Changes in the histological architecture of hepatocytes and ovarian tissues during growth, maturation spawning and post-spawning phases in *Puntius sarana* (Hamilton, 1822)

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Abstract

The present investigation dealt with the cytological status of hepatocytes and correlated them with the seasonal changes of ovarian activities in female *Puntius sarana* (Hamilton, 1822). The hepatocyte of female was provided with distinct nucleus and dense basophilic cytoplasmic granules. Different germ line cells were recognized on the basis of size and histoarchitecture of the cells. During growth and maturation phases it was found that the density of cytoplasmic granules of hepatocytes was increased in number as well as the nucleus became hypertrophied. These features were well correlated with the occurrence of cortical alveolus and yolk granule stage in the ovary. During spawning phase the sparse cytoplasmic granules were encountered in the hypertrophied hepatic cells. This was because of the dynamic cytological activities during vitellogenesis and occurrence of mature follicles in the ovary. It was concluded that the cytological changes in the hepatocytes and ovarian activities correlate well during growth, maturation spawning and post-spawning phases in female *Puntius sarana*.

Keywords: Histology, Hepatocytes, Ovarian tissues, Growth, Maturation, Spawning, Post-spawning, *Puntius sarana*

1. Introduction

The reproductive process is an essential part of the study the biology of species [11]. As such an understanding of the gonadal development and reproductive cycles of fishes is of fundamental importance for the conservation of natural stocks and for fish culture purposes. Various phases of gonadal development of fishes have been studied to clarify the dynamics and regulation of oogenesis [2]. 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 [3,4,5,6,7,8,9]. The gradual increase in total weight and associated morphohistological changes in gonad of the fish from the pre-spawning season is, therefore, intimately associated with the transfer of various nutrients from the body muscles and liver as well as with the proliferation of various germ line cells formed by the active process of gametogenesis [10, 11]. Banaee and Naderi [12] opined that an increase of hepatosomatic index (HSI) during maturation phase may indicate the increased activity of liver during vitellogenesis and vitellogenin synthesis.
Therefore, it would be interesting to study in details the functional aspects associated with the changes in the morphohistology of the ovary on the one hand and the variation in the histological aspects of the liver on the other in relation to growth, maturation, spawning and post-spawning phases of *Puntius sarana* (Hamilton, 1822).

### 2. Materials and Methods

Adult live female specimens of *P. sarana* (length 21-24 cm and weight 100-150 g) were procured from khari river which was 64 km. away from Burdwan, West Bengal during the second week of every month from January to December 2013. The fishes were acclimatized for 5 days period by feeding artificial diet daily. Data on total body weight and ovarian weight of 10 fishes were taken to calculate the mean gonadosomatic index (GSI) using the following formula:

\[
\text{GSI} = \frac{[\text{Total ovary weight/(Body weight-Weight of the ovaries)}]}{100}
\]

Likewise data on total body weight and liver weight to 10 fishes were taken to calculate the mean HSI using the following formula:

\[
\text{HSI} = \frac{[\text{Total liver weight/(Body weight-Weight of the liver)}]}{100}
\]

#### 2.1. Histological Methods

After decapitation of the fish the fragments of liver and ovary were fixed in aqueous Bouin’s fluid for 18 hours for histological studies. Paraffin sections were cut at 4µm thickness and stained with Delafield’s haematoxylin-eosin, Mallory’s triple stain and iron-alum haematoxylin for ovary and liver tissues. 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.

### 3. Results

Histologically the liver of *P. sarana* was composed of parenchyma covered by a thin capsule of connective tissue. Hepatocytes varied from polyhedral to round shape and were irregularly arranged surrounding a central vein. Each hepatocyte contained a round central nucleus and basophilic cytoplasm (Figs.1, 3). Hepatic cells showed changes during different reproductive phases.

#### 3.1. Oogenesis

Histologically the germinal epithelium of ovarian cavity projected into ovigerous lamellae where development of new crops of oogonia took place. The sequence of oocyte maturation in *P. sarana* had been divided into six developmental stages based upon the cytological characteristics of the cells, viz; oogonia (stage I), early and late perinucleolus stage (stage II and stage III), yolk vesicle stage (stage IV), yolk granule stage (stage V) and mature follicle (stage VI).

#### 3.1.1. Oogonia (6 µm × 8 µm to 12 µm ×10 µm)

Oogonia were present either singly or in small nests within the lamellar epithelium. An oogonium was made up of a large nucleus (4 µm to 5 µm) with chromatid threads (Figs. 2, 5).

#### 3.1.2. Early Perinucleolus oocyte (23 µm × 28 µm to 50 µm × 60 µm)

This stage consisted of a large oval centrally placed nucleus and contained about 8 to 10 basophilic nuclei together with fragmented chromatin (Figs. 2, 9).

#### 3.1.3. Late Perinucleolus oocyte (84 µm × 100 µm to 90 µm × 105 µm)

This stage was characterized by the appearance of cortical alveoli along the periphery of the ooplasm. Rounded nucleoli having variable sizes and number of nuclei and condensed chromatins. A thin layer enclosing the zona radiata was also appearing in this stage (Figs.11, 12).

#### 3.1.4. Yolk vesicle oocyte (112 µm × 132 µm to 124 µm × 145 µm)

The yolk vesicles finally cover the entire ooplasm of stage IV oocyte. Most of the vesicles were empty but some of them were filled with homogenous materials. The oocyte was enveloped with a zona radiata, middle multinucleated zona granulosa layer and outermost theca (Fig. 4).

#### 3.1.5. Yolk granules oocyte (220 µm × 250 µm to 280 µm × 340 µm)

In this vitellogenic oocyte stage migration of germinal vesicle from the centre of the egg towards the periphery started. Formation of yolk globules took place and as a result the cell volume and diameter increased considerably. The granulosa cells were more prominent with distinct nuclei (Figs. 5, 6).

#### 3.1.6. Mature follicle (380 µm × 400 µm to 450 µm × 500 µm)

The yolk granules coalesced and remained packed to form homogenous yolk mass. The nuclear membrane lost its identity and the nucleus (germinall vesicle) was eccentric in position with irregular outline. The thickness of the theca, zona granulosa and zona radiata reduced considerably (Figs.8, 9).

#### 3.1.7. Atretic oocytes:

Sometimes the developing oocytes failed to attain maturity were called atretic oocytes. These were characterized by irregular shaped, disintegrated nuclei and liquified yolk granules (Fig. 4).

#### 3.2. Sequential changes in the hepatocytes and ovarian cells during different reproductive phases

In the present investigation the histioarchitecture of hepatocytes, GSI, HSI, morpho-histological characteristics
of oogenetic cells were found to undergo changes during growth, maturation, spawning and post-spawning phases.

### 3.2.1. Growth Phase (December to February)
In this phase the cytoplasm of the liver cells accumulated dense homogenous granules. Nuclei were found in the centre of the hepatic cells. The sinusoid endothelium was present in between hepatic cells (Fig. 1). Primary oocytes (stage I and stage II) were present in the ovary (Fig. 2). However, during the end of this period percentage of stage III oocytes increased which showed cortical alveoli. During this phase slight increment of GSI was noticed and during January and February the GSI value increased gradually from 1.86 ± 0.22 to 2.19 ± 1.15. HSI also gradually increased during December (2.20 ± 0.28) and during late growth phase i.e. January and February the HSI increased to 2.78 ± 0.25 to 3.45 ± 0.28 (Table 1).

### 3.2.2. Maturation Phase (March to May)
During this yolk deposition stage the hepatic cells were found slightly enlarged in size and the nuclei were gradually hypertrophied. The sinusoids were well vascularized (Fig. 3). The highest oogenetic activity was found to occur during this phase. Different stages of vitelligenic oocytes were present. However, majority of the developing oocytes were of stage IV and stage V respectively. At the end of this phase the yolk granules of stage V continued to coalesce. Prominent zona granulosa and zona radiata were present. The immature oocytes were found to be decreased in number. A few atretic follicles were also found at this stage (Figs. 4, 5, 6). During the onset of maturation phase in March onwards when the ovary entered into the maturation, GSI gradually increased from 3.12 ± 0.68 to 12.01 ± 2.15 in May. In this phase during the month of March the HSI reached maximum 3.86 ± 0.09 and considerably decreased thereafter in April (1.38 ± 0.16) and May (1.12 ± 0.15) respectively (Table 1).

### 3.2.3. Spawning phase (June to August)
In the spawning phase the hepatic cells showed momentous changes than growth and maturation phases. The hepatocytes were enlarged with depleted cytoplasm and having a hypertrophic nuclei (Fig. 7). However, some of the hepatic cells still showed granulation in the cytoplasm. The vascularisation of the hepatic sinusoids were also reduced (Fig. 7). The ovaries at this stage were full of stage V oocytes and mature follicles. The mature follicles became larger and irregular in shape, the yolk globules condensed and provided with eccentric germinal vesicles (Figs. 8, 9). A few discharged follicles were also observed (Fig. 9). In June the ovary was with full of mature follicles and GSI attended the peak value (16.73 ± 1.86) but in July and August the GSI value showed a declining trend (13.44 ± 2.10 and 8.60 ± 2.32). The significant decreased of HSI was noticed during the entire spawning phase i.e. in the month of June (1.07 ± 0.08), July (0.98 ± 0.26) and August (0.56 ± 0.17) (Table 1).

### 3.2.4. Post-spawning phase (September to November)
The hepatocytes were gradually transformed like that of growth phase having centrally placed nuclei and basophilic granules were started to accumulate in the hepatic cytoplasm. The hepatic sinusoids were present in between hepatic cells (Fig. 10). During this reproductive phase mature ova were few in numbers. The oogonia and early perinucleolar oocytes were appeared along with few late perinuclear oocytes (Figs. 11, 12) in between primary oocytes.

In the post-spawning period i.e. in September onwards the yolky follicles reabsorbed and the ovaries showed a regression state. The GSI was recorded to 3.52 ± 0.27, 1.28 ± 0.34 and 0.92 ± 0.22 during September, October and November respectively. During September the HSI recorded to 1.20 ± 0.08 and gradually increased during October (1.45 ± 0.06) and November (1.52 ± 0.09) (Table 1).

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**Table 1:** Variations in the gonadosomatic index (GSI) and hepatosomatic index (HSI) of female *Puntius sarana*

<table>
<thead>
<tr>
<th>Stages of ovary</th>
<th>Month</th>
<th>Mean GSI ± SE</th>
<th>Mean HSI ± SE</th>
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<tbody>
<tr>
<td>Growth Phase</td>
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<tr>
<td>December</td>
<td></td>
<td>1.48 ± 0.25</td>
<td>2.20 ± 0.28</td>
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<tr>
<td>January</td>
<td></td>
<td>1.86 ± 0.22</td>
<td>2.78 ± 0.25</td>
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<td>February</td>
<td></td>
<td>2.19 ± 1.15</td>
<td>3.45 ± 0.28</td>
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<tr>
<td>Maturation Phase</td>
<td></td>
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<tr>
<td>March</td>
<td></td>
<td>3.12 ± 0.68</td>
<td>3.86 ± 0.09</td>
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<tr>
<td>April</td>
<td></td>
<td>6.52 ± 1.60</td>
<td>1.38 ± 0.16</td>
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<tr>
<td>May</td>
<td></td>
<td>12.01 ± 2.15</td>
<td>1.12 ± 0.15</td>
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<td>Spawning Phase</td>
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<tr>
<td>June</td>
<td></td>
<td>16.73 ± 1.86</td>
<td>1.07 ± 0.08</td>
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<tr>
<td>July</td>
<td></td>
<td>13.44 ± 2.10</td>
<td>0.98 ± 0.26</td>
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<td>August</td>
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<td>8.60 ± 2.32</td>
<td>0.56 ± 0.17</td>
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<tr>
<td>Post-spawning and Resting Phase</td>
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<tr>
<td>September</td>
<td></td>
<td>3.52 ± 0.27</td>
<td>1.20 ± 0.08</td>
</tr>
<tr>
<td>October</td>
<td></td>
<td>1.28 ± 0.34</td>
<td>1.45 ± 0.06</td>
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<tr>
<td>November</td>
<td></td>
<td>0.92 ± 0.22</td>
<td>1.52 ± 0.09</td>
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**Fig 1:** Hepatic cells (arrow heads) of growth phase with centrally placed nucleus and dense cytoplasm. Note hepatic sinusoids (arrows) in between hepatic cells (HE) × 400X.

**Fig 2:** Oogonia (arrows), oocyte I (OI) and early perinucleolus oocyte (OII) during growth phase (HE) × 150X.
**Fig 3:** Enlarged hepatic cells with prominent nucleus and vacuolated cytoplasm (arrow heads) surrounding central vein (CV) during maturation phase. Note hepatic sinusoids (broken arrows) in between hepatic cells (MT) $\times 400$X.

**Fig 4:** Maturation stage of ovary showing oocyte IV (O IV) stage with yolk vacuoles and germinal vesicle (arrow) covered by germinal epithelium. Note the presence of atretic follicle (AF) and oogonia (arrow heads) (HE) $\times 150$X.
Fig 5: Oocyte V (OV) stage with yolky granules (solid arrows) and prominent germinal layer (broken arrow) during the end of maturation phase. Note oogonia (OO) (arrow heads) in between OV (HE) × 150X.

Fig 6: Higher magnification of OV with condensed yolk granules (arrows) during end of maturation phase. Note zona granulose layer with prominent nuclei (arrow heads) (HE) × 600X.
**Fig 7:** Enlarged hepatocytes (broken arrows) with vacuolated cytoplasm during spawning phase. Some of the hepatocytes still showing granular cytoplasm (arrow heads). Solid arrows indicate sinusoids with vascularization (MT) × 400X.

**Fig 8:** Higher magnification of mature follicle (MF) with eccentric germinal vesicle (arrow head) and condensed yolk granules (arrows) during spawning phase (MT) × 400X.
**Fig 9:** Mature follicles (MF) showing prominent germinal vesicle (arrow) during spawning phase. Note the presence of discharged follicle (DF), oogonia (OO) and oocyte I (OI) (arrow heads) during this phase (HE) × 150X.

**Fig 10:** Showing parenchymal arrangement of liver having prominent hepatic cells (arrow heads) with distinct nucleus during post-spawning phase. Solid arrows indicate hepatic sinusoids (HE) × 400X.
Fig 11: Early perinucleolar oocyte (OII) and late perinucleolar oocyte (OIII) during post-spawning phase. Note some regressed mature ova in between (MT) × 150X.

Fig 12: Oogonia (OO) (arrow heads) oocyte I (OI) and oocyte III (OIII) and adjoining blood vessels (BV) during post-spawning phase (IA) × 400X.
4. Discussion
In the present study the relationship of HSI with GSI in the fish, *P. sarana* during growth, maturation, spawning and post-spawning phases indicate marked differences. The lowest GSI and HSI values were noticed during the end and beginning of post-spawning phase when the ovaries were found in regressed condition containing mainly primary oocytes. However, GSI increased marginally but remained almost stationary during the growth phase whereas HSI gradually increased during the post-spawning phase and sharply rose at the end of growth phase. This may be due to the gradual proliferation of late perinucleolar oocytes in the ovary and accumulation of various nutrients in the hepatic cells of liver. Sudarshan and Kulkarni [13] also reported high HSI value during beginning of preparatory phase in *Notopterus notopterus*. GSI increased rapidly from the end of the maturation phase and continued up to spawning phase due to slow accumulation of yolk granules and proliferation of vitellogenic oocytes. On the contrary, HSI reached maximum during early maturation to beginning of spawning phase. The highest values of HSI indicate heavier liver probably due to the synthesis and accumulation of various nutrients during vitellogenesis process. Such a rhythm of changes of HSI and GSI in *N. notopterus* has also been reported by Sudarshan and Kulkarni [13]. The HSI sharply declined in the spawning phase which might be due to the release of nutrients to the blood and then transported into the mature oocytes during complete process of vitellogenesis. Mukherjee et al. [14] and Mandal [15] reported that adequate food availability helped the female fish in recruitment of vitellogenic oocytes and in maintaining the maturation process in the ovary. However, GSI value declined prominently from September onwards due to discharge or reabsorption of yolky oocytes. Singh and Singh [16] studied the relationship between HSI and GSI in the fish *Heteropneustes fossilis* and found that high HSI during preparatory and post-spawning and low levels during pre-spawning and spawning.

Histologically the liver of *P. sarana* showed hepatic parenchymal arrangement consisted of hepatic cells along with sinusoids which were arranged around a central vein. This histarchitecture of liver resembled that described for the stripped weak fish *Cynoscion guatucupa* [17] and for rainbow trout [18].

In the present investigation oogenesis occurred in two stages, the division of oogonia and the transformation of the resting oocytes into mature oocytes. In *P. sarana* it was observed that oogonia after development from the germinal epithelium of the ovigerous lamellae passed through a number of maturation stages before it become a mature ovum. This involved complex changes in the cytology of the nucleus and cytoplasm. The formation of yolk globules in the late perinucleolus oocyte in *P. sarana* began in the periphery of the developing ooplasm which gradually moved to the centre of mature follicles through different stages of development. Bisht and Joshi [19] and Kapoor [20] observed similar pattern of yolk deposition in *Schizothorax richardsonii* and *Puntius ticto*. In the present study the mature follicles were enveloped by the multinucleated zona granulosa, outermost theca and inner zona radiata. In the late developing oocytes, it may be assumed that through zona radiata the essential nutrients were transported from the granulosa layer to the ooplasm for building up the yolk globules of the mature oocytes. Similar observations have also been made by Bromage and Cumanaratunga [21] and Shabanipour and Heidari [22] in the ovary of rainbow trout and *Liza aurata*.

In the present investigation the maturational activity in the ovary reached to the highest during the late maturation and early spawning phase when oocyte diameter as well as GSI rose up. At the same period the hepatocytes were found to be enlarged along with hypertrophy of nuclei and were accompanied with the depletion of cytoplasmic granules. This feature might be due to the uptake and accumulation of various nutrients in the oocytes and yolk precursor vitellogenin from hepatocytes. Sinha and Pal [20] opined that the stored protein, lipid and carbohydrate were shifted from liver and body muscle to the ovary to ensure proper growth and development of oocytes. de Vlaming et al. [23] reported that principal events responsible for the growth of the oocytes involved sequestration of hepatically derived protein precursor, vitellogenin which took part in the formation of yolk protein. Aida et al. [25] opined that the liver cells in female ayu (*Plecoglosus altivelis*) showed an activated state of female specific plasma protein (FSPP) synthesis following ovarian maturation. They also stated that upon synthesis in the liver cells, this protein is probably released into the blood and then transported into the oocytes during the process of ovarian maturation in ayu.

5. Conclusion
Cytological changes in the hepatocytes are correlated with the reproductive phases in *P. sarana*. It is found that during growth and maturation phases the density of cytoplasmic granules of hepatocytes is increased as well as the nucleus becomes hypertrophied. These features are well correlated with the occurrence of cortical alveolus and yolk granule stage in the ovary. During spawning phase the cytoplasmic granules are sparse in the hypertrophied hepatic cells. This is due to the dynamic cytological activities like vitellogenesis and occurrence of mature oocytes in the ovary. However, no significant alterations are noticed in the hepatocytes during post-spawning phase due to the appearance of new germ cells in the ovary.

6. Acknowledgements
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