

# Dimorphic distribution of progestins and cortisol in the brain of Indian stinging catfish *Heteropneustes fossilis* (Bloch) during different reproductive phases

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**Abstract:** In the brain, steroids are synthesized *de novo* from cholesterol through mechanisms which are not dependent on peripheral steroidogenic glands. These steroids, which are termed as neurosteroids accumulate within brain of several vertebrates including various teleost species. Distribution of steroids remain conserved after removal of peripheral steroids. Still a comprehensive study on the distribution of neurosteroids is lacking in the seasonal breeding teleosts. In the present study, our objective was to measure progestins (pregnenolone, progesterone, and 17-OH-progesterone) and cortisol in brain of male and female Asian stinging catfish *Heteropneustes fossilis*, throughout their reproductive phases, using specific ELISA kits. Catfish *H. fossilis* is a seasonal breeder which serve as an excellent model to investigate physiological responses of neurosteroids and their regulatory mechanism. Our study showed that there was significant differential distribution of pregnenolone, progesterone, 17-OH-progesterone and cortisol levels with respect to season and breeding phases of the catfish. Progestins and cortisol levels are present in both male and female and varied differentially in a

phase dependent manner. Among all progestins, value of pregnenolone was maximum followed by progesterone, cortisol and 17-OH-P<sub>4</sub>. The study suggested that seasonal changes in the progestins and cortisol of catfish brain may be independent of peripheral steroidogenic gland and might be important in inducing behaviour or morphological changes associated with breeding phases for regulation of reproduction.

**Keywords:** brain, catfish, cortisol, neurosteroids, progestins

## I. INTRODUCTION

Steroids are mainly secreted by the gonads and cortex region of adrenal gland, regulating diverse processes of the tissues, including gonads, brain, liver and skeletal muscles (Chaube and Mishra, 2012). They cross blood brain barrier due to their lipophilic nature. These derivatives of cholesterol act on glial cells and neurons, helps in survival, regulation and neuronal connectivity, in the brain and spinal cord (Pellegrini et al., 2016; Diotel et al., 2018). Various scientists have reported an extensive steroid metabolism occurring in the brain and its

regions, suggesting presence of enzymes necessary for steroid biosynthesis (Diotel et al., 2018). Studies on mammalian and non-mammalian vertebrates have shown that neurosteroids can affect multiple neuroendocrine functions through intracellular receptors, which regulates protein synthesis (Callard et al., 1978; Zohar et al., 2009; Pellegrini et al., 2016; Diotel et al., 2018). Recently, it has been shown that the teleost brain produces neurosteroids, and received global attention (Okubo et al., 2011; Chaube and Mishra, 2012; Chaube et al., 2015; Mishra and Chaube, 2016; Mishra and Chaube, 2017).

Besides studies on key steroid biosynthesizing enzymes, other steroid hormone levels have been reported in various vertebrate species such as pregnenolone, progesterone, its derivatives which crosses the blood-brain barrier and concentrated in brain of mammals (Robel et al., 1987), aves (Usui et al., 1995) and amphibians (*Cynops pyrrhogaster*; Inai et al., 2003). Measurement of steroid levels allows comparisons between blood and brain, which are useful in determining which tissues are synthesizing or metabolizing steroids (Hojo and Kawato, 2018). Glial cells are considered to play major role in neurosteroid formation and metabolism. Oligodendrocytes and astrocytes are the primary site for steroid biosynthesis (Hojo et al., 2009; Konkle and McCarthy, 2011). In catfish, the gonads and adrenal gland are the main site for steroid biosynthesis, little is known about its biosynthesis in brain. There is growing evidence indicating that neurosteroids might modulate neurogenesis in the developing or adult central nervous system and this can lead to sexual differentiation of certain structures which are involved in sexual behaviour and neuroendocrine control of reproduction (Morris et al., 2004). Further, there is lack of knowledge on the distribution of these steroids in the brain throughout its reproductive cycle in seasonal breeding teleosts.

Numerous studies by various researchers reported that steroidogenic enzymes and steroid metabolism (Okubo et al., 2011; Mishra and Chaube, 2012, 2016, 2017; Chaube et al., 2015) exist in various teleost group. Progesterone *de novo* synthesis in the nervous system and cerebellum has been recognized in studies on humans and several species e.g. rat, mouse, bird, ovine fetus, newt (Petraatos et al., 2000). Seasonally breeding teleosts like catfish serve as an excellent model for understanding functional significance of neurosteroids. However, from our previous studies on catfish *H. fossilis*, it has been reported that catfish brain is the site of steroid biosynthesis as we have reported that key steroid biosynthesizing enzyme  $3\beta$ -HSD and aromatase is localized in the different regions of the catfish *H. fossilis* brain (Chaube and

Mishra, 2012; Chaube et al., 2015; Mishra and Chaube, 2016; Mishra and Chaube, 2017) and this may be involved in the regulation of reproductive activity through its differential distribution.

It is hypothesized that besides other peripheral steroids, neurosteroids can be synthesized *de novo* (Corpechot et al., 1981; Chaube and Mishra, 2012; Chaube et al., 2018). It has been reported recently that these hormones are the signals that modulate neural function and development, growth, maturation and differentiation (Mishra and Chaube, 2016; Hojo and Kawato, 2018). In the present study, our objective was to measure progestins (pregnenolone, progesterone, and 17-OH progesterone) and cortisol hormone in the catfish *H. fossilis* brain during different reproductive phases. Catfish *H. fossilis* (Bloch) is a seasonal breeder of economic importance in the aquaculture sector and it is also good model for reproductive physiology study. It has been well established that brain - pituitary -gonadal axis is important for the regulation of reproduction in most teleost species. The data obtained from this study will provide information related to changes in levels of neurosteroids/local progestins and cortisol hormone, its role and significance with respect to different seasons in both male and female catfish.

## II. MATERIALS AND METHODS

### A. Chemicals

Pregnenolone (FR E-2700), Progesterone (FR E-2500), 17-OH-Progesterone (FR E-2800), and Cortisol (MS E-5000) were purchased from Labor Diagnostika Nord GmbH & Co. KG; Germany. Diethyl ether SRL (Sisco Research Laboratories Pvt. Ltd, Mumbai, India). Nanopure water (Barnstead International, Dubuque, and IO, USA) and HPLC grade methanol (SRL, Mumbai, India).

### B. Collection and acclimatization of animals

*H. fossilis* is a fresh water, air breathing catfish whose reproductive cycle is divided in to five phases: resting or quiescent (November - January), preparatory or early vitellogenic (February–April), prespawning or late vitellogenic (May - June), spawning or vitellogenic (July- August) and post spawning or post-vitellogenic (September - October) phases. Sexually mature male and female catfish (30-60g) were purchased from a local fish market from Varanasi and were maintained in tanks with circulating water under natural conditions for two weeks and fed daily with goat liver *ad libitum*. After the acclimatization, brain tissues were collected

by decapitation between 9.00-11.00am (Chaube and Joy, 2002), and were stored at  $-80^{\circ}\text{C}$  till processed for assay. All procedures performed in animals were approved by the Institutional Animal Care and Use Committee (F.Sc./IAEC/2016-17/113S) of Banaras Hindu University, Varanasi. Intensive care was given to prevent cruelty of any kind.

### *C. Experimental Design*

#### *1) Steroid hormone extraction and estimation*

Both male and female catfish were sacrificed and whole brain (wt. 80-90 mg;  $n=5$ ) was collected. Brain tissues were homogenized and processed for steroid extraction by following protocol described in Chaube et al., (2020). After steroid extraction samples were processed for estimation of steroid hormones by using the LDN GmbH, ELISA kit according to the manufacturer's protocol and absorbance was taken at 450 nm using a Multiscan EX (Thermo Electron Corporation, USA) ELISA reader.

#### *2) Pregnenolone ( $P_5$ ) assay*

The assay was carried out using an ELISA kit according to manufacturer's instruction. Briefly, samples were reconstituted and 50  $\mu\text{L}$  sample and pregnenolone ( $P_5$ ) standard (0, 0.1, 0.4, 1.6, 6.4, 25.6 ng/ml) were transferred to the anti-Pregnenolone Immunoglobulin G-coated plate. The immunoreactions were started by adding 100  $\mu\text{l}$  of Pregnenolone-HRP conjugate into each well, followed by incubation at  $37^{\circ}\text{C}$  for 1 h. The content from each plate was removed and washed with 300  $\mu\text{l}$  of wash buffer, 5 times. Next 150  $\mu\text{l}$  of TMB substrate was dispensed into each well and incubated at room temperature for 10-15 min in dark. Color development was stopped by adding 50  $\mu\text{l}$  of stop solution (1 mol/L or 1M sulphuric acid). Absorbance was taken at 450 nm using a Multiscan reader (Thermo Electron Corporation, USA).

#### *3) Progesterone ( $P_4$ ) assay*

The assay was carried out using an ELISA kit for  $P_4$ . The procedure of assay was as described in Chaube et al., (2020). Absorbance was taken at 450 nm using a Multiscan reader (Thermo Electron Corporation, USA).

#### *4) 17-OH- Progesterone (17-OH $P_4$ ) assay*

The assay was carried out using an ELISA kit for 17-OHP. Briefly, 50  $\mu\text{l}$  each of 17-OHP standard (0, 0.15, 0.5, 1.5, 3, 7.5, 20 ng/ml) and samples were transferred to the anti-17-OH- $P_4$  Immunoglobulin G-coated plate. The immunoreactions

were started by adding 150  $\mu\text{l}$  of 17-OHP HRP conjugate, followed by incubation at  $37^{\circ}\text{C}$  for 1 h. The content from each plate was removed and washed with 300  $\mu\text{l}$  of distilled wash buffer, 5 times. Next 150  $\mu\text{l}$  of TMB substrate was dispensed into each well and incubated at room temperature for 10-15 min in dark. Color development was stopped by adding 50  $\mu\text{l}$  of stop solution (1 mol/L or 1M sulphuric acid). Absorbance was taken at 450 nm using a Multiscan reader (Thermo Electron Corporation, USA).

#### *5) Cortisol (F) assay*

The assay was carried out using an ELISA kit for cortisol. The procedure of assay was as described in Singh and Chaube, (2019). Absorbance was taken at 450 nm using a Multiscan reader (Thermo Electron Corporation, USA).

#### *6 Validation of steroid hormones*

The response-concentration curves for progesterone, cortisol, pregnenolone and 17-OH- $P_4$  were linear over 0.1-60 ng/ml, 0.5-60 ng/ml, 0.1-25.6 ng/ml and 0.1-20 ng/ml range, respectively. The sensitivity of the progesterone, cortisol, pregnenolone and 17-OH- $P_4$  assays was 0.1 ng/ml, 0.4  $\mu\text{g/ml}$ , 0.05 ng/ml and 0.11 ng/ml, respectively. Known concentrations of  $E_2$ , testosterone, progesterone, cortisol, pregnenolone and 17-OHP were processed in the same manner as samples. Percentage recovery of  $P_4$  (15.4 ng/ml added)  $97 \pm 2\%$  ( $n = 5$ ), cortisol (15.3  $\mu\text{g/dl}$  added)  $93.8 \pm 1.6\%$  ( $n = 5$ ), pregnenolone (6.30 ng/ml added)  $98 \pm 2\%$  ( $n = 5$ ) and 17-OHP (16.4 ng/ml)  $91.8 \pm 1.6\%$  ( $n = 5$ ). The coefficients of inter and intra-assay variations were 11.8 %, 12.6 % for  $P_4$ , 5.6 %, 5.76% for cortisol 10.2 %, 11.2 % for pregnenolone and 5.2 %, 7.3 % for 17-OHP.

### III. STATISTICAL ANALYSIS

For statistical analysis SPSS 16.0 software was used (SPSS Corporation, USA). The data were checked for normal distribution by applying Kolmogorov-Smirnov test. The normality distribution and homogeneity of variance were satisfied. The data were expressed as mean  $\pm$  SEM and were analysed with two-way ANOVA followed by Newman-Keuls' multiple post hoc comparison test,  $P < 0.001$  or  $P < 0.05$  was accepted as statistically significant.

### IV. RESULTS

#### *A. Changes in level of pregnenolone ( $P_5$ )*

The study showed an overall significant variation in the P<sub>5</sub> level of brain in both the sexes of catfish *H. fossilis* during different reproductive phases (**Fig.1**; two-way ANOVA,  $F_{\text{Season}}= 7.167$ ,  $F_{\text{Sex}}= 31.46$ ,  $F_{\text{Season} \times \text{Sex}} = 10.97$ ,  $P<0.001$ ). Dimorphic differential variation was observed and it was season dependent in both sexes. In male, highest level was in preparatory and postspawning, moderate in resting and spawning and lowest in prespawning season. There was no significant variation in the level of P<sub>5</sub> during resting, spawning and during preparatory and post-spawning phases of the reproductive stage. In female, highest level was in resting phase followed by spawning, preparatory and post spawning, it was lowest in pre-spawning phase ( $P<0.05$ ).

#### B. Changes in level of progesterone (P<sub>4</sub>)

Our study showed an overall significant variation in P<sub>4</sub> levels of brain in both the sexes of catfish *Heteropneustes fossilis* during different reproductive phases, (**Fig.2**; two-way ANOVA,  $F_{\text{Season}}=102.08$ ,  $F_{\text{Sex}}= 103.01$ ,  $F_{\text{Season} \times \text{Sex}} = 21.7$ ;  $P<0.001$ ). In male there was decreasing trend from resting to post-spawning season. The level was highest in preparatory and prespawning season with no significant difference and lowest in the postspawning. However, there was significant season dependent decrease. In female, P<sub>5</sub> level decreased from resting to postspawning phase ( $P<0.05$ ) in a season dependent manner, highest in resting and lowest in post spawning phase.

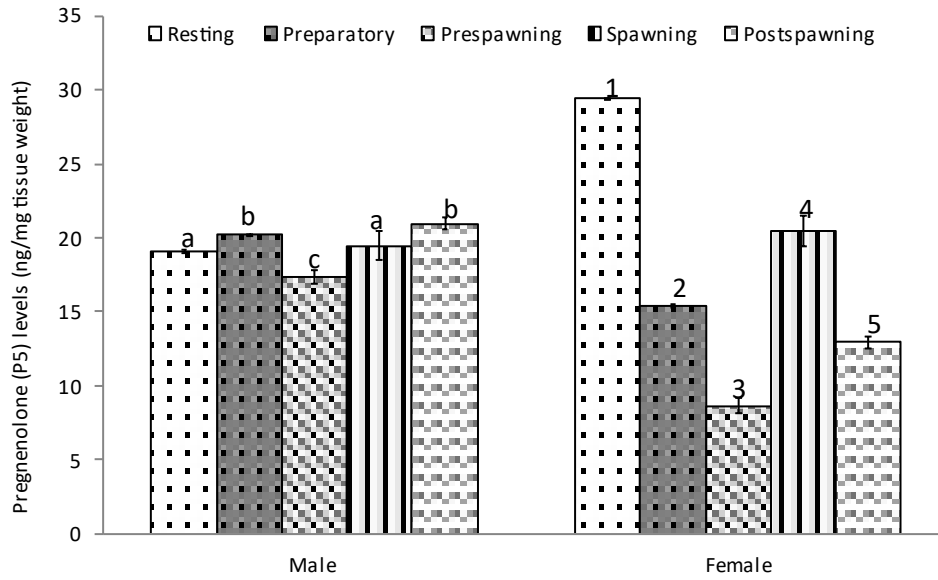
#### C. Changes in level of 17-OH-Progesterone (17-OH-P<sub>4</sub>)

The study showed an overall significant variation in brain 17-OH-P<sub>4</sub> level of both the sexes (**Fig.3**; two-way ANOVA,  $F_{\text{Season}}=136.20$ ,  $F_{\text{Sex}}= 33.6$ ,  $F_{\text{Season} \times \text{Sex}} = 87.32$ ;  $P<0.001$ ). There was differential variation in the level 17-OH-P<sub>4</sub> in both the sexes. In male, it was highest in preparatory phase compared to other phases. In male, it increased significantly with respect to resting phase and was lowest in post-spawning phase. In female, it is highest in preparatory and lowest in resting phase ( $P<0.05$ ). However, in comparison to other steroids the concentration of hormone was less almost negligible in both male and female.

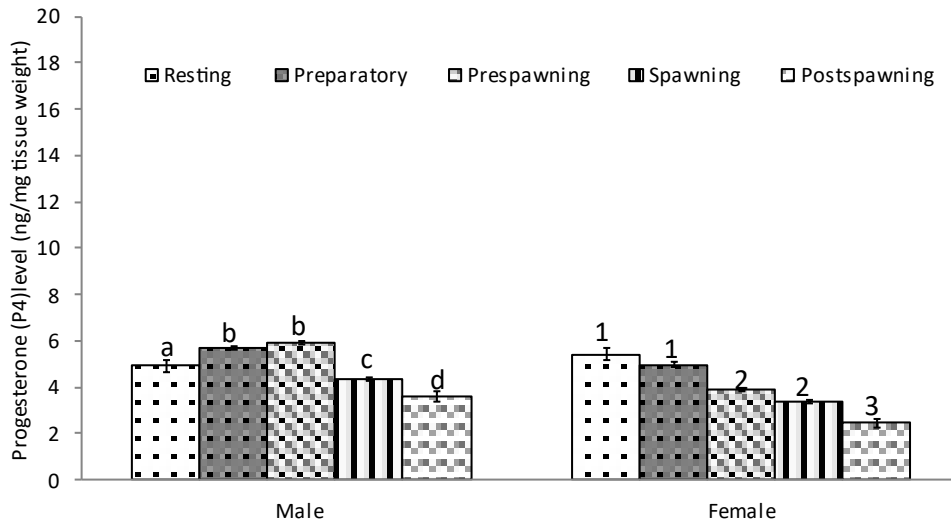
#### D. Changes in level of cortisol

Cortisol level showed an overall significant variation in both the sexes during different reproductive phases (**Fig. 4**; two-way ANOVA,  $F_{\text{Season}}=81.61$ ,  $F_{\text{Sex}}= 74.59$ ,  $F_{\text{Season} \times \text{Sex}} = 18.0$ ;  $P<0.001$ ). In male, there was an increasing trend in level from resting to prespawning season and then decreased during spawning and postspawning phases, highest in prespawning and lowest in postspawning. In female, level decreased from resting to postspawning season significantly ( $P<0.05$ ).

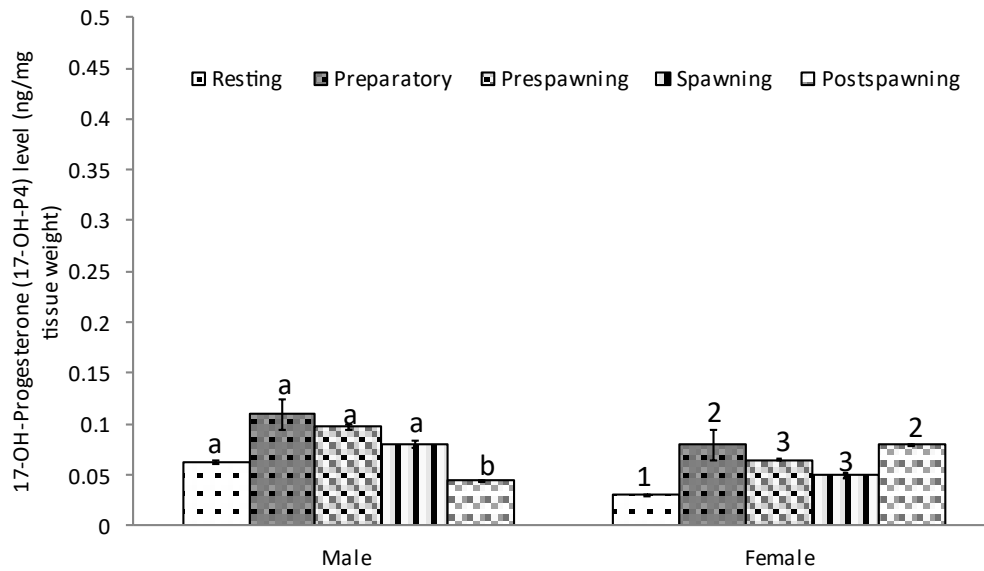
In the present study, we represented our data in histogram to show the trend of steroid hormones with respect to season and sex (Fig. 1 to Fig. 4). Besides this the numerical values were represented in table separately for both male and female. (Table 1: male and Table 2 : female)



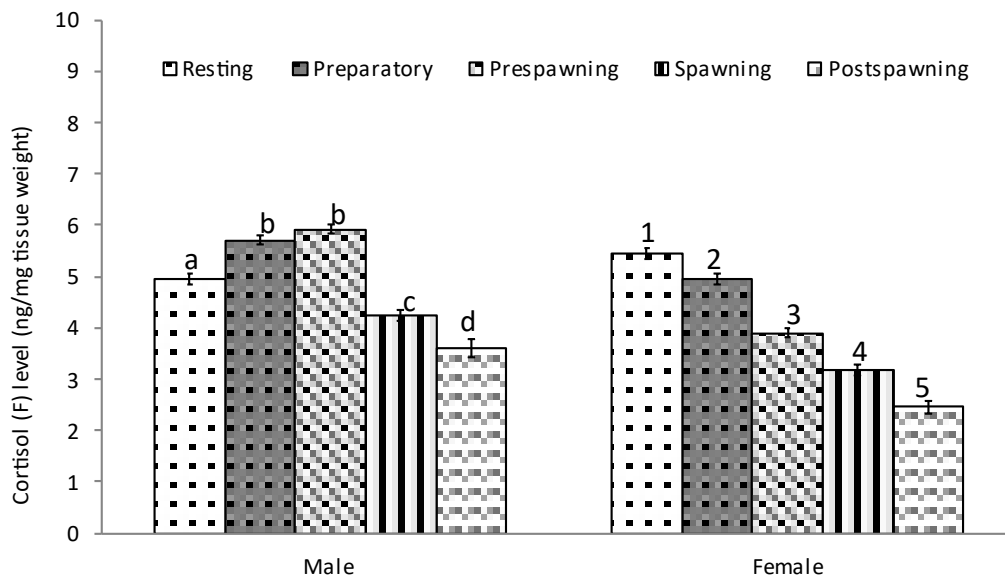
**Fig. 1.** Dimorphic and reproductive stage dependent changes in the pregnenolone (P<sub>5</sub>) levels of catfish *H. fossilis*. Data were expressed as mean ± SEM (N=5). Data were analysed by two-way ANOVA followed by Newman Keuls' test (P<0.05). Different alphabets show significant difference between male and numbers between females.



**Fig. 2.** Dimorphic and reproductive stage dependent changes in the progesterone (P<sub>4</sub>) levels of catfish *H. fossilis*. Data were expressed as mean ± SEM (N=5). Data were analysed by two-way ANOVA followed by Newman Keuls' test (P<0.05). Different alphabets show significant difference between male and numbers between females



**Fig. 3.** Dimorphic and reproductive stage dependent changes in the 17-OH-Progesterone (17-OH-P<sub>4</sub>) levels of catfish *H. fossilis*. Data were analysed by two-way ANOVA followed by Newman Keuls' test ( $P < 0.05$ ). Different alphabets show significant difference between male and numbers between females.



**Fig. 4.** Dimorphic and reproductive stage dependent changes in the cortisol levels of catfish *H. fossilis*. Data were expressed as mean  $\pm$  SEM (N=5). Data were analysed by two-way ANOVA followed by Newman Keuls' test ( $P < 0.05$ ). Different alphabets show significant difference between male and numbers between females.

Table 1: Showing values of various steroid hormones in the brain of male catfish *H. fossilis* during different reproductive phases.

	Pregnenolone	Progesterone	17-OH-Progesterone	Cortisol
Resting Phase	19.09±1.28 <sup>a</sup>	4.9±0.38 <sup>a</sup>	0.063±0.002 <sup>a</sup>	4.94±0.08 <sup>a</sup>
Preparatory Phase	20.18±0.92 <sup>b</sup>	5.68±0.42 <sup>b</sup>	0.11±0.003 <sup>a</sup>	5.71±0.09 <sup>b</sup>
Prespawning Phase	17.35±0.87 <sup>c</sup>	5.92±0.50 <sup>b</sup>	0.097±0.005 <sup>a</sup>	5.92±0.067 <sup>b</sup>
Spawning Phase	19.56±1.02 <sup>a</sup>	4.34±0.62 <sup>c</sup>	0.08±0.001 <sup>a</sup>	4.23±0.74 <sup>c</sup>
Post-Spawning	20.98±1.67 <sup>b</sup>	3.59±0.41 <sup>d</sup>	0.045±0.002 <sup>b</sup>	3.59±0.04 <sup>d</sup>

Table 2: Showing values of various steroid hormones in the brain of female catfish *H. fossilis* during different reproductive phases.

	Pregnenolone	Progesterone	17-OH-Progesterone	Cortisol
Resting Phase	29.39±1.56 <sup>1</sup>	5.4±0.61 <sup>1</sup>	0.03±0.001 <sup>1</sup>	5.45±0.01 <sup>1</sup>
Preparatory Phase	15.38±0.82 <sup>2</sup>	4.95±0.35 <sup>1</sup>	0.08±0.002 <sup>2</sup>	4.96±0.007 <sup>2</sup>
Prespawning Phase	8.65±0.97 <sup>3</sup>	3.87±0.31 <sup>2</sup>	0.065±0.004 <sup>3</sup>	3.9±0.06 <sup>3</sup>
Spawning Phase	20.46±1.05 <sup>4</sup>	3.36±0.17 <sup>2</sup>	0.05±0.005 <sup>3</sup>	3.18±0.01 <sup>4</sup>
Post-Spawning	12.94±0.65 <sup>5</sup>	2.44±0.18 <sup>3</sup>	0.08±0.003 <sup>2</sup>	2.45±0.007 <sup>5</sup>

The numerical value in the table showed that pregnenolone value was in the range of 17.35-20.98, progesterone 3.59-5.92, 17-OH-progesterone 0.045-0.11, and cortisol 3.59-5.92 in the male brain. In female brain the range was as follows:

pregnenolone 29.39-8.65, progesterone 2.44-5.4, 17-OH-progesterone 0.03-0.08, and cortisol 2.45-5.45. The order of measured hormone was pregnenolone>progesterone>cortisol>17-OH-progesterone.

## V. DISCUSSION

Brain, gonads and interrenals (adrenocortical homolog) are steroidogenic sites in teleost, as in higher vertebrates. Teleosts have long been known for their high brain aromatase and 5 $\alpha$ -reductase activities, but recent data now document the capacity of the fish brain to produce a large variety of sex steroids (Coumailleau et al, 2015; Diotel et al, 2018; Kah, 2020). In the present study, we showed presence of progestins and cortisol in the whole brain of male and female catfish *H. fossilis* during different reproductive phases (quiescent to post spawning) which exhibited significant season dependent differential variation. In most teleosts, key steroidogenic enzymes such as 3 beta-hydroxy steroid dehydrogenase (3 $\beta$ -HSD), 17 beta -hydroxysteroid dehydrogenase (17 $\beta$ -HSD), 5 $\alpha$ -reductase and aromatase was reported in brain by using various techniques like immunolocalization, *in-situ* hybridization, mRNA expression and biochemical analysis (Sakamoto et al, 2001; Do Rego et al, 2009; Mishra and Chaube, 2016; 2017). Several reports are available related to the presence of neurosteroids in higher vertebrates including mammals, avians, amphibians (Tsutsui, 2001). However, studies are lacking regarding presence of neurosteroids in lower vertebrates with respect to changes in the reproductive stages mainly in seasonal breeders.

Recently, localization and distribution of 3beta-hydroxysteroid dehydrogenase (3 $\beta$ -HSD) and aromatase in the brain of the catfish *Heteropneustes fossilis* have been demonstrated by using qPCR, enzyme assay and *in situ* hybridization technique (Chaube et al, 2015; Mishra and Chaube, 2017). On basis of this study it was hypothesized that catfish brain may have other steroids (progestins and cortisol) and differential variation of these steroids may be involved in various functions related to reproduction via brain-gonadal axis in catfish. Reports have been available on the differences in neurosteroids level in different strain of mouse brain (Tagawa et al., 2006). Progesterone, is produced in neuron only during neonatal life, which may be involved in the promotion of neuronal and glial growth and initiation of synaptic contact in the cerebellum (Tagawa et al., 2006). Similar to this our study also showed that progestins and cortisol levels are differentially distributed in the brain of catfish through different stages of reproductive cycle. Though, exact nature and mechanism of such variation is not known and study is scarce.

Recently, data presented by various research groups worldwide demonstrated that the brain of adult fish is able to *de novo* synthesize a wide variety of steroids from pregnenolone, suggesting that the substrates available for steroidogenesis can originate from local synthesis within the brain, and also from the conversion of peripherally produced precursors. Similar to this in the present study, pregnenolone concentration was highest among all steroids measured suggesting it as primary substrates for steroid biosynthesis (Diotel et al., 2018)

In our previous investigation, we have reported localization and expression of key steroidogenic enzymes, aromatase (rate limiting enzyme which converts androgen to estrogen), and 3 $\beta$ -HSD (enzyme which catalyzes dehydrogenation and isomerization of the  $\Delta^5$ -3 $\beta$ -hydrosteroids (pregnenolone and dehydroepiandrosterone) into  $\Delta^4$ -ketosteroids (progesterone and androstenedione, respectively) in the brain and its different regions in the catfish *H. fossilis* suggesting catfish brain as a site of steroidogenesis (Chaube et al., 2015; Mishra and Chaube, 2016; 2017). Further, 3 $\beta$ -HSD in preoptic area was reported in the frog *Rana ridibunda* (Do Rego et al., 2000) African lungfish *Protopterus annecteus* (Mathieu et al., 2001) and in zebra fish *Danio rerio* (Sakamoto et al., 2001).

Pregnenolone (P5) is an important hormone in neurosteroidogenesis, and is known to affect nerve cell growth and cognition function in vertebrates. In the present study, P5 showed significant dimorphic seasonal pattern in brain and its concentration was higher in brain than plasma (data not shown) throughout reproductive stages in both sexes. Female brain has more pregnenolone levels than male, highest in resting (quiescent) followed by spawning season and lowest in prespawning season. However, males have shown no varied changes from resting to prespawning season in levels of pregnenolone. Similar to our result, pregnenolone level were also higher in female brain than ovary and plasma in *Xenopus laevis* (Takase et al., 2013).

Seasonal changes in pregnenolone and progesterone levels in brain was reported in male newts, independent of peripheral steroidogenic glands and higher concentration of progestins in brain localization and biochemically enzyme activity of 3 $\beta$ -HSD in preoptic area, which are known to be involved in reproductive behaviour have been documented in urodele (Malacarne and Giacomini, 1980) and anurans species (Wada and Gorbman, 1997). In both the sexes of *Rana nigromaculata*, a seasonal breeding amphibian, concentration



of pregnenolone sulphate was found highest during active season, i.e. breeding phase (female) and post breeding phase (male) and low during quiescent season, i.e. hibernating phase and levels of pregnenolone were also different in brain from that of plasma (Takase et al., 2013). Significant dimorphic and differential variation of P5 in the catfish *H. fossilis* suggests its possible role in regulating neuroendocrine functions, which further needs detailed investigation.

P<sub>4</sub> (progesterone) is synthesized from pregnenolone and/or 17- $\alpha$ -hydroxypregnenolone, the cholesterol metabolites, that has been demonstrated by Matsumoto et al, (2003) and may act through non-genomic pathway in vertebrate brain via the involvement of progesterone membrane receptor (mPR $\alpha$ ). In catfish brain, progesterone level was highest in male than female and showed decreased pattern from preparatory to post spawning season in both the sexes. Levels of P<sub>4</sub> was dropped significantly in spawning and postspawning phase, similar results were reported in male catfish *H. fossilis* (Chaube and Mishra, 2012), which showed cessation of breeding and dormancy of brain – pituitary gonadal axis. Constant P<sub>4</sub> level were also detected by RIA, in quail brain during development and in adult, supported by mRNA expression study of 3 $\beta$ -HSD and enzyme activity, were involved in constant conversion of pregnenolone to progesterone in brain (Ukena et al., 2001). It was shown that progesterone is involved in many brain functions including certain behavioural patterns, adaptation to stress and neuroprotection (Gonzalez et al., 2002). In the present study, catfish brain contains progesterone in significant amount with significant season-dependent changes in both the sexes, suggesting its important role throughout the reproductive cycle. 17-OH-P<sub>4</sub> level was lowest in comparison to other steroids suggesting its metabolism from pregnenolone or progesterone.

The stress hormone cortisol is known to affect several aspects of cognition, including memory retention. Cortisol, the predominant corticosteroid in teleosts modulate the activity pattern of a hindbrain–spinal vocal pattern generator that directly establishes the temporal features of midshipman vocalizations (Bass and Ramage-Healey, 2008), in stressed and non-stressed rainbow trout Johansen et al., (2011) quantified mRNA expression of GR1, GR2 and MR in different brain regions. Cortisol is the main glucocorticoid in salmonid fish (Patiio et al., 1987) and has major effects on several tissues in the fish body, including the brain. This steroid hormone mediates the stress response and, in mammals, has been shown to play important roles in memory

and learning (Lupien et al., 1997). In the present study, cortisol was significantly higher in the prespawning and spawning phases in male brain than in female *H. fossilis* (Chaube and Mishra, 2012). Cortisol is implicated in a neuromodulatory role, regulating the main neural populations of the caudal telencephalon/anterior preoptic region and diencephalon that are involved in the regulation and secretion of gonadotropins and spawning behaviour (Satou et al., 1984). Besides these functions it has been reported that brain cortisol may be involved in establishing relation between stress axis and neurogenesis (Sorensen et al., 2013; Sadoul et al., 2018) and may be also responsible for sex determination and in regulation of fish physiology and behaviour (Olivotto and Geffroy, 2017)

## CONCLUSIONS

Thus, our present investigation showed that progestins and cortisol in the catfish *H. fossilis* brain might play vital role in regulating reproductive behaviour and other activities during quiescent and spawning phases. Further, there is need for more physiological experiments to be done in future, to verify exact function of these neurosteroids. However, it seems that these steroids are responsive to changes in the reproductive stages in both sexes and might be important in inducing behaviour and /or morphological changes associated with seasonal breeders.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

SM is a recipient of UGC-BSR Research Fellowships for meritorious students, Department of Zoology, Institute of Science, Banaras Hindu University. The partial financial assistance of DST-FIST and UGC-CAS for infrastructural support is acknowledged. This work was partially supported by a UGC Research grant (SR/34-436/2008) to RC, New Delhi.

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