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Hormonal Control of Testicular Cholesterol Levels in the Catfish, *Clarias batrachus* (Linn.)—Siluroidea

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The action of gonadotropins on steroidogenesis are studied by determining the testicular cholesterol levels in the intact catfish, *Clarias batrachus* Seasonal variations in the total testicular cholesterol level are determined. The lowest level of cholesterol is observed during the 'phase of functional maturity' (spawning period) and the highest level is observed during the 'phase of depletion '(post spawning period) and 'phase of slow spermiogenesis' (early preparatory period). Exogenous administration of mammalian gonadotropins (oFSH, oLH and HCG), oPRL and homoplastic pituitary extract during the 'phase of functional maturity' results in significant elevation of cholesterol levels, thereby providing the necessary substrate for steroid biosynthesis. The results indicate that cholesterol content in the testis is an index of the fluctuations of the gonadal hormone levels, that are expected during the different phases of reproductive cycle.

Introduction

The effect of pituitary hormones on cholesterol levels in gonads is reported in diverse groups of animals such as Aves (Vogel 1957 and Maung 1976), rat (Eik-Nes 1964), mice (Bartke 1969, Simmons 1969) and rabbit (Hall 1963). Dufau et al. (1974) have reported the steroidogenic action of gonadotropic hormones in amphibia and fish. Incorporation of C^{14} into gonadal steroids has been reported recently in the dogfish (Kime 1978). Not much is known regarding the role of pituitary hormones on the testicular cholesterol levels, which is an important parameter of steroid biosynthesis, in fishes. With this end in view the present investigation on the catfish, *Clarias batrachus* was undertaken.

Materials and Methods

The fish, *Clarias batrachus* were collected from tanks around Bangalore city and brought alive to the laboratory. They were maintained in the laboratory for a week before experimentation.

Experiment 1: Body weight, testis weight and seminal vesicle weight in the period extending from June to December of two successive years (1976 and 1977) were recorded soon after collection. Gonosomatic index was calculated from the data and the

exact period of the 'phase of functional maturity' of the testis was evaluated.

Total and free cholesterol levels in the testis were also determined for a 2-year period. Cholesterol was extracted with acetone-alcohol (1:1) and the extract was divided into equal parts for total and free cholesterol estimations respectively. Total cholesterol was determined in one half following alkaline hydrolysis, digitonin precipitation and color development by Liebermann-Burchard reaction (Sperry & Wabb 1950). Free cholesterol was determined in the similar manner but digitonin precipitation was carried out before saponification. The ratio of total cholesterol to free cholesterol was determined.

Experiment 2: Fish were divided into two batches with five groups each for hormone treatment. Hormones were administered intramuscularly in 0.9% NaCl solution (table 1). Fish were decapitated one day after the last injection. Testes and seminal vesicles were dissected out and weighed. Total and free cholesterol in the testes were determined as outlined above. Statistical evaluations were made using regression analysis and the significance of differences was tested by Students 't' test.

Results

Experiment 1: Four distinct periods are discernable in the annual cycle in *Clarias batrachus* (Lahri 1967). The testes increase in weight gradually from June (0.1955g/100g Body wt) and attain the maximum size in August (0.3883g/100g Body wt) and this period between June–August is termed the 'phase of functional maturity'. The weight of the seminal vesicle also follows a

Table 1 *Experimental protocol to study the effect of diverse hormones on the testicular cholesterol levels in* C. batrachus

Batch	Group	Treatment				
		Daily dose of hormones administered for 3 days				
		PRL	HCG	P.E.	LH	FSH
I	Control	—	—	—	—	—
	1	0.25 mg	—	—	—	—
	2	0.25 mg	25.0 IU	—	—	—
	3	—	—	0.16 mg protein	—	—
	4	—	—	—	0.25 mg	—
	5	—	—	—	—	0.25 mg
	Group	Daily dose of hormones administered for 7 days				
II	6	0.25 mg	—	—	—	—
	7	0.25 mg	25.0IU	—	—	—
	8	—	25.0IU	—	—	—
	9	—	—	—	0.25 mg	—
	10	—	—	—	—	0.25 mg

*Each group consists of 5 fish; *PRL*, ovine prolactin (NIH-P-S12); *HCG*, Human Chorionic gonadotropin (ANTUITRIN-'S' Parke, Davis & Co, USA; *Pi.E*, Homoplastic pituitary extract; *LH*, ovine luteinizing hormone (NIH-LH-S 20); *FSH*, ovine follicle stimulating hormone (NIH-FSH-S 12);

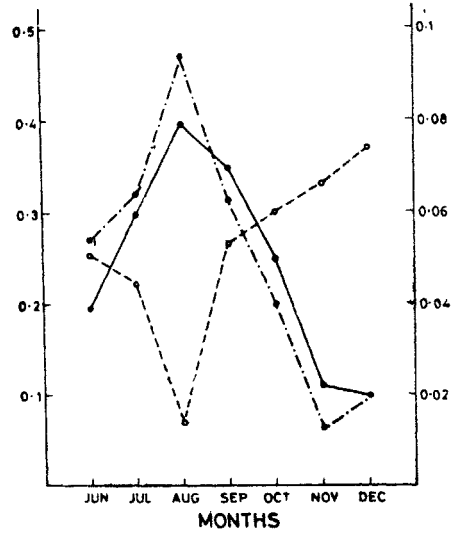

Figure 1 Cyclical changes in testis weight, seminal vesicle weight, and testicular cholesterol levels in the catfish, *Clarias batrachus* (Linn.)

similar pattern (0.0541g/100g Body wt in June and 0.0942g/100g Body wt in August). The weight of testis and seminal vesicle shows a significant direct correlation with a correlation coefficient equal to 0.714 (figure 1 and figure 2).

Total testicular cholesterol expressed as mgs/100mgs testis wt has an inverse relationship with the testicular weight (figure 1). A decline in the cholesterol content (0.2741) is observed during May-June, the 'phase of rapid spermiogenesis' followed by a steep decrease showing the lowest level (0.0657) in the month of August. Soon after, the cholesterol content rises sharply in September (0.2655) to levels found in June and thereafter the increase is very gradual. The greatest fluctuation in the cholesterol content (0.2741 to 0.2655) is observed between early July and late September. The amount of free cholesterol is almost half of the total cholesterol in all the cases studied.

Experiment 2: The GSI decreases in all experimental groups (group 1 to group 10) except for an insignificant increase and an insignificant decrease in group receiving a combination of oPRL and HCG for 7 days and group receiving HCG alone for 7 days, respectively. The maximum decrease in the testicular weight (0.1065) is seen in group receiving a combination of oPRL and HCG for 3 days and groups receiving oLH (0.1575) and oFSH (0.1920) for 7 days. There are no significant individual differences between groups receiving oPRL, oLH, oFSH for 3 days and oPRL for 7 days respectively. However there is a significant decrease in these four groups compared to the control fish. The decrease in group receiving oFSH for 7 days is more than that observed in group receiving oPRL for 7 days and this decrease is comparable to that produced in group receiving pituitary extract for 3 days. The weight of the seminal

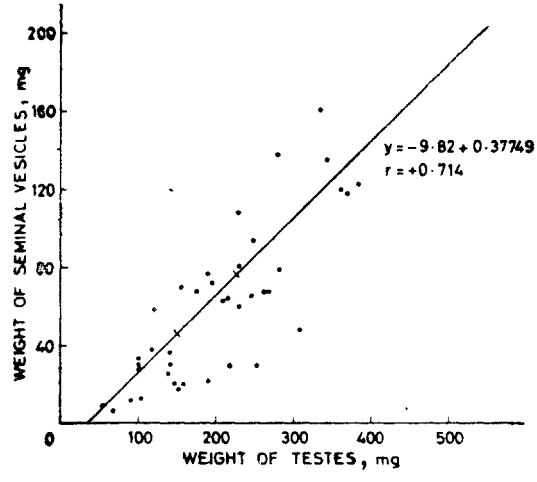

Figure 2 Testis weight *versus* seminal vesicle weight in the catfish, *Clarias batrachus* (Linn). The two variables show highly significant ($p > 0.001$) positive correlation

vesicles expressed in g/100g Body wt also decreases in all experimental groups except in groups receiving oPRL, HCG and a combination of oPRL and HCG for 7 days, where a significant increase in the weight of seminal vesicles is observed($p < 0.02$). The weight of seminal vesicle is maximum in group receiving oPRL for 7 days (figure 3 and figure 4)

Significant accumulation of cholesterol is seen in all the experimental groups and the peaks of testicular cholesterol is observed in groups receiving oFSH, HCG, oLH and oPRL for 7 days, respectively (0.4291, 0.3899, 0.3752 and 0.3160). The levels in groups receiving pituitary extract (0.2482), oFSH (0.2607) for three days and a combination of oPRL and HCG (0.2590) for 7 days are comparable.

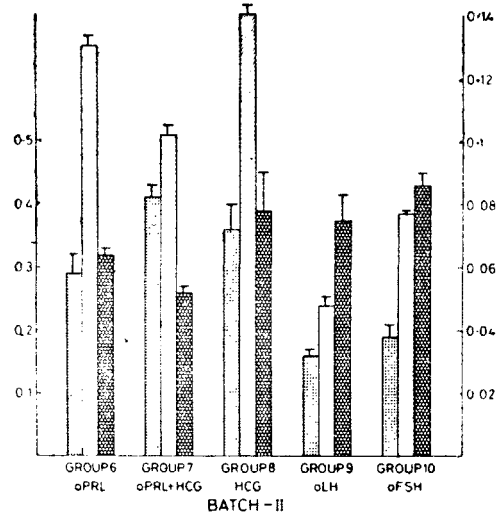

Figure 4 Effect of ovine prolactin (oPRL), ovine prolactin and human chorionic gonadotropin (oPRL × HCG) ovine luteinizing hormone (oLH) ovine follicle-stimulating hormone (oFSH) and human chorionic gonadotropin (HCG) injected for 7 days

(FIG 3)

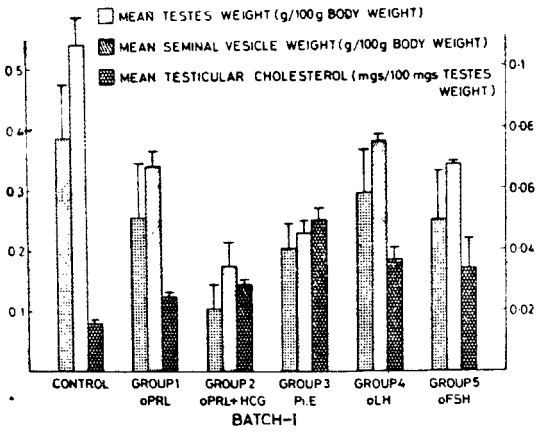

Figure 3 Effect of ovine prolactin (oPRL), ovine prolactin and human chorionic gonadotropin (oPRL+HCG), pituitary extract (Pi.E) ovine luteinizing hormone (oLH) and ovine follicle-stimulating hormone (oFSH) injected for three days

Discussion

Three of the important parameters of testicular function namely testis weight, seminal vesicle weight and testicular cholesterol levels are studied over a period of two years. Testis and seminal vesicle attain the maximum size during breeding season due to the release of the endogenous gonadotropin(s). The correlation between the structure of gonadotrophs and the seasonal changes in the ovary and testis of the catfish *Heteropneustes fossilis* was reported by Sundararaj (1959, 1960). A similar annual cycle in the testis of *Clarias batrachus* is also reported (Lehri 1967). The testicular cycle in this catfish is in conformity with those of other teleosts (Lehri 1967). The seminal vesicles also exhibit seasonal weight changes in direct correlation with the testicular cycle in

Clarias batrachus. *Clarias lazera* exhibits a similar variation (Nawar 1959). The secretory activity and the maintenance of seminal vesicle has been shown to depend on the testicular androgens in *Heteropneustes fossilis* (Nayyar & Sundararaj 1970). It could be assumed here that the weight increments observed prior to and during breeding season is due to the high androgen levels. This level will decrease soon after the act of spawning resulting in a concomittant decrease in gonadal and seminal oxide weights. If the above assumption were to be correct, a corresponding decrease and increase in the precursors of androgens in testis prior to breeding season and postspawning season respectively is to be expected. The results obtained are highly indicative of such a change. Testicular cholesterol is described to be an important precursor for steroidogenesis (Hall 1963, Krum et al. 1964, Major et al. 1967). Cholesterol is present in steroidogene glands and the trophins are known to affect the tissue concentration of this sterol in target organs (Sayers et al. 1944, Levin & Jailar 1948). Perlman (1950) and Hall (196:) have demonstrated that cholesterol can be used as a precursor for testicular steroids. In the present investigation on the testicular cholesterol levels in the different periods of testicular cycle, the lowest level is observed during the peak of spawning season exhibiting the highest GSI and maximum seminal vesicle weight. This shows that all the available precursor stores are utilized for androgen synthesis supporting the aforesaid assumption. Androgen production *in vitro* reflect seasonal differences in quantities of stored precursors in turtle *Chrysemys* (Callard & Ryan 1977). Cell suspension prepared from turtle testis produces immunoreactive testosterone from endogenous precursors *in vitro* (Callard & Ryan 1977). The other possibility of the reduced cholesterol content during the spawning season may be due to the reduced biosynthesizing tissue, the Leydig cell homologue. The peak testicular activity in the spawning period is associated with atropic Leydig cells in the catfish *Heteropneustes fossilis* (Nayyar & Sundararaj 1970). The increase in the cholesterol level observed in the post spawning period may be due to reinitiation of activity. Graig-Bennet (1931) has correlated a seasonal cycle in the interstitial cells of testes of *Gasterosteus aculeatus*, with the secondary sexual characters. Tepperman and Tepperman (1947) and Bartke (1971) have correlated the accumulation of cholesterol to an inhibition of androgen production in testes. Some teleosts lack a true interstitum, however the cells lying at the periphery of the testis lobules are described to be the Leydig cells homologue (Marshall & Lofts 1956). These lobule boundary cells are the source of male hormone in *Clarias batrachus* (Lehri 1967). The changes in cholesterol levels correlate with the rate of androgen synthesis (Bartke 1971). Maximum production can be correlated with the phase of marked lipid depletion seen prior to and during spawning season in *C. batrachus*. Van Oordt and Lofts (1963) demonstrated a close relation between an increase in lipid and cholesterol in the interstitial cells of *Rana temporaria* and a decrease in LH secretion. The phenomenon of cyclical changes involving a seasonal waxing and waning of cytoplasmic lipids in the interstitial Leydig cell is seen in fishes also (Lofts & Marshall 1957, Chan & Phillips 1967). The above results suggest that the cholesterol content in testis also records a regular cyclical change, closely correlated with the testicular cycle, in response to the endogenous gonadotropin(s) in *C. batrachus*.

With a view to understand the specific role of the diverse hormones in the maintenance of cholesterol levels and in turn

the androgenesis in the testis, ovine gonadotropins (oFSH, oLH), ovine prolactin, HCG and homoplastic pituitary extracts were exogenously administered into intact fish during the 'phase of functional maturity'. This period was chosen as the cholesterol content is least in this period with peaks of GSI and seminal vesicle weight. Plasma testosterone increases following gonadotropin administration in *Heteropneustes fossilis* (Truscott et al. 1978). In the present investigation the testicular cholesterol levels increased in all experimental groups. oFSH, oLH, HCG and oPRL administered for 7 days brought about a highly significant increase in the cholesterol level. A gonadatropin will promote almost an instantaneous increase in the concentration of testosterone and precursors of testosterone in blood (Eik-Nes 1964), Accumulation of cholesterol is more in oFSH treated fish than oLH treated fish. This explains the important role played by oFSH in providing the precursor available for further androgenesis. This level is almost equal to what is observed in the late post spawning period in *C. batrachus*, when the granulating basophils are known to be more numerous releasing large amount of FSH in *H. fossilis* (Sundararaj 1959). Since in the recent years only one type of gonadotropin has been identified in fishes this aspect of the response to oFSH needs to be further looked into. oFSH brings about an elevation in circulating androgens in both intact and hypophysectomised turtles (Lance et al. 1977). oPRL given alone resulted in a significant accumulation of cholesterol but when HCG is given in combination, the levels are much reduced and comparable to those produced in fish receiving pituitary homogenate. Since HCG is like LH in its activity (Dorfman 1972) it can be presumed that this reduction in the cholesterol content is due to its further cleavage and utilization. Bartke (1971) showed that LH causes depletion of esterified cholesterol in testes of mice. Prolactin is also known to potentiate the effect of testosterone by increasing the concentration of cholesterol in testes of intact and hypoyhysectomised mice (Bartke 1969, 1971). It can be said that oPRL can increase the production of testicular androgen by making more cholesterol available for the conversion into sex steroids. The decrease in fish receiving a combination of oPRL and HCG may be due to an increased responsiveness to LH brought about by oPRL, thereby reducing the cholesterol content. Bartke (1971) demonstrated a synergistic action of PRL and LH on the production of testicular androgens. A lower level observed in fish treated with pituitary extract is due to the combined action of the hormones present in the extract. The pituitary dosage equivalent to 0.16 mg of protein is more potent than mammalian gonadotropins as only injections were sufficient to elevate the level to that produced with mammalian hormones injected for 7 days. The maximum gonadotropic potency of pituitary of *H. fossilis* is during the spawning and early postspawning periods (Sundararaj 1959).

A decrease in testes and seminal vesicles weight is observed in all the experimental groups and it seems that the weight decrease may be due to accumulation of fat generally and may not be due to any fall in testosterone level. The levels of gonadal hormones or its metabolites is to be measured for a better understanding. However from the results it is clear that the exogenous hormones administered have a definite effect on testis weight, seminal vesicle weight and testicular cholesterol levels in catfish, *Clarias batrachus*. Further it is seen that the condition of these parameters of reproductive function are similar to what is encountered during the late post spawning period.

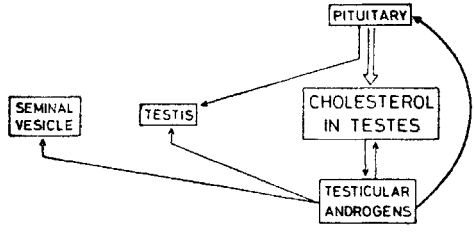

Figure 5 Schematic representation of the probable role of the pituitary hormones in the maintenance of testicular cholesterol levels in the catfish, *Clarias batrachus* (Linn.)

In all the experimental groups the percentage of free cholesterol increased as compared to controls and this ratio did not vary much individually. In mouse testes it is the concentration of esterified cholesterol that fluctuates with apparent changes in the rate of androgen synthesis and not the concentration of free cholesterol (Bartke 1971).

It can be postulated that the control of testicular cholesterol levels which is the major precursor for steroid production is mediated by the release of the gonadotropin(s) from the pituitary. A direct control of cholesterol levels in the testes is also manisfested by the circulating steroid hormone levels (figure 5).

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