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A comparison of steroid profiles in the testis and seminal vesicle of the catfish (*Heteropneustes fossilis*)

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ABSTRACT

In the present study, distribution of steroid hormones (estradiol-17β (E_2), testosterone (T), corticosteroids and progestins) in the testis and seminal vesicles (SV) of the catfish *Heteropneustes fossilis* were investigated in two seasons (pre-spawning and spawning phases) of the reproductive cycle. The data showed that the levels of the steroid hormones varied significantly in both reproductive phases and exhibited organ-related differences. Sex steroid levels were highest in the pre-spawning phase and lowest in the spawning phase. The concentration of E_2 and cortisol was highest in the testis. However, E_2 level was higher in the SV compared to the testis in the pre-spawning phase and during spawning phase there was no significant difference in the E_2 level of testis and SV. Seminal vesicle and testis recorded higher levels of cortisol in the spawning phase. The concentration of corticosterone was significantly higher in testis during pre-spawning phase and in seminal vesicle during the spawning phase. 21-deoxycortisol was higher in the pre-spawning phase in the SV but not in the testis and deoxycorticosterone was significantly higher in the pre-spawning phase than spawning phase. Progesterone was high in the pre-spawning phase and low in all tissues in the spawning phase. 17-P_4 concentration was the highest in both SV and testis during spawning phase. In the testis 17, 20β-DP concentration was the highest in the pre-spawning phase. In contrast, in the SV, 17, 20β-DP was lowest during pre-spawning phase. This study shows high levels of corticosteroid profile in the testis and SV. The physiological significance of the steroids other than T is not clear at present. However, knowledge of seasonal profile of key steroid hormones in the male catfish may be helpful in monitoring reproductive capability and important for fish breeding in captivity.

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1. Introduction

Steroids are major hormonal messengers synthesized by gonads and adrenal cortex and play key role in sexual and tissue differentiation, metabolism, osmoregulation and germ cell growth, maturation and release. These are degraded largely in the liver to form water soluble metabolites, which are excreted out. The hormonal steroids have tissue-specific distribution, estrogens (C-18) are female hormones produced mainly in ovaries, androgens (C-19) are male hormones produced by the testis and corticosteroids (C-21) are synthesized by the adrenal cortex (interrenals in teleosts). Regardless of their structurally specific tissue distribution, the steroids have a common pathway of biosynthesis and metabolism as indicated by the presence of intermediates or metabolites which occur in smaller amounts. In recent years, it has been demonstrated that the steroid hormones occur not only in conventional endocrine glands but in a variety of other tissues such as brain, liver, adipose tissue, bone, skin and act in a paracrine or autocrine manner [1–6].

In teleosts, as a result of GTH-I (gonadotropic hormone-I) stimulation, the testis or ovary respond by synthesizing the steroid precursor pregnenolone from cholesterol, followed by the successive production of (among others) progesterone (P_4), 17-hydroxyprogesterone (17-P_4), androstenedione and testosterone (T) [7]. During vitellogenesis, the enzyme aromatase converts a portion of the available T into estradiol-17β (E_2). However, as ovarian maturation approaches, a switch to GTH-II synthesis in the pituitary gland results in a decline of T and E_2 synthesis, and a concomitant surge in the production of maturational progestogens [8,9] occurs. Steroid levels are generally low during the non-reproductive period, but increase gradually throughout gametogenesis and decline abruptly thereafter. The predominance of T, 11-

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ketotestosterone (11-KT) and E_2 in initiating and regulating seasonal reproductive events are most intensively studied. Corticosteroids are mainly synthesized in the interrenal tissue. Recent studies have shown that female and male gonads (ovary, testis, and seminal vesicle) may have the capacity to produce main corticosteroids, enzymes responsible for their synthesis and supports the hypothesis of gonadal corticosteroidogenesis [10–12].

Accessory sex organs in the form of seminal vesicles (SVs), testicular glands and testicular blind pouches are present in catfishes (Siluriformes) and gobies and blennies (Perciformes). Biochemical studies have shown that the SV is capable of both steroid biosynthesis and glucuronide formation [13]. SVs in teleost like catfish have both exocrine and endocrine like functions [14–17]. The SVs showed marked annual variations in their structure and secretory activity that can be correlated with the testis [18]. In both *C. batrachus* (walking catfish) and *H. fossilis* (stinging catfish), total proteins, hexosamines and fructose showed significant annual variations with their concentrations increasing during gonadal recrudescent phase and decreasing during gonadal quiescent phase with the exception of glucose [19,20]. Furthermore, the concentrations of these variables except glucose can be positively correlated with plasma testosterone titre. However, there are studies lacking on the distribution of steroid hormones (E_2, T, progestins and coticosteroids) which play important role in regulating reproductive status and spawning behaviour.

Catfish *Heteropneustes fossilis* is a freshwater air breathing, seasonal breeder. It is popularly known as 'stinging catfish', belongs to a group of fish having characters close to primitive bony fishes [21]. It is an economically edible fish and its flesh which is rich in protein and iron but poor in fat content. It is a popular fish among local people and ideal for the derelict and waste water aquaculture. It has been used as a fish model for studying various aspects of endocrine physiology.

In captivity, *H. fossilis* produce ripe gametes, but fail to show breeding behaviour, sperm release and oviposition. The objective of this study was to investigate possible difference in steroid level between pre-spawning and spawning fish. It was also our main aim to evaluate variances of steroid levels between the SV and testis. This analysis can provide valuable insights with regard to the functions of the steroids in the male reproduction such as certain testicular steroids can induce spermiation and certain steroid glucuronides function as sex pheromones implying importance in breeding and captive reproduction.

2. Materials and methods

2.1. Collection and acclimatization of animals

In the present study, 120 sexually mature male catfish (30–40 g; length = 15 ± 3.5 cm) were purchased from local fish markets during pre-spawning (May; 13.5L: 10.5D, 28 ± 2 °C; GSI: 1.52 ± 0.03 g%) and spawning (July; 13.0L: 11.0D, 29 ± 2 °C; GSI: 2.43 ± 0.06 g%) phases and were maintained in tanks (1m×1m×0.2 m) with circulating water under normal photoperiod and temperature for 48 h after arrival to overcome stress due to transportation and were fed with minced goat liver *ad libitum* twice daily till the day of the experiment.

Maturity status of the fish was checked by calculation of Gonadosomatic index (g%).

$$\text{Gonadosomatic Index (g\%)} \frac{\text{Gonad weight of fish (g)}}{\text{Fish weight (g)}} \times 100$$

Testes exhibit initiation of spermatogenesis in the mid-preparatory period, but significant increase in weight of the testes accompanied by active spermatogenesis occurs during the pre-spawning phase. In the spawning phase, the testes are maximally enlarged and their seminiferous tubules are packed with spermatozoa. Following spawning, the testes gradually regress in the post-spawning phase. The seminal vesicles also show initiation of secretory activity during the preparatory period but their recrudescence lags behind that of the testes by about a month. The seminal vesicles attain maximum weight and secretory activity during the spawning period. Thereafter, the seminal vesicles regress successively (22).

After acclimatization for one week, in each reproductive phase fish were euthanized by treating with MS 222 and sacrificed by decapitation between 9.00 and 11.00 h (local time) to collect testes and seminal vesicles. The tissues were storedat −80 °C, till processed for steroid extraction. All the experiments were performed in accordance with the guidelines of the Animal Ethics Committee of Banaras Hindu University, Varanasi and all care was taken to prevent cruelty of any kind.

2.2. Chemicals and reagents

Steroid standards: Estradiol-17β (E_2), Testosterone, Cortisol, Corticosterone, 21-deoxycortisol, 17,20β-dihydroxy-4-pregnene-3-one (17,20β-DP), Progesterone (P_4), 17α-hydroxy-4-pregnene-3, 20-dione (17-P_4) and deoxycorticosterone were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. HPLC grade methanol and degassed and filtered nanopure diamond water (Bernstead; U.S.A) were used throughout chromatography.

2.3. Steroid extraction

Testes and seminal vesicles of 20 fish were pooled to make one sample (N = 5). All tissues were thawed and homogenized separately in 4 vol of cold PBS (0.02 M, phosphate buffered saline, pH-7.4) with an ultrasonic homogenizer (XL-2000 Microson, Misonix, USA) 0 °C for 5–10 s. The homogenates were centrifuged at 5000 g for 20 min at 4 °C and extracted with 3 vol of diethylether, three times. The ether phase was collected, pooled, evaporated and dried under nitrogen gas, and then stored at −20 °C, until processed for chromatography/ELISA.

2.4. Steroid hormone assay

Briefly, 25 μl each of standard and samples were pipetted into the anti − E_2 IgG-coated plate wells and anti − T IgG-coated plate wells, respectively. Estradiol-17β (E_2) and Testosterone (T) were assayed by ELISA (Diametra, Italy) according to the manufacturer's protocol [23]. Other steroids (progestins and corticosteroids) were assayed by HPLC using the protocol standardized by Ref. [24]. For analysis, the ether evaporated and dried samples were reconstituted separately in 100 μl methanol and 20 μl were injected into the system and eluted for 30 min. The samples were analyzed in triplicate. The samples were also co-chromatographed with known concentrations of the standards in a mixture and the elution pattern was compared with that of the steroids. Chromatograms for blank were run with the vehicle (methanol and mobile phase) to check any interference in the elution of the steroids. The blank eluted first before the steroid peaks appeared. The differences in the peak area between the standard and standard with the sample in chromatograms were recorded with the help of Shimadzu Class VP-Series software and concentrations were calculated.

2.5. Inter and intra assay for steroid hormones

The coefficients of inter and intra-assay variations were 9.1%,

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