Introduction

The banded gourami, *Trichogaster fasciata* (Bloch and Schneider, 1801) is an important wetland fish of West Bengal. They inhabits mostly in the muddy water adjacent to the localities or paddy fields covered with weeds or surface vegetation. Although this fish is not famous for commercial culture, but still this fish is popular in the rural area of West Bengal to fulfill the nutritional requirements of poor people. Due to its colourful morphology, the banded gourami is now becoming one of the popular ornamental fish in India. As this species reside nearby water bodies of human inhabitant, conservation of this fish is also important for maintaining rural health to control mosquito transmitted diseases like Malaria, Dengu, Chikungunya etc. due to feeding on mosquito larvae. No statistical data is available in relation to the population dynamics, stock of status of this species; but unavailability in market indicates that the population of this species is declining rapidly. Habitat destruction, household pollutant emission, pesticide contamination from nearby agricultural fields and other anthropogenic activities are the main cause for declining of this species. Presently, the status of *T. fasciata* in the IUCN red data book is 'least concern'.

To overcome the depletion of the banded gourami in their natural habitat, both restoration of their ecological factors and development of intensive production techniques are equally important. The success of this technological development requires a comprehensive understanding of their reproductive cycle, sex hormonal status, hormonal regulation of gonadal development and maturation. Surprisingly, except some scanty information on the biology (Welcomee, 1988; Behra *et al*., 2005; Bindu *et al*., 2014) and captive breeding for aquarium (Hossain *et al*., 2010; Abujam *et al*., 2015), no study has yet been made on seasonal gonadotropin hormone levels in the plasma and their regulatory role in controlling gonadal development, aromatase activity and other key regulating factors activity for reproduction, throughout the year, particularly during breeding season in this ecologically important fish.

In female teleost, pituitary gonadotropins appears to be the regulator of gonadal development, vitellogenesis, maturation, ovulation and spawning. Teleost pituitary gonadotropins, now considered as FSH and LH have been shown to stimulate steroid production by testicular and ovarian tissues from various teleosts (Suzuki *et al*., 1988; Swanson *et al*., 1989; Van Der Kraak *et al*., 1992; Planas *et al*., 1993; Planas and Swanson, 1995; Tanaka *et al*., 1995; Kagawa *et al*., 1998; Pramanick *et al*., 2013), despite differences in steroidogenic potency. In

females, during vitellogenesis, gonadotropins stimulate the production of 17β-estradiol (E_2). E_2 stimulates hepatic vitellogenin synthesis (Fostier *et al*., 1983), as well as vitelline envelop synthesis (Hyllner *et al*., 1991; Hyllner and Haux, 1995). Vitellogenin then incorporate into oocyte by vitellogenic gonadotropin, FSH. The final maturation of the oocyte in most teleosts is controlled by progestins, either 17,20β-P (Salmonid, cyprinid etc.) (Goetz *et al*., 1983; Nagahama *et al*., 1986; Yaron, 1995; Bhattachryya *et al*., 2000; Sen *et al*., 2002) or 17,20 β, 21-trihydroxy-4-pregnen-3- one (17,20β,21-P) (eg. Scianid fish) (Thomas and Trant, 1989; Trant *et al*., 1986). Testosterone (T) circulates in the plasma of female as well as in male and this steroid is a substrate for E_2 production (Kagawa *et al*., 1982) and may play role in maintaining the oocyte once vitellogenesis is completed (Kime, 1993).

It is already established that aromatization of androgens to estrogens is catalyzed by cytochrome P450 aromatase (P450arom) which is a product of *cyp19a1* gene. Expression of *cyp19* genes are essential for gonadal and brain development (Roy Moulik *et al*., 2016; Tchoudakova *et al*., 2001; Zhao *et al*., 2008). Unlike mammals, teleosts has two forms of *cyp19*; *cyp19a1a* which is responsible for ovarian differentiation and development (Roy Moulik *et al*., 2016; Pal *et al*., 2018) and *cyp19a1b* is involved in neuronal development (Forlano *et al*., 2001). Changes in P450 aromatase enzyme activity or expression of *cyp19a1* genes is the key regulators of the gonadal production of E_2 during different stages of reproductive cycle in teleosts (Chang *et al*., 1997). Thus, to understand the role of aromatase in different developmental stages of oocyte it is essential to find out the seasonal variation pattern of aromatase expression in the ovary at various developmental stages. Since little information is known in this aspect, present study examined the localization pattern of expressed aromatase in different reproductive stages of ovary in *T. fasciata*.

In mammals, it is well known that steroidogenic factor-1(SF-1) plays a crucial role in signaling procedure to regulate the *cyp19a1* gene in granulosa cells (Simpson *et al*., 2002; Hu *et al*., 2001; Leers-Sucheta *et al*., 1997). SF-1 activity is also essential for aromatase gene expression during the oocyte developmental process in fish as reported by many authors (Dalla Valle *et al*., 2002; Kazeto *et al*., 2001; Yoshiura *et al*., 2003; Das *et al*., 2013). Therefore, to elucidate the cooperative mechanism (if any) of SF-1 and aromatase, present study examined the SF-1 localization in the *T. fasciata* ovary in each developmental stages.

Therefore, the overall objectives of the present study were a) to measure the level of FSH and LH throughout the year in relation to the gonadosomatic index (GSI); b) to characterize the ovarian developmental stages throughout the year and c) to find out the expression pattern of *cyp19a1a*, localization of aromatase and SF-1in different developmental stages of ovary. Also find out their participation in the folliculogenesis process of this fish species.

Materials and Methods

Animals

Adult female *T. fasciata* (length is around 10 cm) at different reproductive stages were collected throughout the year from a local fisherman and were kept in laboratory aquaria (50 L capacity: Size – 30"x18"x15") at 23 ± 1°C for 2 days to acclimatize them to the laboratory environment. They were fed with commercial fish food (Shalimar fish food, Bird and Fish food manufacturer, Mumbai) twice a day. Before sacrifice, fishes were deeply anaesthetized with MS 222 (150 mg/L), ovary and blood samples were collected for normal histology, immunohistochemistry and for extraction and estimation of steroids respectively.

Chemicals

Labeled steroids, [^{3}H] estradiol-17β (specific activity 75.0 Ci/mmol) and [^{3}H] testosterone (specific activity 95.0 Ci/mmol) were purchased from Amersham Biosciences. E_2 antibody was a generous gift from Gordon Niswender (Colorado State University, Fort Collins, Colorado, USA). Cocktail T (for scintillation) was purchased from Spectrochem Pvt. Ltd. Anti aromatase antibody was procured from Abcam (ab215443), SF-1 rabbit polyclonal antibody (sc-28740) and goat anti–rabbit secondary antibody (sc-2004) were purchased from Santa Cruz Biotech (St. Louis, MO, USA). 17β-Estradiol (E_2), 17α,20β-Dihydroxy-4-pregnen-3-one (17,20β-P), Testosterone(T), 17α-hydroxyprogesterone(17α OH-P), progesterone(P) were purchased from sigma-aldrich in HPLC grade. All other chemicals were used of analytical grade.

ELISA

LH and FSH were assayed using Fish specific Enzyme-linked Immunosorbent Assay Kit (ELISA kit) (Bioassay Technology Laboratory; FSH – E0039F1; LH- E0040F1) following manufacturer's instruction manual. The assay range of the kit was 2-100 pg/ml and the sensitivity was 1.14 pg/ml. Intra- and inter-assay variations were <8% and <10% respectively. Before starting the protocol, plasma samples were prepared by centrifugation of blood for 20 min

at 3000 RPM. For ovarian samples, 100 mg of follicles were homogenized with 1 ml of phosphate buffer (pH 7.4), centrifuged at 3000 RPM for 20 min. In both cases, 50 µl of supernatant was used for the assay. 50µl of media sample was used directly for the blank.

Histology and Immunofluorescence Imaging

Ovarian samples were fixed in 4% paraformaldehyde in phosphate buffer (pH 7.4). Fixed tissues were dehydrated in increasing concentrations of ethanol (50, 70, 90 and 100%) series, cleared in xylene and embedded in paraffin blocks at 60-62ºC. Blocks were sectioned with a rotary microtome at 8 µm thickness. Tissues were stained with Hematoxylin and Eosin following regular protocols and observed under bright field microscope.

For immunofluorescence staining to localize *cyp19a1a* and SF-1, ovarian sections were deparafinized in xylene, rehydrated, washed in PBS and blocked in NP 40 for one hour at room temperature (protocol adapted from Majumder *et al*. (Majumder *et al*., 2012)). Then, sections were incubated in primary antibody at 1: 200 dilutions (rabbit anti SF-1) for overnight at 4°C. Fluorescein conjugated secondary antibody (Alexa fluor 488: A11034 for green and Alexa fluor 568: A11036 for red) was used. To stain nuclei, DAPI (0.05µg/ml) (ab104139) was used. Imaging was performed in a Leica upright microscope and images were analyzed with provided software.

Measurement of aromatase activity

Aromatase activity was estimated by *in vitro* conversion of [^{3}H] T to [^{3}H] E_2 following the method of Chan and Tan (Chan and Tan, 1986) as described by Pramanick *et al.*(Pramanick *et al*., 2013). 100 mg ovarian tissue of different stages were incubated in the 1 ml Idler's medium with 140 pmol [^{3}H] T (1 x 10^6 CPM, sp. activity 95.0 Ci /mmol) for 6 h at 23 ± 1°C. After termination of the incubations, steroids were extracted, and subjected to thin layer chromatography (TLC) for separation of T and E_2. Both T and E2 eluted, re-extracted and counted in liquid scintillation counter with cocktail T. Aromatase activity was measured by calculating the percent conversion of T to E_2.

RNA isolation, cDNA synthesis and Realtime-PCR for gene expression study

After decapitation of each stage fish, 100 mg ovarian tissues were fixed at 1 ml of TRI reagent (Promega). Total RNA from was extracted by following the manufacturer's instruction and the method described by Paul *et al*., 2010. After quantification in a Nanodrop spectrophotometer, cDNA synthesis was carried out with 2.0 µg of Dnase-treated total RNA

using Revert Aid M-MuLVreverse transcriptase (MBI; Fermentas, USA) and a mix with oligo (dT) and random primers (Promega), according to the manufacturer's protocols. Quantitative real time PCR was performed by using prepared cDNA diluted 1:20 for *cyp19a1a* gene and 1: 1000 for housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The total reaction volume was 20 µl contained 10 µl of SYBR Green, 5µl of cDNA and 500nM of forward and reverse primers. Reactions were run in a Bio-Rad PCR system maintaining following protocol: 50 ºC for 2 min, 96 ºC for 10 min, 45 cycles of 95 ºC for 30 s and 62 ºC for 1 min. All samples were run in triplicate. Expression level was calculated and presented as fold-change after normalizing the value obtained from GAPDH gene. Primers were used for both the gene presented in Table 1.

Table 1: Primers used in realtime -PCR (primer designed by primer 3 interactive primer design software).

Gene Product	Forward primer (5'-3')	Reverse primer (5'-3')	Size of amplicon (bp)
Cyp19a1a (EF687713.1)	TGTCCGCTGAGGATGTCAAG	CAGCTTAGGAAGGTCTGCGT	188
GAPDH (AJ870982)	ATTGCCCTCAACGACCACTT	GCGTATTATGGGGGATGGCT	232

Statistical analysis

Three fishes of each reproductive stage were sacrificed in each year. Consecutive 3 years data were pooled and expressed as mean ±SEM. So, all the data point represents the pooled data of 9 fishes. Statistical differences were calculated by one way ANOVA and Bonferoni's multiple tests, using SPSS (Chicago). The level of significance was chosen at $p < 0.05$.

Results

Ovarian histology

Histological observation of *Trichogaster fasciata* ovaries summarized in the Table 2 and Fig. 1. It showed the different developmental stages of the ovarian cycle throughout the year. Depending on the diameter of the ovarian follicles, deposition of yolk granules and cortical granules in the cytoplasm of the oocytes, coalescence of lipid droplets and location of germinal vesicle (GV), we categorize the stages as resting stage, preparatory stage, previtellogenic stage, vitellogenic stage, post-vitellogenic stage, post-GVBD stage and post-spawning stage. In December and January ovary contains resting stage oocytes; in February, March and April preparatory stage oocytes were arranged inside the lamella and lean inside the ovarian cavity from germinal epithelium; in May, oocytes develop rapidly to the previtellogenic stage; in June oocytes rapidly enter into the vitellogenic stages, yolk deposition and huge amount of vitellogenin is seen inside the oocyte; in July-August, oocytes develops further and enter into the postvitellogenic stages either with centrally located germinal vesicle or with migratory GV; in September GVBD occurs, oocytes are going to ovulate and spawning occurs; in October-November post-spawning stage appears with flaccid ovary and scattered small unspent ova; in December, ovary enter in resting phase with small number of scattered oocytes.

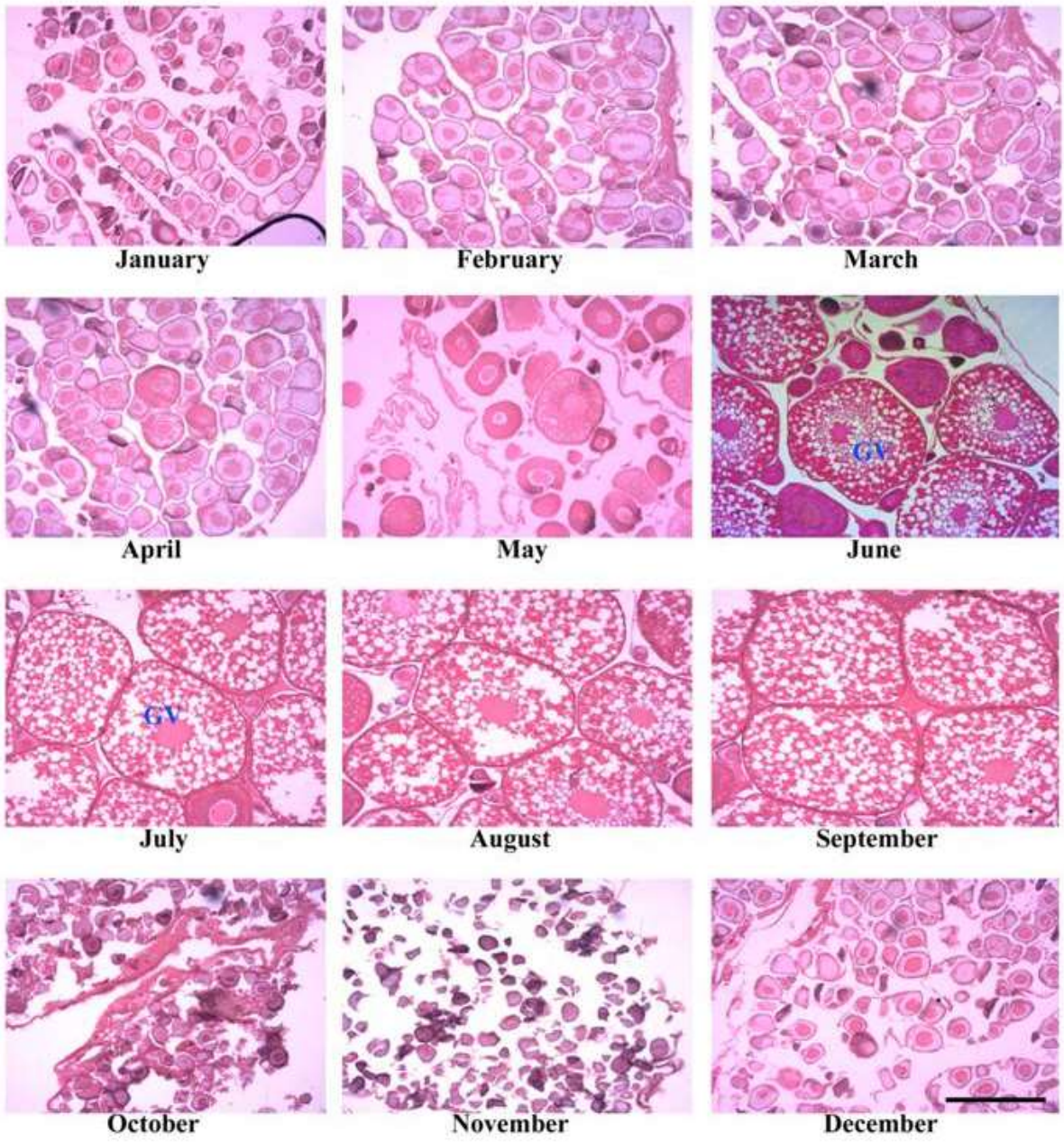

Fig. 1 Histological appearance of oocytes throughout the year. T.S. of ovary (collected from 3 fishes for each stage × 3 years) were stained in H.E. staining and observed in the bright-field microscope. Characteristic of different stages of oocyte were summarized in Table 2. Scale bar in 200μm.

Ovarian stage	Month	Macroscopic structure of ovary	Microscopic structure of the Oocytes
Resting stage	December and January (Stage-1)	Ovaries are very small and transparent whitish color, eggs were not visible.	Oocytes were very small with little cytoplasm. Nucleus was relatively dark stained. Under microscope they appear as irregular cells.
Preparatory stage	February to April (Stage-2)	Very small size, Thread like, transparent, oocyte was not visible in naked eye.	Individual ova are transparent with very little yolk granules in the cytoplasm.
Previtellogenic stage	May (Stage-3)	Thickness just higher than preparatory phase ovary. Ovaries were pale yellow colour and presence of blood vessel.	Early previtellogenesis with large germinal vesicle developing; middle previtellogenesis characterized by developing nucleoli late previtellogenesis characterized by appearance of lipid vacuoles.
Vitellogenic stage	June (Stage-4)	Ovary larger size than previous stage. Fully yolked contain yellow colour egg. Eggs were visible in naked eye. Rich blood vessel present.	Vitellogenic oocytes have increased in size and in number of lipid vacuoles. Cytoplasm of the oocytes was filled up with densely packed yolk granules. The germinal vesicle (GV) was centrally located.
Postvitellogenic stage	July and August (Stage-5)	Ovary larger, round oocytes were present in the gonad. Deep	Oocyte become more rounded. Lipid vacuoles concentrate around periphery.

		yellowish colour. Rich blood vessel present.	Centrally located GV or migrating GV present.
Post GVBD stage	September(Stage-6)	Ovary larger, deep yellowish colour, round oocytes were present in the gonad. Ovary shrunken and transparent oocyte visible.	Fully develop size of oocyte. Lipid vacuoles are concentrated around the oocyte periphery. GVBD completed in most of the oocytes.
Post Spawning stage	October and November (Stage-7)	Ovary long and degenerative, transparent in colour.	A few residual ova scattered in connective tissue. Majority of the ova are very small in size.

Table 2: Maturity stages of the ovary throughout the year.

Gonado-somatic Index (GSI)

Seasonal changes of gonado-somatic index (GSI) of *T. fasciata* is recorded and presented in Fig 2. It was observed that in resting and preparatory stage, GSI values were 1.65±0.43 and 1.73±0.37 respectively. In previtellogenic and vitellogenic stage, which comes in immediate next two consecutive months (i.e. May and June), the GSI values were found to be 5.2±0.91 and 6.1±0.85. It was noted that during this stage the accumulation of yolk granules are occur into the oocyte. In post vitellogenic and post GVBD stage (during the month of July, August and September), the GSI value was 7.45±0.55 and 7±0.43. However, GSI value decreased drastically (2.75±0.4) in post spawning stage during the month of October and November.

Annual profile of gonadotropins (FSH and LH)

The study measured the levels of both gonadotropins i.e. GTH-I (FSH) and GTH-II (LH) by ELISA. Results presented in the Fig 3 shows single peak for both FSH and LH in the month of June and August respectively, when the oocytes were in vitellogenic and postvitellogenic stages. In resting stage, plasma FSH levels were 2.0±0.21 and 2.8±0.51 and LH levels 2.0±0.21

and 1±0.23 ng/ml were observed during the month of December and January respectively. In preparatory stage the plasma FSH levels were 4.2±0.63, 5.1±0.95 and 6.9±1.2 ng/ml and LH levels were 1.0±0.67, 3.0±1.3 and 6.1±0.92 ng/ml during the month of February, March and April respectively. In previtellogenic stage plasma FSH and LH levels were 8.13±0.53 and 7.8±2.1 ng/ml respectively in the month of May. During vitellogenic stage plasma FSH and LH levels were 9.1±0.23 and 11.3±1.11ng/ml respectively in the month of June. In this phage FSH level reached its highest value. In post vitellogenic stage, plasma FSH become decreased (7.2±0.51 and 3.0±0.54 ng/ml) and LH level reached its highest valve i.e. 14.1±1.6 and 16.2±0.91 ng/ml respectively during the month of July and August. However, FSH and LH levels were shown to decreased sharply at post GVBD and post spawning stage.

Expression of cyp19a1a and aromatase activity in the different developmental stages of the ovary

Variations in expression of *cyp19a1a* in the different stages of ovary are shown in Fig. 4A. In preparatory stage *cyp19a1a* very little expression was found; but in vitellogenic it shows its maximum expression. In post vitellogenic and post-GVBD stages relatively lower expression were detected and expression of *cyp19a1a* was at not-detected level in the post-spawning stage of ovary.

Aromatase activity of *T. fasciata* ovary shows the similar pattern like *cyp19a1a* expression depending on the developmental stages of the ovary. Fig. 4B clearly shows that aromatase activity reaches its maximum level at the vitellogenic stage and its activity lowering down gradually.

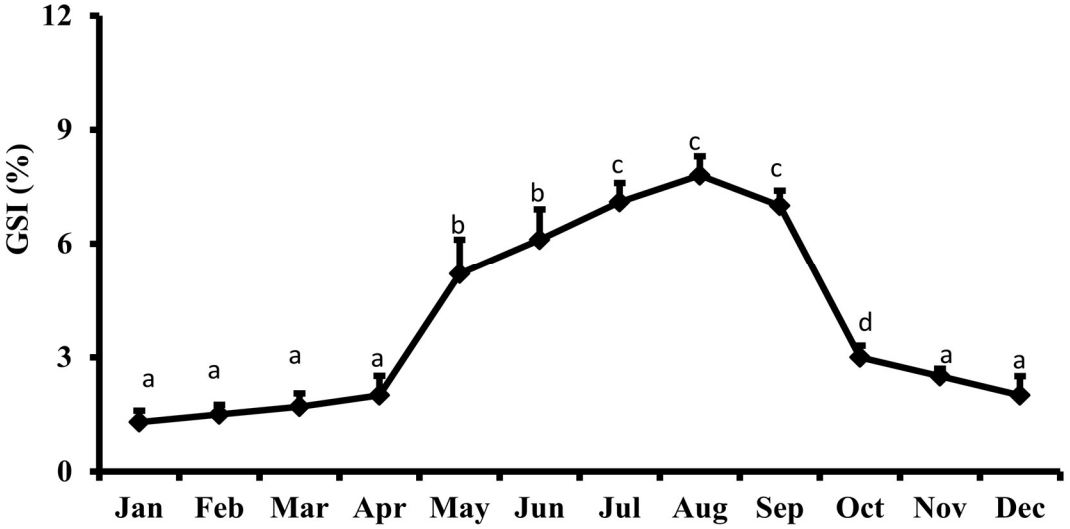

Fig. 2 Variations in gonadosomatic index (GSI), throughout the year. Each point shows the mean ±SEM of nine observations (three for each year × three years). Points on a given line associated with common letters are not significantly different. (ANOVA and Bonferonni's multiple range tests; $p < 0.05$).

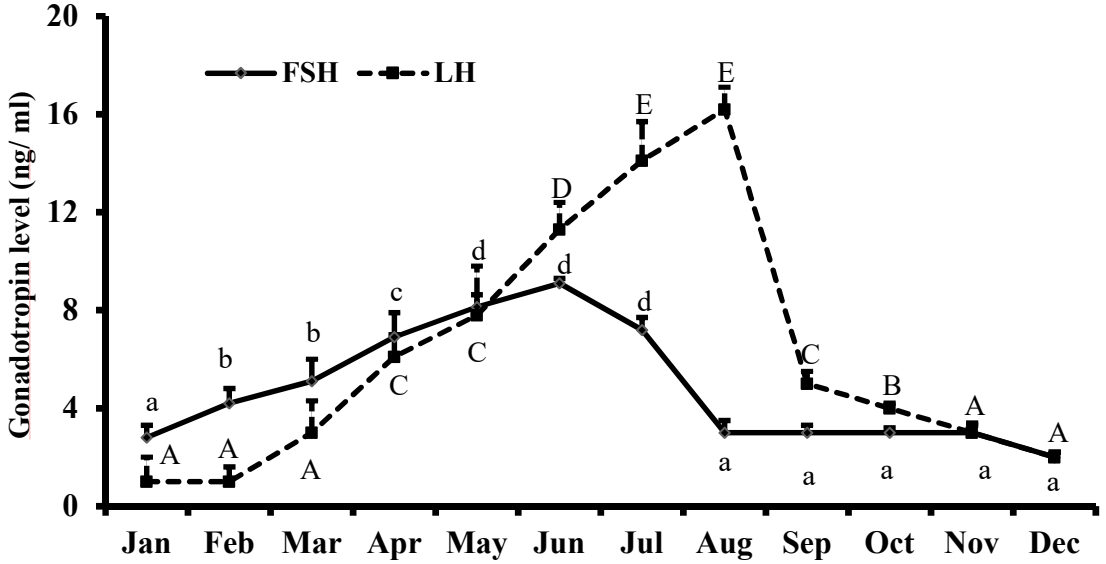

Fig. 3 Variations in plasma gonadotropins (FSH and LH) throughout the year. Each point shows the mean ±SEM of nine observations (three for each year × three years). Points on a given line associated with common letters are not significantly different. (ANOVA and Bonferonni's multiple range tests; $p < 0.05$.) FSH: Follicle stimulating hormone; LH: Luteinizing hormone.

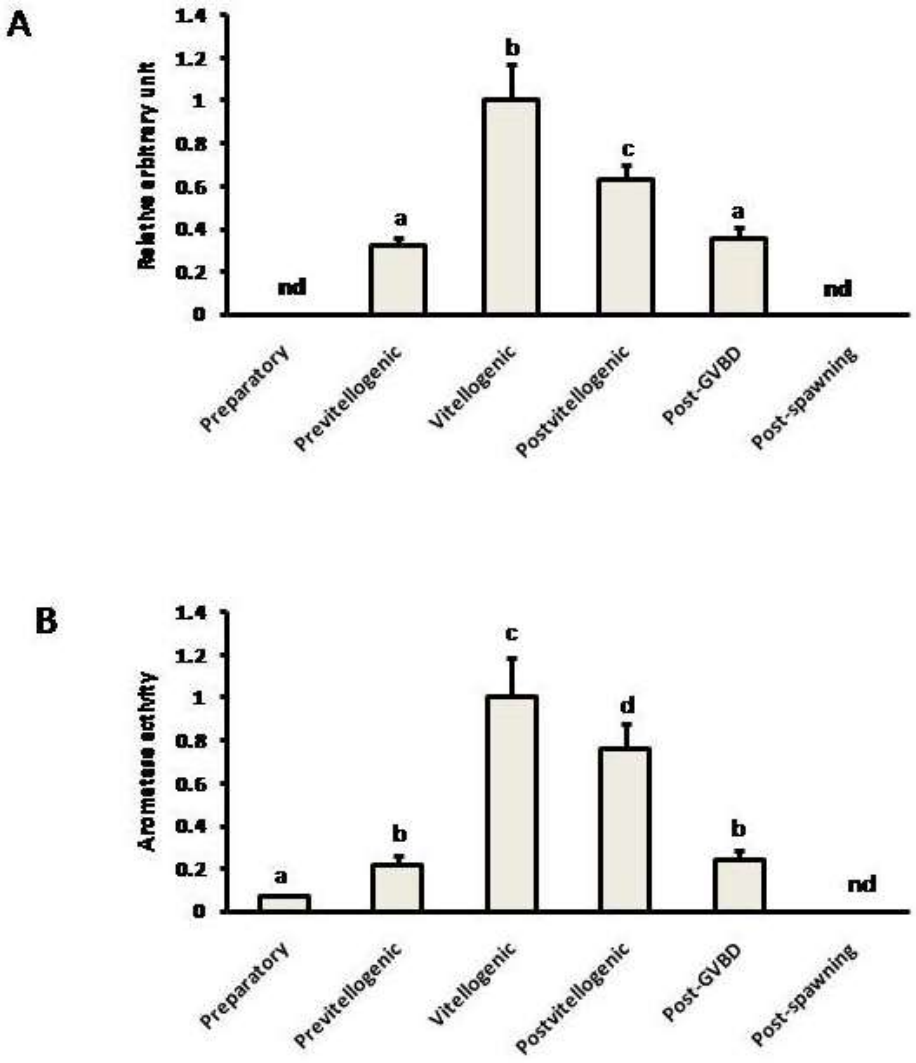

Fig. 4 Expression of *cyp19a1a* and aromatase activity in the different developmental stages of the ovary. (A) Ovarian follicles Total RNA was isolated from ovarian follicles collected from different stages, cDNA were prepared and real time PCR was performed using ovary specific primer of *cyp19a1a* gene. The value obtained in the vitellogenic stage was set to 1 and the other values were measured by comparison to that value. The GAPDH was used as housekeeping gene. (B) Aromatase activity was measured by the conversion of [^{3}H] testosterone to [^{3}H] E_2 in different stages of follicles of *T. fasciata*. Ovarian follicles were incubated in the presence of [^{3}H] testosterone (1×10^6 c.p.m., 154 pmol) for 6 h. The amount of converted [^{3}H] E_2 (pmol/mg tissue homogenate) was measured, the value obtained in the vitellogenic stage was set to 1 and the other values were measured by comparison to that value. Each value represents the mean ± SD of nine fish. Different letters are significantly different from each other ($p \leq 0.05$).

Immunohistochemical localization of aromatase and SF-1 protein in the different stages of the ovary

Using ovary specific antibody of aromatase, present study tried to localize the aromatase protein in the different stages of ovary. Results (Fig. 5A) shows that high amount of aromatase was localize in the cellular layer of oocytes in the vitellogenic stage. The amount of aromatase was lower down in postvitellogenic and post-GVBD stages and were absent in the preparatory and post-spawning stages of the ovary.

SF-1 does not show the similar pattern like aromatase. More or less similar amount of SF-1 was found in cellular of the vitellogenic, post-vitellogenic and post-GVBD stages oocytes (Fig. 5B). However, SF-1 was absent in the preparatory and post-spawning stages.

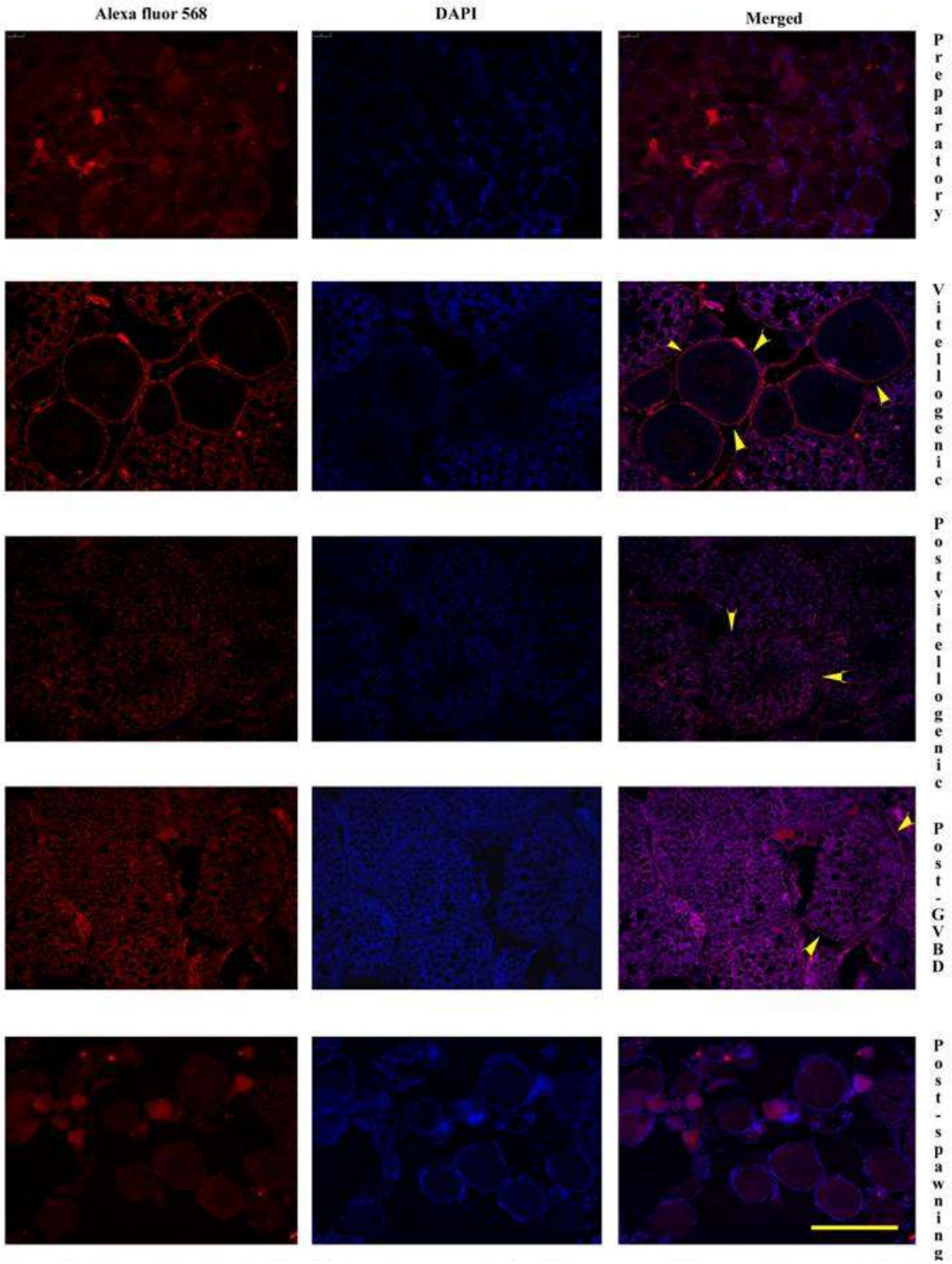

Fig. 5A Immunohistochemical localization of aromatase in the different stages of the ovary. Aromatase is localized in the follicular cells surrounding the oocyte (arrow). Fluorescein labeled secondary antibody of aromatase appears as red colour (left column) and DAPI stained nuclei appear as blue (middle column). Right side column is the merged photograph of these two. High amount of aromatase localization was found in vitellogenic stage follicles which are lower down in the postvitellogenic and post-GVBD stages. Scale bar in figure is 200 µm. The labels in the right side of each horizontal panel indicates the stages of the ovary.

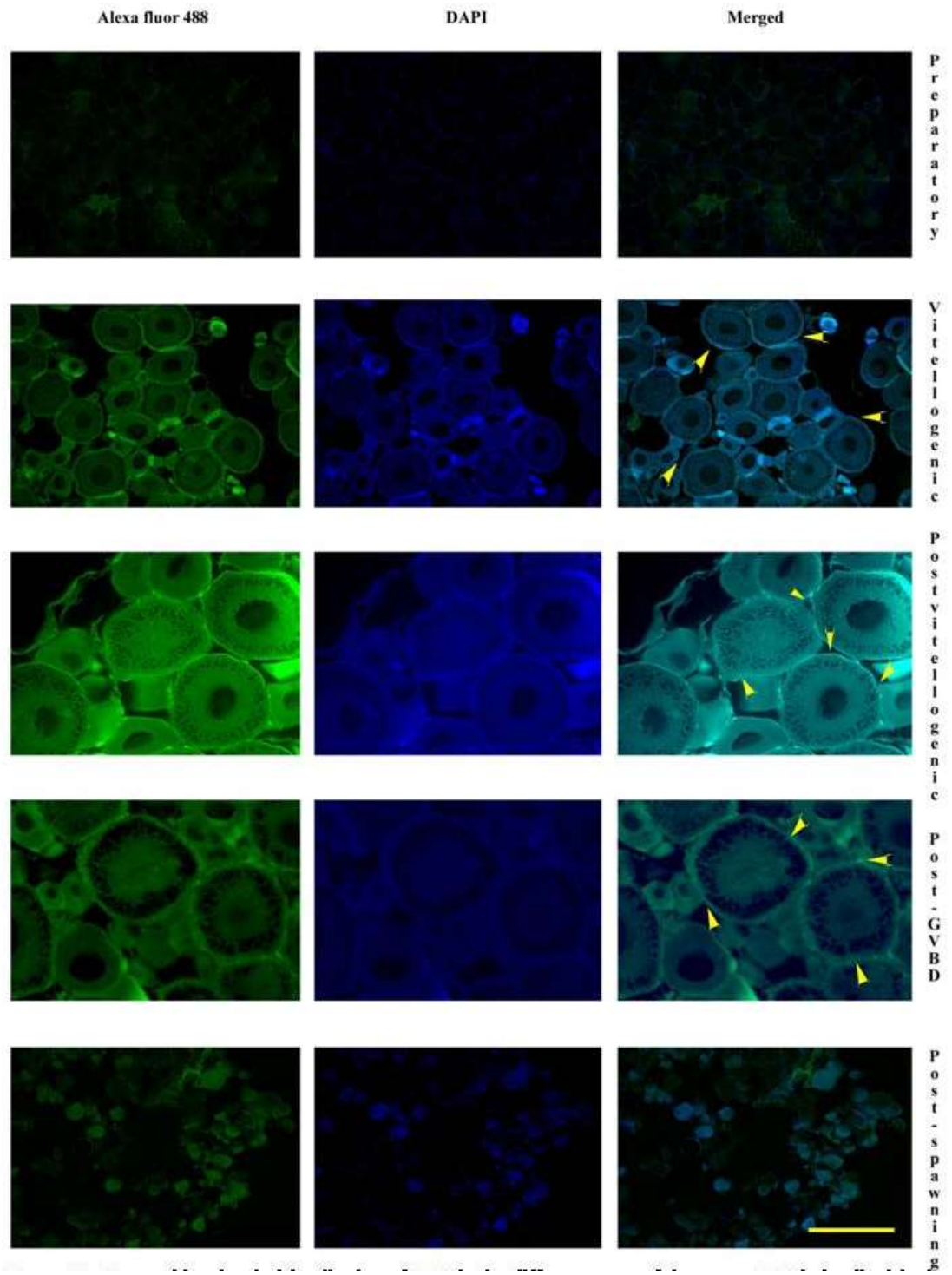

Fig. 5B Immunohistochemical localization of SF-1 in the different stages of the ovary. SF-1 is localized in the follicular cells surrounding the oocyte (arrow). Fluorescein labeled secondary antibody of SF-1 appears as green colour (left column) and DAPI stained nuclei appear as blue (middle column). Right side column is the merged photograph of these two. High amount of SF-1 localization was found in vitellogenic, postvitellogenic and post-GVBD stage follicles. Scale bar in figure is 200 μm. The labels in the right side of each horizontal panel indicates the stages of the ovary.

Discussion

The present study describes the histological features of the ovaries throughout the year and records the plasma profiles of both gonadotropins i.e. FSH and LH of *T. fasciata*. Furthermore, for the first time, this study shows the histochemical localization of aromatase in correspondence to the *cyp19a1a* gene expression and SF-1 in the different developmental stages of the ovary.

The monthly variation of GSI is correlated with its maturity stages and is one of the indicator of the spawning periodicity of the teleost. The GSI value of *T. fasciata* shows increased value from the month of April and reached to its single peak in the month of August. This value decreased abruptly in the month of October, which indicates the onset of spawning in that month. In this study, the developmental stages of the oocytes were divided into seven stages on the basis of their macroscopic and microscopic characteristics (Fig. 1 and Table 2). Similar types of developmental stages were found in other fishes also (Nagahama, 1983; Mayer *et al*., 1988; West, 1990; Treasurer, 1990; Fishelson *et al*., 1996; Unal, 1999; Verma, 2013). Co-appearance of post-GVBD stage oocytes and single GSI peak in the month of September indicates the single spawning season of *T. fasciata*.

This study documented the seasonal cycles of gonadotropins (FSH and LH) in plasma as they relate to gonadal development and reproductive behavior in natural population of *T. fasciata*. Fig. 2 presented a graphic summary of this cyclicity. Results clearly showed that in the month of January and February, when the ovary of female *T. fasciata* was very small and contained mostly immature stage follicles; both gonadotropins (FSH and LH) levels were very low in this stage. From March, concentrations of both gonadotropins in plasma began to rise in correspondence with slightly increased GSI and FSH reached their highest value in May-June, coinciding with the prepondance of vitellogenic follicles in the ovary. After July, GSI continued to increase further and reached highest value in August and at this stage plasma LH increased further. In the month of September, GSI showed a moderately increased value and plasma LH reaches its maximum peak in this time. From October onwards, GSI decreased drastically along with FSH and LH.

Thus, increased FSH levels were found in early stages of folliculogenesis whereas LH surge occurs after the postvitellogenic stages. This result indicates that early stage of folliculogenesis and steroidogenesis of *T. fasciata* like other fresh water teleosts is regulated by

GTH-I which is comparable to FSH; maturation and ovulation is regulated by GTH-II which is LH-like. Thus, our results supports the two gonadotropin theory in regulating folliculogenesis and steroidogenesis in female *T. fasciata* like other fishes already reported by many authors (Suzuki *et al*., 1988; Planas *et al*., 1993; Planas *et al*., 2000; Ko *et al*., 2007; Paul *et al*., 2009; Pramanick *et al*., 2013).

Aromatase activity is essentially important for the conversion of T to E_2 in the ovarian developmental processes in teleost (Guiguen *et al*., 2010; Roy Moulik *et al*., 2016; Miao *et al*., 2011). Present study shows that aromatase becomes highly active in the vitellogenic stage; in postvitellogenic and post-GVBD stages this activity becomes quiescent, and in spawning stage it was inactivated again just like the preparatory stage. Both localization and gene expression study of aromatase shows the similar type of results which corroborated with the published data in other fishes (Wong *et al*., 2006; Barney *et al*., 2008; Roy Moulik *et al*., 2016; Pal *et al*., 2018; Cheshenko *et al*., 2008; Kishida and Callard, 2001; Kagawa *et al*., 2003).

Localization of SF-1 does not correlate with the aromatase expression and localization. SF-1 is found in the vitellogenic, postvitellogenic and post-GVBD stages also. It is reported that ovarian *cyp19a1a* contains SF-1 regulatory element in the promoter region (Callard *et al*., 2001; Kazeto *et al*., 2001), which promotes the expression of StAR protein and steroidogenic enzyme like P450 aromatase in gonad (Roy Moulik *et al*., 2016; Pal *et al*., 2018; Hu *et al*., 2001; Das & Mukherjee, 2013).

The observed results in the present study regarding SF-1, *cyp19a1a* and E_2 synthesis supports the idea that in vitellogenic stage, SF-1 regulates the *cyp19a1a* expression and E_2 synthesis. As in vitellogenic stage, FSH is a key gonadotropin, so FSH may have significant role in aromatase activity and E_2 production. Relatively lower amount of aromatase localization in the postvitellogenic and post-GVBD stages were observed in immunohistochemistry study does not correlate with the SF-1 which is highly visible in both postvitellogenic and post-GVBD stage like the vitellogenic stage follicles. The high amount of SF-1 in both postvitellogenic and post-GVBD stages indicate that SF-1 activity may be regulated by both FSH and LH.

This study, for the first time reported about the changes of gonadotropins (FSH and LH) in relation to the ovarian stages of *T. fasciata*. Furthermore, this study first reports about the localization of SF-1 and expression of *cyp19a1a*, aromatase activity and its localization in the different stages of the teleost ovary. All these preliminary data about the reproductive biology of

T. fasciata may be useful to understand the interaction between gonadotropins, steroids, aromatase and SF-1 in teleost fishes and may help to experiment further by using this fish as model organism.