

Research Article

Histological organization and surface ultrastructure of ovaries of *Gudusia chapra* during different phases of reproduction (Teleostei: Clupeidae)

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Abstract: Cytological status of ovarian activities in *Gudusia chapra* (Hamilton, 1822) were studied during different phases of reproduction. An asynchronous development was observed in the ovaries. Variations in the ovary weight and gonadosomatic index (GSI) were also observed. Diameter of oocytes were investigated during growth, maturation and spawning phases. It was further revealed that peak of spawning period was during March to April, with a second spawning time in July.

Keywords: Histology, Ovary, Gonadosomatic index, Growth, Maturation, Spawning.

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Introduction

A thorough knowledge on the reproductive process is an essential part for successful culture and mobilization of seed resources of the cultivable freshwater fish. Accordingly, an understanding of the gonadal development and reproductive cycles of the cultivable fishes is necessary for successful management and increase in production of these fishes.

Various phases of gonadal development of fishes have been studied to clarify the dynamics and regulation of oogenesis (Polts & Wootton 1984). Most of the teleost are seasonal breeders and ovaries show marked variations in shape, size and volume and pattern of oocyte development. Some teleosts exhibit asynchronous ovary containing oocytes of all stages of development which breed many times during their breeding season

(de Vlaming 1980; Hilwa et al. 2011). It is known that the ovarian cycle in majority of freshwater teleosts which are seasonal breeders undergo remarkable changes during various periods of the season (Srivastava & Srivastava 1984; Manna & Bhattacharya 1993; Singh et al. 2008; Yin et al. 2012; Chakrabarti & Hazra chowdhury 2014).

Histological studies offer the scope to understand of cellular kinetic of gonad, recruitment, development and reabsorption of gonadal cells and finally in staging the maturity state of the gonads. Furthermore, studies of the surface ultrastructure of female germ cells in comparison with histology bring out more information regarding the ovarian development. However, ultrastructural studies of egg surface in teleost are limited (Lonning & Davenport 1980; Mooi 1990; Breining &

Britz 2000; Lee et al. 2008).

Gudusia chapra is very much popular as food fish due to its palatable qualities and a crucial source of micronutrients essential in preventing malnutrition in rural communities and some information on the biology of this clupeid fish is available. It has been showed that the algae constituted the major food item in the stomach of *G. chapra* followed by crustacean, protozoan and insects (Rahmatullah et al. 1995; Mondal & Kaviraj 2010; Phukan et al. 2012). Afroz (1998) indicated that *G. chapra* breeds twice in a year, first during March to April and the second during August to September. But, detailed information on its reproductive biology has not been provided.

Therefore, the objectives of the present study were to examine female germ cells at various stages of development in the ovary of *Gudusia chapra* (Clupeiformes: Clupeidae) based on detailed histological findings and scanning electron microscopy (SEM).

Materials and Methods

Adult female specimens of *G. chapra* (length 9.0-15.5 cm and weight 10.30 to 35.5 g) were procured from particular area of Panchet reservoir, Dhanbad district, Bihar during the second week of every month from January to November, 2015. Soon after the collection the ovaries were dissected out from each fish. Data on the total body weight and ovarian weight of ten fishes were taken to calculate the mean Gonadosomatic index (GSI) using the following formula:

$$\text{GSI} = \frac{\text{Total ovary weight}}{\text{Body weight} - \text{Weight of the ovaries}} \times 100$$

Histological methods. After decapitation of the fish following the guidelines given by the Institutional Ethical Committee the ovaries were removed carefully and fragments of ovaries were fixed in aqueous Bouin's fluid for 18 h. The fixed ovaries were washed in 70% ethanol, dehydrated in ascending series of ethanol, then cleared in benzene and

embedded in paraffin wax (56-58°C melting point). Sections of 4µm thickness were stained with Delafield's haematoxylin-eosin and Mallory's triple stain. From the histological preparations of the ovaries, the diameter of various oogenetic cells and their nuclei were measured with the help of reticulo-micrometer and ocular-micrometer respectively.

Scanning Electron Microscopical (SEM) preparation. The ovarian tissues were perfused with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) initially for 15 min. The adhering blood, fat on the surface was removed by 0.5% Tween 40 solution. After being rinsed in 0.1 M cacodylate buffer (pH 7.4), the tissues were infiltrated with 2.5% glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.4) for 24 hr at 4°C. After proper fixation, the tissues were rinsed in the same buffer and post fixed in 1% osmium tetroxide in 0.1M cacodylate buffer at room temperature. The tissues were rinsed with cacodylate buffer, dehydrated through ascending series of acetone, followed by isoamyl acetate and subjected to critical point drying method. The ovarian tissues were mounted on metal stubs, coated with gold-palladium and scanned in Hitachi, S-530 SEM.

Results

Gonadosomatic index (GSI). In the present study it has been observed that the values of GSI in *G. chapra* follow two peaks during April and subsequently in July. However, during the onset of growth phase in January increment of GSI started and recorded to be 2.5±0.32. During the period of first maturation phase i.e. in February and early March the GSI value increased gradually from 3.8±0.74 to 5.2±0.13. Subsequently from late March onwards when the ovary entered into the spawning phase, GSI gradually increased from 6.0±0.20 to a peak value 6.80±1.15. Again in May and June i.e. during second maturation phase the GSI value showed a declining trend (4.77±0.25 to 5.90±0.4). During second phase

of spawning in July, ovary was full of mature follicles and the GSI was 6.5 ± 0.015 followed by 4.26 ± 0.09 in August (Table 1).

Table 1. Variations in the GSI of female *Gudusia chapra*.

Maturity stages of the ovary	Months	Mean GSI \pm SE
Growth phase	January	2.5 ± 0.32
1 st Maturation phase	February	3.8 ± 0.74
1 st Spawning phase	Late March	6.0 ± 0.20
2 nd Maturation phase	May	4.77 ± 0.25
2 nd Spawning phase	July	6.5 ± 0.15

Histology and Scanning electron microscopy (SEM). On the basis of nucleocytoplasmic ratio and the mean oocyte diameter for each developmental stage, the sequence of oocyte maturation in *G. chapra* was divided into five distinct developmental stages viz. Oogonia (Stage I), perinucleolus stage (Stage II), cortical alveolus or early maturing oocyte (Stage III), yolk vesicle oocytes or late

maturing oocytes (Stage IV) and mature follicles (Stage V).

Oogonia (Stage I): An oogonium was made up of well-defined spherical nucleus with chromatin threads and sometimes contained two or three nucleoli (Fig. 1). The diameters ranging from 10×14 to $19 \times 23 \mu\text{m}$. Under SEM views nests of oogonia were found to be adhered with peritoneal surface of the ovary (Figs. 3, 4).

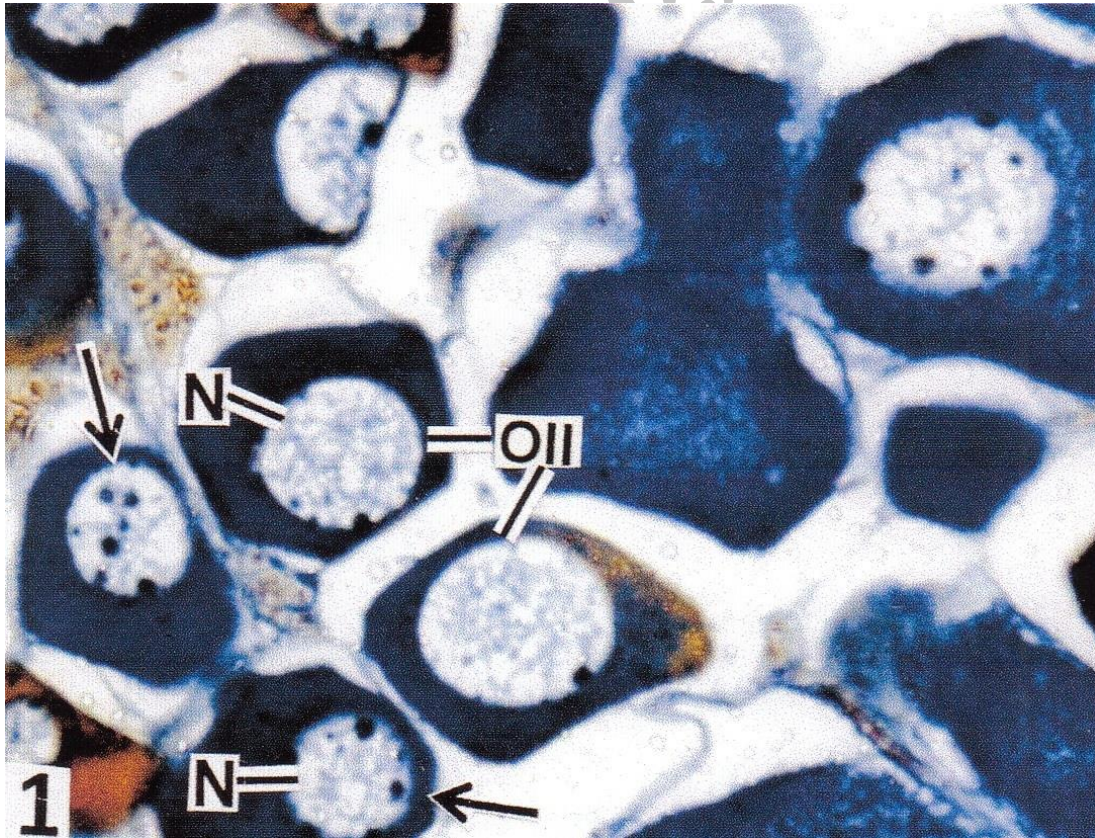


Fig. 1. Growth phase showing early perinuclear oocytes (OI) (arrows) and oocytes II (OII) having prominent nucleus (N) (MT) $\times 400$.

Perinucleolar oocyte (Stage II). Oocyte size increased and was more voluminous with basophilic cytoplasm with a diameter ranging from 28×34 to $40 \times 48 \mu\text{m}$. The centrally placed nucleus ranging from 14 to 16 μm and 114 the nucleoli increased to 10 to 15 in

number (Figs. 1, 2). Under SEM observation the follicles were anchored in the loose stromal connective tissue. Vascular elements of the follicles could be seen on the apical hemisphere of the oocytes (Figs. 3, 4).

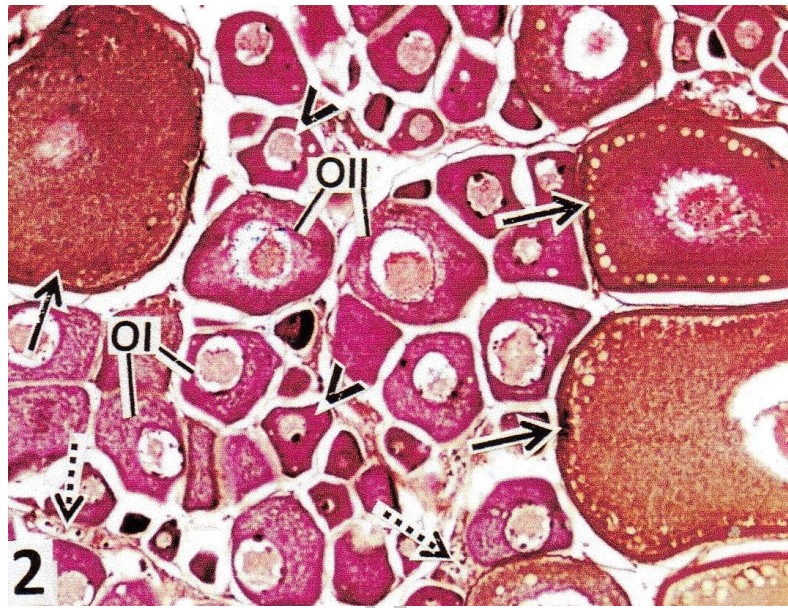


Fig. 2. Late perinucleolar oocytes (solid arrows) along with early perinucleolar oocytes (OI) and OII during the end of the growth phase. Note the presence of blood vessels (BV) (broken arrow) in between oocytes. Arrow heads indicate oogonia (H & E) $\times 400$.

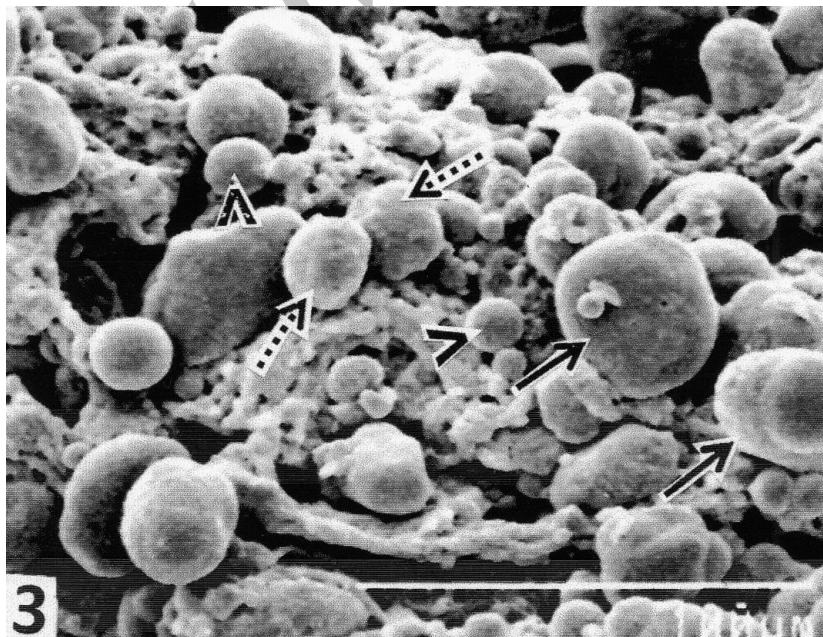


Fig. 3. Oogonial cells (arrow heads), OI (broken arrows) and OII (solid arrows) during growth phase (SEM) $\times 1000$.

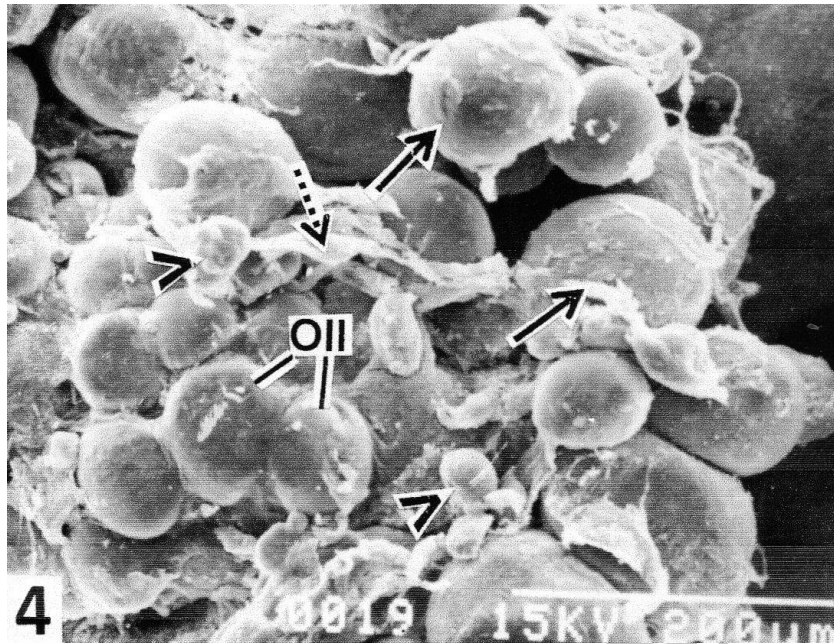


Fig. 4. Oogonial cells (arrow heads), OI (broken arrows) and OII (solid arrows) during the end of growth phase. Broken arrow indicates blood vessels adjacent to oocytes (SEM) $\times 400$.

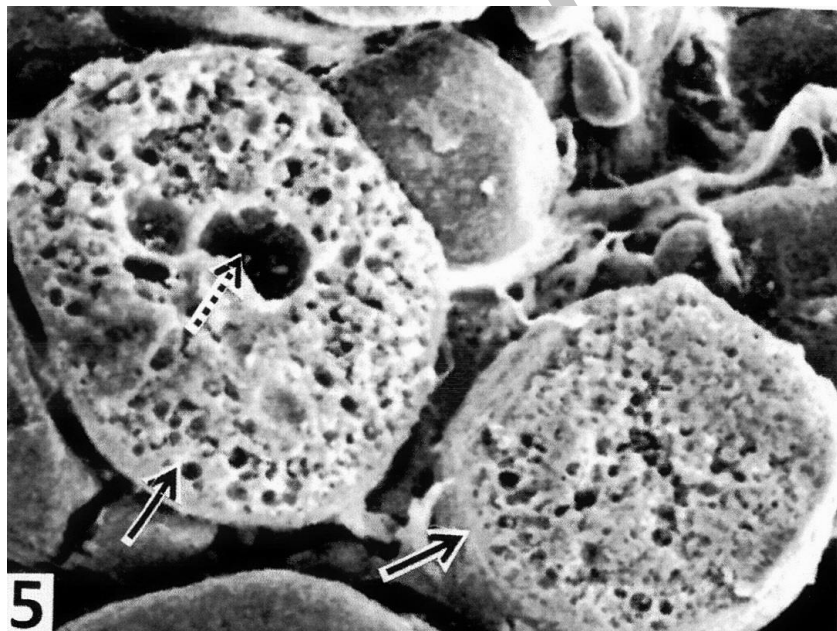


Fig. 5. Showing cut surface of oocytes V during maturation phase having prominent germinal vesicle (broken arrow) and germinal layers (solid arrows) (SEM) $\times 1000$.

Cortical alveolus stage or early maturing oocytes (Stage III): This stage was characterized by the appearance of cortical alveoli along the peripheral region of the ooplasm. The diameter ranging from 88×102 to $90 \times 110 \mu\text{m}$. The cytoplasm gradually lost its basophil. Nucleus was large and spherical

having condensed chromatin materials. A thin follicular layer enclosing the zona granulosa was also appearing in this stage (Fig. 2).

Yolk vesicles or late maturing oocyte (Stage IV). The oocytes at this stage increased in size and cortical alveoli occupied the entire ooplasm. The diameter of oocytes ranged from

145 × 170 to 180 × 220 μm. The thickness of theca, middle multinucleated zona granulosa and zona radiata increased gradually. Most of the vesicles were empty but some of them were filled with homogeneous material (Figs. 6, 7). Under SEM observation most of the yolk vesicles were found encircling the germinal vesicle (Fig. 5). The germinal layers

were also evidenced (Fig. 5). The outer surface of theca provided with various diameter of pores measuring about 0.2 to 0.3 μm in diameter (Fig. 9). The well developed granulosa cells are lined over zig-zag zona radiata which are attached with yolk granules (Fig.10).

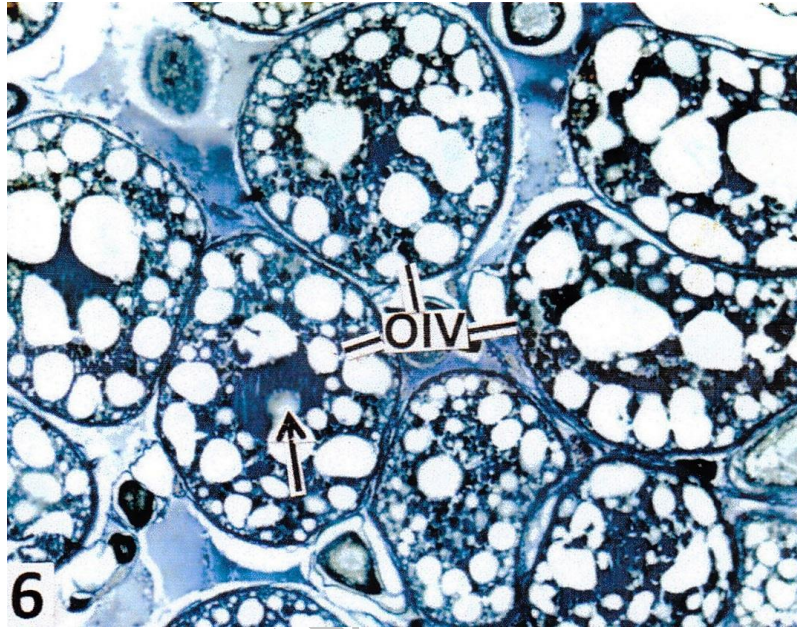


Fig. 6. Maturation phase showing oocyte IV (OIV) stage with yolk vesicles and prominent germinal vesicle (arrow) (MT) × 400.

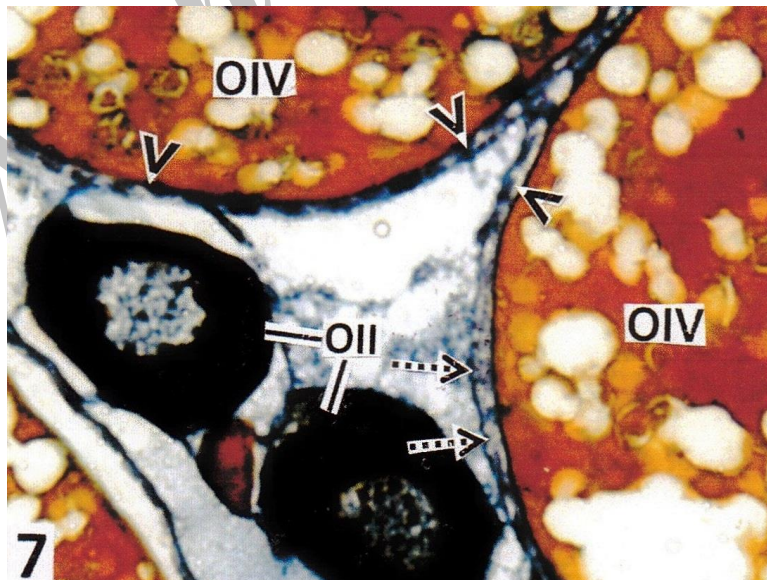


Fig. 7. End of the maturation phase showing oocytes IV with yolk granules (YG). Note theca (broken arrows), zona granulosa (ZG) cells (arrow heads) and thin zona radiate of oocytes V. Note OII in between oocytes V (MT) × 600.

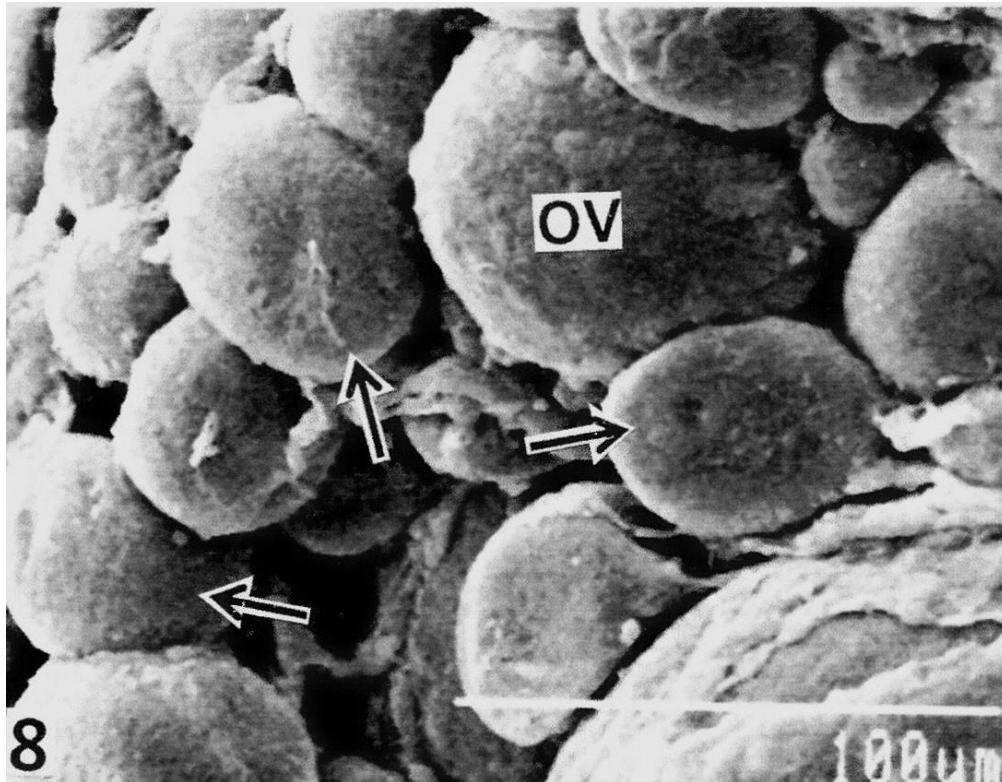


Fig. 8. Different dimensions of oocyte V (OV) and oocytes IV (arrows) (SEM) $\times 100$.

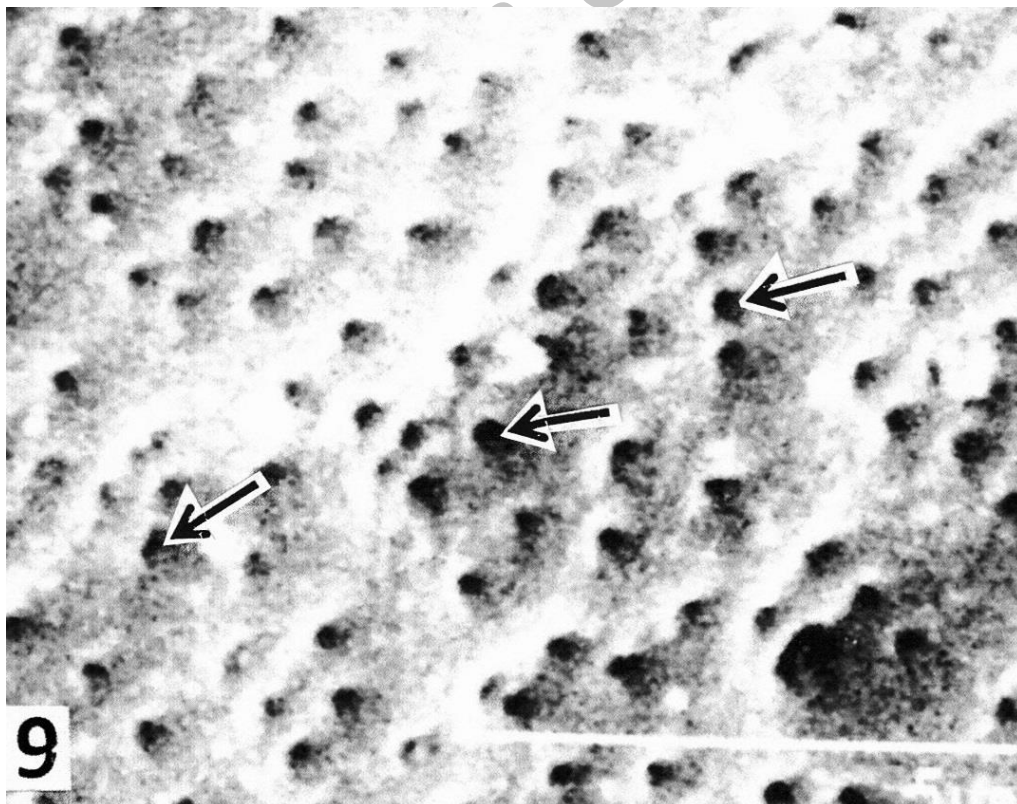


Fig. 9. Outer surface of mature follicle during maturation phase showing various dimensions of pores (SEM) $\times 6000$.

Mature follicle (Stage V). Ova at this stage were more or less spherical or elongated in shape and attained an average diameter of 300×350 to $380 \times 450 \mu\text{m}$. Yolk granules occupied the entire oocyte and the nucleus has disintegrated and was found to be eccentric in position with irregular outline (Fig. 11). The thickness of the theca, zona granulosa and zona radiata reduced considerably. Under

SEM views mature follicles were found to be adhered in between different sizes of oocyte IV (Fig. 8). Each mature follicle was provided with depressed micropyle (Fig. 13). The cut surface of oocyte V stage also provided with fused yolk granules. The germinal vesicle was clearly discernible among the yolk granules (Fig. 12).

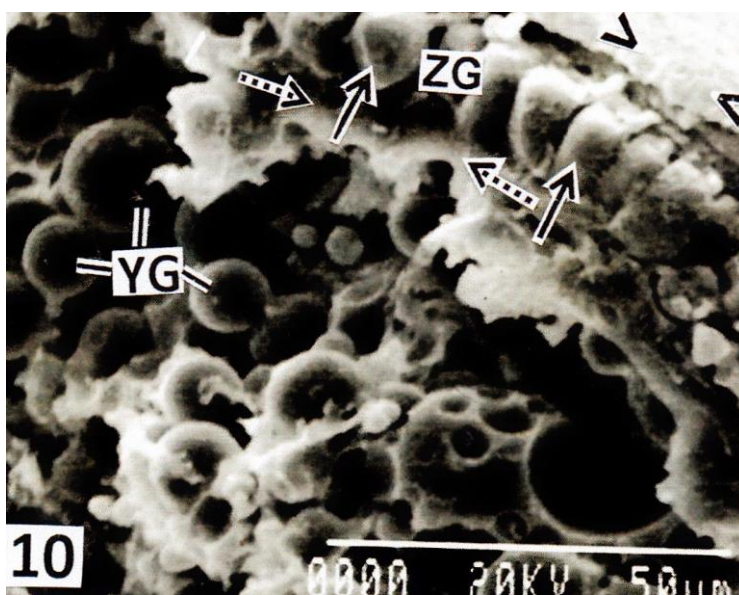


Fig. 10. Cut surface of mature oocyte during end of maturation phase showing outer theca (arrow heads), middle zona granulosa (ZG) cells (solid arrows) and inner zona radiata (broken arrows). Note prominent yolk granules (YG) within ooplasm (SEM) $\times 6000$.

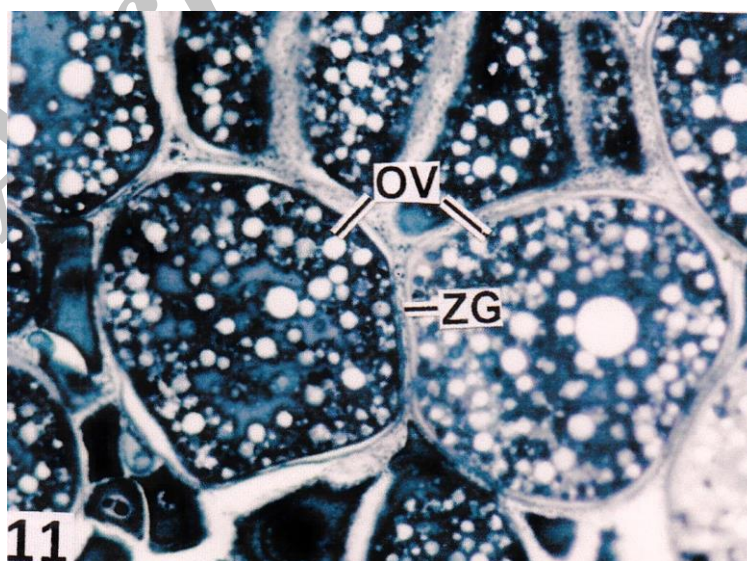


Fig. 11. Mature follicles with compact yolk granules (YG) and eccentric germinal vesicle (solid arrow). Arrow heads indicate zona radiata (MT) $\times 600$.

Sequential changes in relation to oogenesis during growth, maturation and spawning phases:

Growth phase (January). Primary oocytes at all stages were present in the ovary. Dominant cell types in this period were the early perinucleolar oocytes. However, the percentage of late perinucleolar oocytes increased at the end of this period which showed cortical alveoli (Figs. 1-4).

Maturation phase (February to early March and May to June). The highest oogenetic activity had been found to occur during this phase when cytoplasmic materials increased and vitellogenesis started in majority of the oocytes. However, majority of the oocytes were in stage IV (Figs. 5-8). Prominent

follicle granulosa cells and zona radiata were present (Fig. 10). From early March, May and June the yolk granules continued to coalesce. Subsequently with the advancement of maturity, the immature oocytes were decreased in number.

Spawning phase (Late March to April and July to August). The predominant cell types in this period were the mature follicles of stage V but a few resting primary oocytes were common (Fig. 11). The mature oocytes became larger, the yolk globules broke up and follicles were provided with eccentric germinal vesicle. At the end of August, the relative abundance of mature follicles was reduced though discharged follicles are seen (Fig. 12).

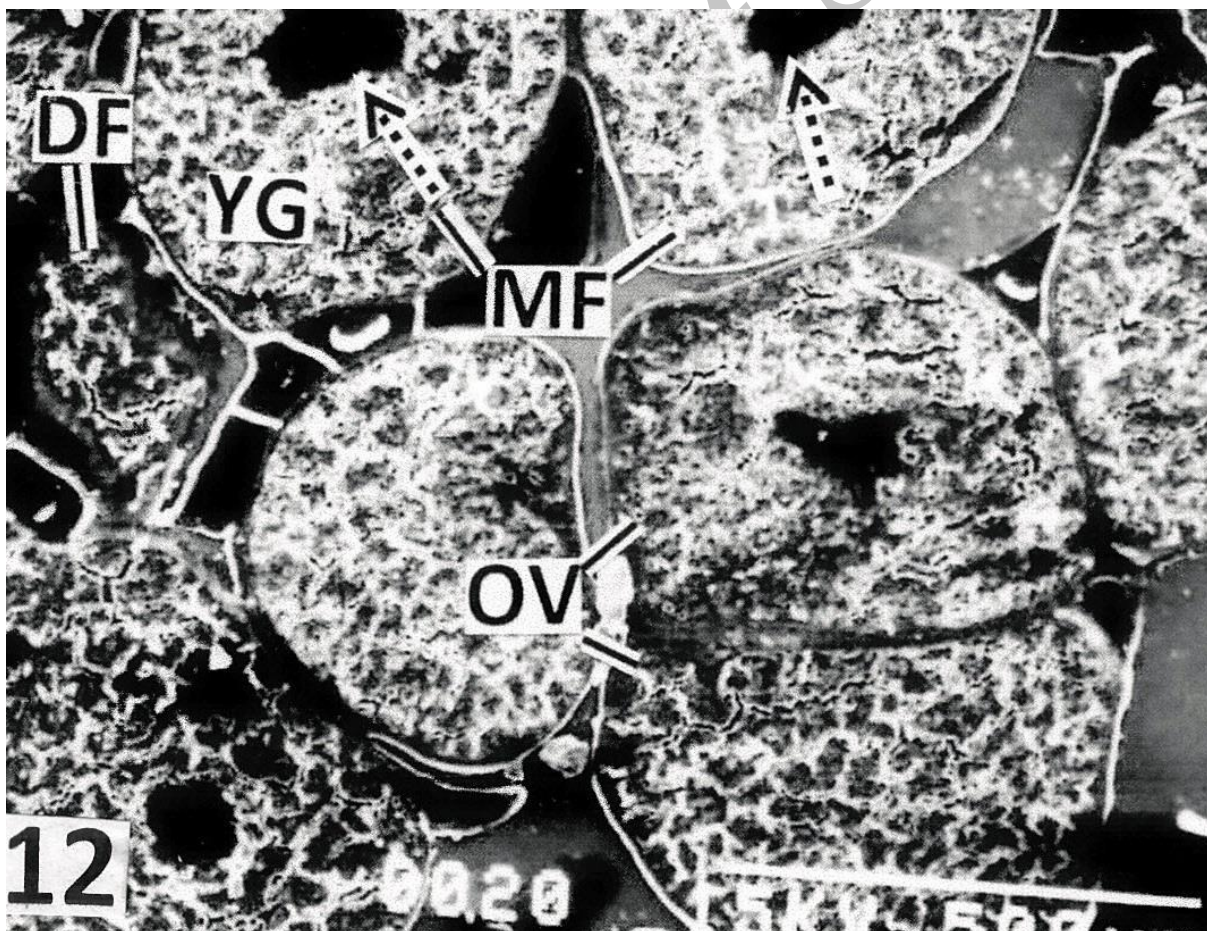


Fig. 12. Cut surface of mature follicle (MF) and oocytes V (OV) showing fused yolk granules (YG) and eccentric germinal vesicles (broken arrows) during spawning phase. DF indicates discharged follicles (SEM) $\times 1500$.

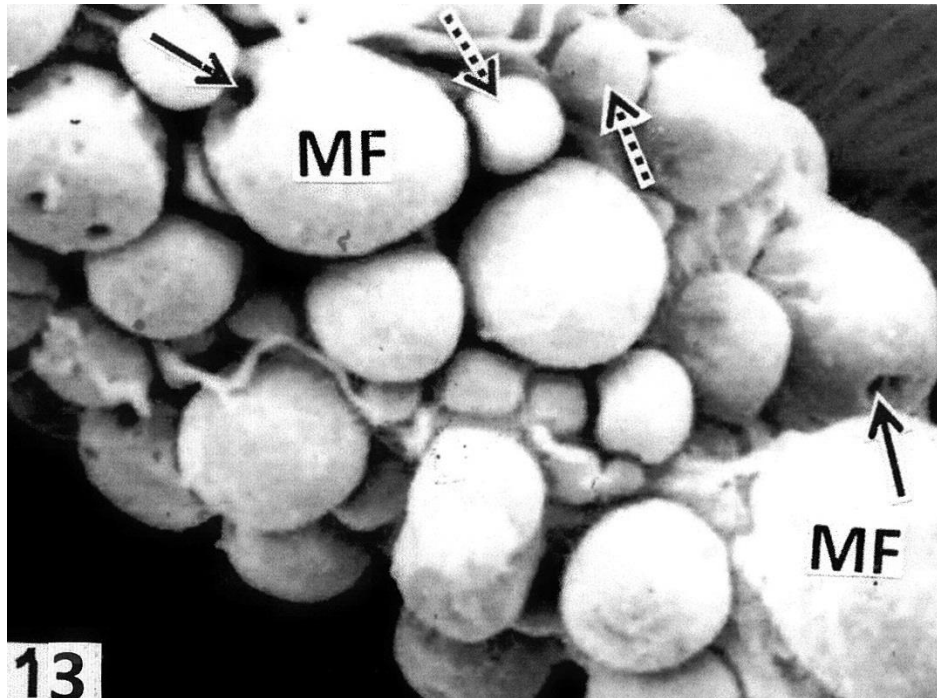


Fig. 13. Mature follicles (MF) with distinct depressed micropyle (solid arrows) during spawning phase. Note few immature follicles (broken arrows) in between MF (SEM) \times 150.

Discussion

Investigation concerning the ratio of ovary mass in proportion to body mass (GSI) is one of the indicators of the process of oogenesis (Fujita et al. 1997; Koya et al. 1998). In the present investigation, it has been observed that GSI value in *G. chapra* varies greatly during different months. It has been noticed that lowest GSI value has been registered during growth phase. This may be due to the gradual proliferation of early and late perinucleolar oocytes. However, during the month of February, early March, May and June GSI increased rapidly due to maximum growth and proliferation of vitellogenic oocytes. Garg & Jain (1984) reported that adequate food availability help the fish in recruitment of vitellogenic oocytes and in maintaining the maturation process in the ovary. Further, the GSI in the ovary of *G. chapra* gradually increased and reached to maximum during April *i.e.* first spawning phase followed by second spawning phase in July when the ovaries are packed up with mature follicles. This finding keeps uniformity of the view of

some workers (Abu Hakima 1987; Mukherjee et al. 1989; Mandal 2000). However, Afroz (1998) indicated that *G. chapra* breeds twice in a year, first during March to April and the second during August to September. Rahman & Haque (2008) reported two spawning peaks of *G. chapra*, one in March and the other in July as indicated by the peaks of gonadosomatic index (GSI) has been widely used as indicator of the fish spawning season, but its use in reproductive biology studies is more suitable when it is associated with macroscopic and histological techniques (Ghasemian et al. 2015)

In the present study, based on diameter of the oocytes in the ovary, yolk vesicles, vitellogenic oocytes, five stages of ovarian development were observed. Similar five developmental stages have also been reported in the ovary of *Aphanius farsicus* (Monsefi et al. 2007). In *G. chapra* each oogonium passed through a number of maturation stages before it becomes a ripe ovum. Using light and scanning electron microscopy, it has been found that the trophoplasmic growth period

begin with vacuolization followed by the deposition of yolk. During the maturation process the formation of yolk globules in the oocyte which started in the periphery of the developing ooplasm gradually moves towards the centre of the ovum. Kapoor (1977) and Afonso-Dias & Hislop (1996) observed similar pattern of yolk deposition in *Puntius ticto* and *Lophius piscatorius*. In the present study, in *G. chapra* the yolk vesicle oocyte and mature follicles were enveloped by outer theca, middle multinucleated zona granulosa and inner zona radiata. It may be assumed that granulosa and zona radiata layer are relatively active in the synthesis and/or transport of essential substances from granulosa layer to the ooplasm for building up protoplasm of oocyte. Similar observation has also been made by Shabanipour & Heidari (2004) in the mature ovary of *Liza aurata*. Using light microscopy and SEM, it has been observed that in mature follicles migration of germinal vesicle having invisible nuclear membrane was an event associated with the onset of final maturation of oocyte. Mylonas et al. (1995) also reported the dissolution of nuclear wall in mature oocyte in American shad, *Alosa sapidissima*.

In the present investigation oogonia were found throughout growth, maturation and spawning phases and their abundance tended to be somewhat higher in January. During maturation and early spawning phases the ovarian activity reached to the higher due to significant increase in the vitellogenic oocytes along with increase of oocyte diameter as well as GSI. de Vlaming et al. (1980) reported that principal events responsible for the growth of the oocytes involved sequestration of hepatically derived protein precursor, vitellogenin which look part in the formation of yolk protein. An important event that has been observed in the present study during spawning phase is the germinal vesicle breakdown which leads the oocyte towards the final maturity stage.

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مقاله پژوهشی

سازمان دهی بافتی و ساختار سطحی تخمدان ماهی *Gudusia chapra* (ماهیان استخوانی عالی: شگ-ماهیان) در طی فازهای مختلف تولیدمثلی

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چکیده: در این مطالعه، وضعیت سلول شناختی فعالیت‌های تخمدان ماهی *Gudusia chapra* (Hamilton, 1822) در طی فازهای مختلف تولیدمثلی مورد مطالعه قرار گرفت. تکوین غیرهمزمان در تخمدان‌ها و گوناگونی اندازه تخمدان و نمایه گنادی-بدنی (GSI) این ماهی مشاهده گردید. تغییر قطر تخمک‌ها در طی رشد، بلوغ و تخم‌ریزی بررسی شد. اوج دوره تخم‌ریزی این ماهی در طی ماه‌های مارچ و آوریل بوده و دومین تخم‌ریزی در ماه جولای رخ داد.

کلیدواژه‌ها: بافت شناسی، تخمدان، نمایه گنادی-بدنی، رشد، بلوغ، تخم‌ریزی.