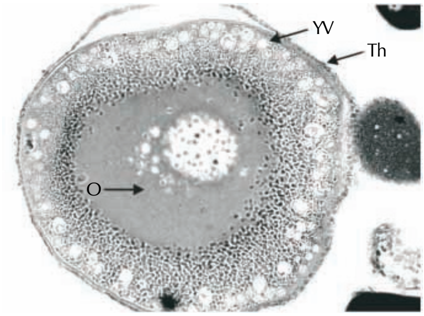

Figure 7: Photomicrograph of section of an oocyte showing yolk vesicle (YV) in the peripheral area with outer layer of theca (Th) and centrally placed ooplasm (O) X200

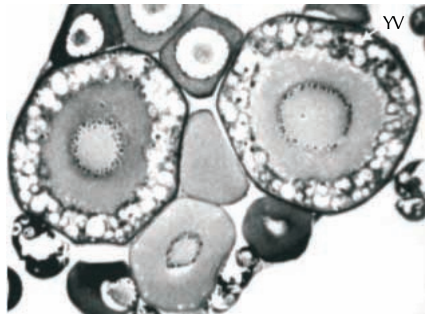

Figure 8: Photomicrograph of section of an oocyte showing an increase in size and number of yolk vesicle (YV) at peripheral region of the oocytes X200

Figure 9: Photomicrograph of section of ovary showing yolk (Y) deposition in the maturing oocyte along with presence of deeply stained nucleus (N) X200

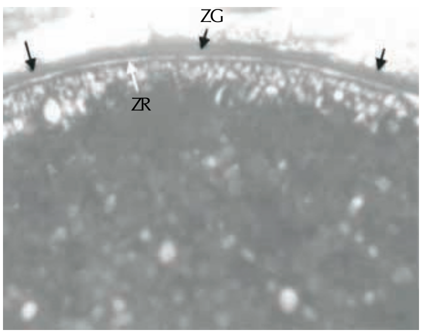

Figure 10: Photomicrograph of section of the matured oocyte (type IV) showing outer zona granulose (arrow) and inner zona radiate (arrow) X200

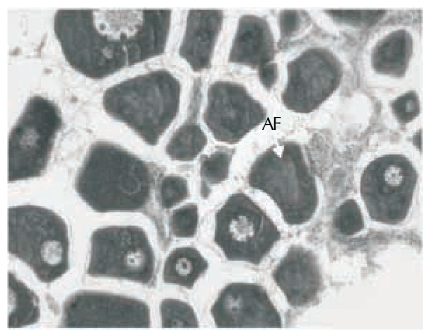

Figure 11: Photomicrograph of section of the ovary showing the atretic follicle (AF, type-V) X200

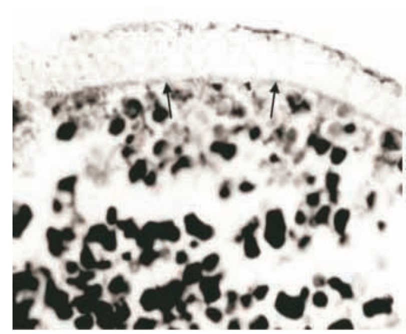

Figure 12: Photomicrograph of section of an oocyte showing pores (arrow) of ruptured zona radiate X200

James (1946) and Cooper (1952) have suggested the projection of ovigerous lamellae from the tunica albuginea of connective tissues. In *Labeo rohita* it has been observed that during preparatory phase there is a gradual increase in GSI (1.89 $\pm$ 0.24) as maturation proceeds and new oogonia grow to become oocytes at different stages. The oocytes are held in ovigerous lamellae which protrude in the cavity of the ovary. Large numbers of oocytes are yolkless; some of them belong to perinucleolar stage (type-II oocytes) where nuclei move to the periphery of the nucleus. This phase of growth did not bring any marked influence on ovarian weight in *Labeo rohita*. The oocytes of *Labeo rohita* show a period of growth from preparatory to prespawning although this growth is steady during resting phase (Table 1), as the oocytes during this phase are in primary growth phase during which only cytoplasmic growth takes place, this cytoplasmic growth thus does not result in much increase in diameter of oocytes or in gonadosomatic index of the ovary of fish.

During preparatory phase, a sharp increase in diameter of oocytes and gonadosomatic index is first observed when considerable quantity of yolk is added within the oocytes. In *Labeo rohita*, the ovaries are dominated by oocytes at perinucleor stage (type-II oocytes) with large nuclei and many nucleoli of various sizes. The nucleoli play an important role in vitellogenesis (Malhotra, 1963) but extruded nucleoli do not take part in the process of yolk formation (Chaudhary, 1951). In *Labeo rohita* many nucleoli of various sizes are seen in the oocytes which are at early perinucleolar stage. The size of nuclei decreases with developing stages of the oocytes. The growth of previtellogenic oocytes is characterized by increase in the size of nucleus or germinal vesicle, increase in number and size of nucleoli, formation of acellular zona pellucida between oocytes surface and single layered follicular epithelium or granulose and vitellogenic oocytes are characterized by formation of cortical alveoli and yolk (Guraya, 1993). In *Labeo rohita* granulose layer is distinctly visible in type-IV oocytes and cortical alveoli are apparent in type-III oocytes.

During prespawning phase, *Labeo rohita* shows rapid increase in the GSI (10.42 $\pm$ 0.79). The growth during this phase is mainly due to formation of yolk vesicles and deposition of yolk. Such changes in the prespawning phase have been reported in the ovaries of several teleostean species (Burton and Idler, 1984). However, the yolk nucleus has been considered as a mass of lipid beside the nucleus, which later on detaches from the nucleus and migrates towards the periphery of the oocytes (Nayyar, 1964). In *Labeo rohita* during this phase, oocytes proliferate and all types of oocytes are visible except the matured ones. Yolk vesicles appear in type-III oocytes. Guraya (1986) has described vitellogenic oocytes by the formation of cortical alveoli and yolk where yolk consists of protein yolk bodies and fatty yolk globules.

In *Labeo rohita*, the yolk nuclei initially arise in vicinity of the nuclear membrane in young oocytes, but later on migrate towards the periphery of the ooplasm. This peripheral migration of yolk nucleus may be associated with the processes of yolk

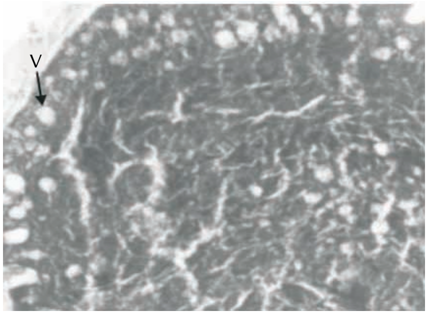

Figure 13: Photomicrograph of section of an oocyte having rounded vacuoles (arrow) containing granular product along its periphery X200

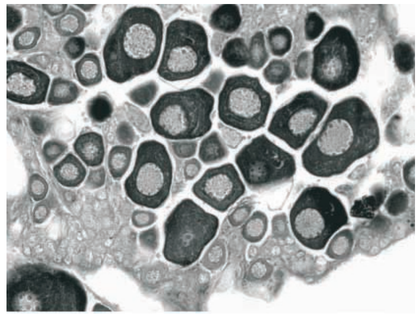

Figure 14: Photomicrograph of section of the ovary showing a large number of immature (type I and II) oocytes during Resting phase X200

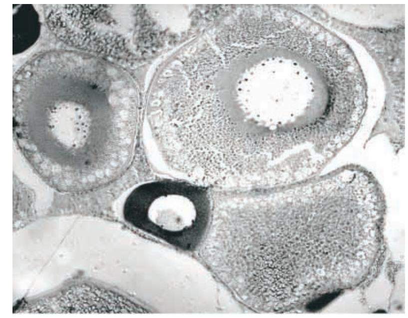

Figure 15: Photomicrograph of section of the ovary showing yolk vesicle stage (type-III) of oocyte during the Preparatory phase X200

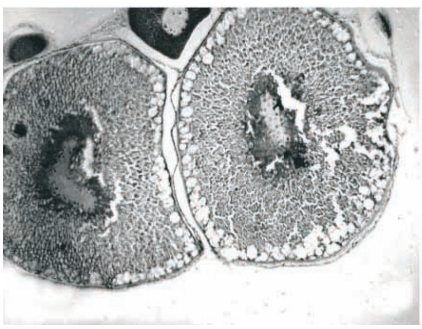

Figure 16: Photomicrograph of section of the ovary showing maturing oocytes (type-III) during the Prespawning phaseX200

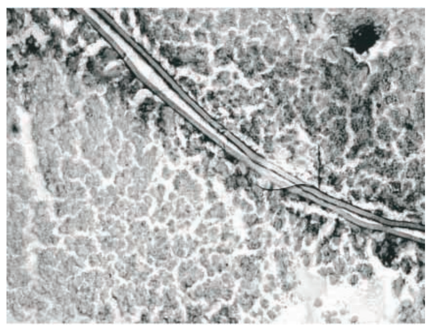

Figure 17: Photomicrograph of section of the ovary showing matured oocytes (type-IV) during the Spawning phase X200

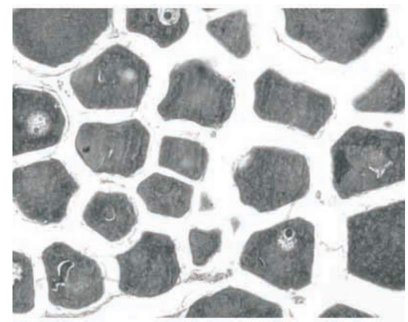

Figure 18: Photomicrograph of section of the ovary showing atretic follicle (type-V) during the Postspawning phase X200

formation. As the oocytes mature, their basophilia increases and they acquire a vitelline membrane and follicular layer (Brackevelt and McMillan, 1967). The vitelline membrane is also called as zona radiate (Lehri,1968) and zona pellucid (Wiebe, 1968). In *Labeo rohita* an outer layer of zona granulosa and an inner layer of zona radiate becomes distinct in type-IV oocytes.

In the spawning phase, GSI of *Labeo rohita* attains a maximum peak (16.49 $\pm$ 1.70). The ovaries during spawning phase are filled with yolk laden oocytes (type-IV oocytes) which become so large that interfollicular space is obliterated and septa are stretched to their fullest capacity. Very few immature oocytes are also visible along the peripheral region of the ovary. Towards end of this phase the ovary decreases in weight not only due to ovulation or discharge of the eggs, but also due to degeneration of oocytes which is referred to as atresia. Similar condition is also reported in many other teleost species such *Ophicephalus punctatus* (Belsare, 1962) *Ictalurus punctatus* (Eleftherion *et al*., 1966) *Heteropneustes fossilis* (Vishwanathan and Sundararaja, 1974).

The resorption of oocytes involves rupturing of zona pellucida, hypertrophy of the cytoplasm and its contents and breakdown of muscular theca (Rastogi, 1966). In *Labeo rohita* atresia is characterized by hypertrophy of the granulosa cells or by granulosa and thecal cells. Follicular atresia (type-V oocyte) in the fish ovary is of common occurrence during the prespawning, spawning and postspawning periods (Saidapur, 1978) as evidenced by the presence of degenerating mature yolky eggs in the ovaries. In *Labeo rohita* such follicular atresia (type-V oocyte) is noticed in prespawning only and on a large scale in postspawning phases. During this phase GSI also goes down (3.08 $\pm$ 0.34). Mature vitellogenic eggs in the ovaries are affected by atresia mostly during and after spawning in snowtrout *Schizothorax plagiostomus* (Agrawal and Singh, 1990) and in *Eucalia inconstans* (Brackevelt and McMillan, 1967). According to Guraya (1993) these eggs must have developed atresia during the prespawning period but failed to ovulate and thus continued to persist in the postspawning ovaries. All these features are observed in atretic follicles of *Labeo rohita* in postspawning seasons.

Results of the present study hopefully would contribute knowledge to the research on the process of the oogenesis of the *Labeo rohita*. It spawn only once in a year in the month of July and August with highest gonadosomtic index.

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