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TESTICULAR STEROIDOGENESIS AND SECONDARY SEXUAL CHARACTERS FOLLOWING TESTOSTERONE PROPIONATE TREATMENT IN A TROPICAL FRESHWATER FISH *MYSTUS (M) VITTATUS* (BLOCH)

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Markandey Misra and Kamleshwar Pandey (1985) Testicular steroidogenesis and secondary sexual characters following testosterone propionate treatment in a tropical freshwater fish *Mystus (M) vittatus* (Bloch). *Bull. Inst Zool., Academia Sinica* **24**(2): 187-194. The testosterone propionate injection does not initiate remarkable change either in testicular histology or secondary sexual structures after one week treatment in *Mystus (M) vittatus*. But after the second week of treatment the secretory activity of Leydig cells is manifested in the increase of their size and number. Their secretion has also activated the spermatogenesis by increasing the number of germ cells. The signifcant change in secondary sexual characters after the second week of treatment shows that the secretion of Leydig cells is now also involved in the development of these characters and show further enhancement after the expiry of the third week. Lastly, the high percentage of spermatids in the lobules and well developed secondary sexual characters after fourth week indicate that the exogenous hormone in the *M. (M) vittatus* has activated the Leydig cells which in turn has accelerated steroidogenesis and secondary sexual characters (SSC).

The testes of teleost fishes have shown frequent stimulation of spermatogenesis when treated with testosterone, or methyl testosterone (Pickford and Atz, 1957; Dodd, 1960 and Hoar, 1965). These techniques were less successful in the analysis of endocrine control of spermatogenesis and more so in the analysis of endocrine control of the secondary sexual characters. Results with the androgen treatment on intact male fish have met a number of reversal of opinion (Eversole, 1941 and and Dodd, 1960). Burger (1941) injected testosterone propionate into hypophysectomized male Fundulus and concluded that its effect on the gametogenic function of the testes are not impressive. Those who have described the effect of androgen administration on the secondary sexual characters in the hypophysectomized teleost, have completely isolated the hormone produced by the activated gland. Lofts *et al.* (1966) have, however, shown the development of nuptial colouration which was attributed to the direct action of the

androgen in the hypophysectomized *Fundulus heteroclitus*. Present study has been undertaken to testify the results of the Burger's experiment by using testosterone propionate in the intact fish on which the results are highly conflcting.

MATERIALS AND METHODS

M. (*M*) *vittatus* males possess a well developed genital papilla and a spear-shaped thickening at the base of the caudal fin near the level of lateral line (Swarup and Swaroop, 1975). Male fishes were collected mostly from "Ramgarh Tal" Gorakhpur and brought alive to the laboratory and acclimatized from three weeks prior to the commencement of the experiments. They were fed with the liver and living earthworms at the rate of 5% of their body wight.

Selected eighty specimens were weighed and an exogenous hormone testosterone propionate was injected 3 mg/100 g of fish body weight intraperitonealy once in a week using an one ml tuberculin syringe and twenty six gauge needle. Controls were injected sterile olive oil on the same pattern. Both the groups of fishes were equally fed half an hour before changing the water of aquaria. For histological and histochemical studies groups of fifteen fishes were sacrificed for four weeks at the regular weekly intervals and small pieces from the anterior, middle and posterior regions of the testes were fixed in—

(1) Bouin's fixatives (Aqueous, Alcoholic)
(2) Picro-mercuro-formol (Pandey, 1979)
(3) Baker's formol calcium.

Tissues sectioned at 5 μm to 8 μm were stained with

(1) Delafield haematoxylin counterstain with Eosin
(2) Heidenhain's haematoxylin
(3) Heidenhain's Azan

Sections from Baker's Formaldehyde calcium fixed tissues were stained in Sudan Black B.

Mean and stained error (M$\pm$SE) were calculated for various testicular parameters. Student's '*t*' test (Campell, 1974) was used to test significant differences between experimental and their respective control groups at $p<0.05$ or less.

RESULTS

First week experimental

The interlobular septa measure 8.80$\pm$0.56 μm average in thickness containing 8.5$\pm$0.27% interstitial Leydig's cells of 2.81$\pm$0.06μm average diameter (Table 1) Leydig's cells possess granular cytoplasm with a nucleus in the centre.

The average length of genital papilla and caudal thickening has been recorded 0.55$\pm$0.01 cm and 0.52$\pm$0.01 cm respectively (Table 2).

Control

The average thickness of interlobular septa has been recorded 9.10$\pm$0.73 μm. The number and size of interstitial Leydig's cells has been recorded 8.7$\pm$0.33% and 2.75$\pm$0.07 μm respectively (Tabble 1). They are marked with granular cytoplasm and prominent nucleus in the centre.

The average length of genital papilla and caudal thickening has been recorded 0.53$\pm$0.01 cm and 0.01$\pm$001cm respectively (Table 2).

Thus no noticeable change occurs after first week of testosterone propionate injection in *M.* (*M*) *vittatus*.

Second week experimental

The interlobular septa (7.50$\pm$0.75 μm average thickness) contains an increased number and size (10.1$\pm$0.28% and 2.89$\pm$0.06 μm average in diameter respectively) of interstitial cells (Table 1). The cytoplasm of the Leydig's cells is more granular with a well marked central nucleus.

A significant ($p<0.001$) increase in the average length of genital papilla and caudal thickening (0.59$\pm$0.003 cm and 0.55$\pm$0.01 cm respectively (Table 2) has been observed in this week.

TABLE 1

Showing weekly numerical changes in germ cells Interstitial Leydig's cells along with their size and thickness of interlobular septa following testosterone propionate (3 mg/100 g) of body weight administration in *M.* (*M*) *vittatus*

Observations Time (weeks)		Thickness of interlobular septa (μm)	Diameter of IC (μm)	No. of IC (%)	No. of SMC (%)	No. of PS (%)	No. of SS (%)	No. of Sptd/Sptz
First	Experimental	8.80±0.56	2.81±0.06	8.5±0.27	14.5±0.32	15.0±0.38	10.0±0.31	52.0±0.35
	Control	9.10±0.73	2.75±0.07	8.7±0.33	11.3±0.58	12.0±0.40	14.0±0.41	54.0±0.60
Second	Experimental	7.50±0.75	2.39±0.06	10.1±0.28	8.0±0.35	8.9±0.24	9.0±0.20	64.0±0.47
	Control	9.00±0.72	2.75±0.07	8.7±0.34	12.0±0.53	13.0±0.43	13.3±0.43	53.0±0.38
Third	Experimental	6.66±0.34**†	2.95±0.05**†	13.2±0.36**†	5.0±0.31	4.0±0.17	6.8±0.26	71.0±0.81
	Control	8.80±0.43	2.76±0.05	8.8±0.36	12.6±0.34	11.0±0.35	12.6±0.49	55.0±0.84
Fourth	Experimental	5.20±0.45**†	3.20±0.04**†	14.0±0.33**†	2.0±0.13	2.1±0.10	1.9±0.10	80.0±0.58
	Control	8.60±0.39	2.81±0.07	8.9±0.29	11.0±0.52	12.1±0.34	13.0±0.56	55.0±0.49

Weekly numerical changes in the germ cells and Interstitial Leydig's cells (Mean±SE) of ten repllcates following testosterone propionate administration.

**† $p<0.001$ significantly different from corresponding control group when t-test was applied.

Abbreviations: SMC=Sperm mother cell, PS=Primary spermatocyte, SS=Secondary spermatocyte, IC=Interstitial Leydig's cells, Sptd=Spermatid, Sptz=Spermatozoa.

TABLE 2

Showing weekly changes in the secondary sexual characters of *M.* (*M*) *vittatus* following testosterone propionate (3 mg/100 g of body weight) administration

Observations Time (weeks)		Average length of genital papilla (cm)	Average length of caudal thickening (cm)
First	Experimental	0.55±0.01	0.52±0.01
	Control	0.53±0.01	0.51±0.01
Second	Experimental	0.59±0.003**†	0.55±0.01**†
	Control	0.54±0.01	0.52±0.01
Third	Experimental	0.62±0.006**†	0.58±0.01**†
	Control	0.55±0.01	0.53±0.01
Fourth	Experimental	0.66±0.01**†	0.61±0.01**†
	Control	0.56±0.01	0.54±0.01

Weekly changes in secondary sexual characters (Mean±SE) of ten replicates of *M.* (*M*) *vittatus* following testosterone administration.

**† $p<0.001$ significantly different from corresponding control when *t*-test was applied.

Control

The interlobular septa (9.00±0.72 μm average in thickness) contain the interstitial Leydig's cells of similar number, size and cytological features corresponding to those of the previous week (Table 1). Likewise the SSC do not show any change.

The average length of genital papilla and caudal thickening has been recorded 0.54±0.01 cm and 0.52±0.01 cm respectively (Table 2).

Based on the above observations it may be inferred that the testosterone propionate has accelerated the activity of the Leydig's cells as is evident from their increasing size and number. The significant change in the SSC in this week shows that the secretion of Leydig's cells is now additionally involved in the development of SSC.

Third week experimental

The interlobular septa have significantly ($p<0.001$) declined in thickness (6.66±0.34 μm average). The number (13.2±0.36%) and the size (2.95±0.05 μm average in diameter) of interstitial cells (Table 1) show significant increase ($p<0.001$) and ($p<0.01$) respectively. The cytological features of Leydig's cells are more or less similar to those of the specimens treated during the previous week.

The average length of genital papilla and caudal thickening has significantly ($p<0.001$) increased 0.60±0.006 cm and 0.58±0.01 cm respectively (Table 2).

Control

The interlobular septa (8.80±0.43 μm average in thickness) incorporates 8.8±0.36% of interstitial cells. Their average diameter has been recorded 2.76±0.05 μm (Table 1). The detailed cytological features of the Leydig's cells are similar to those recorded in the previous week.

An insignificant slight increase in the average length of genital papilla and caudal thickening (0.55±0.01 cm and 0.53±0.01 cm respectively) has been recorded in this week (Table 2).

It may be asserted that the testosterone propionate has accelerated the activity of the Leydig's cells. Thus the enhanced secretion has initiated the development and growth of SSC increasing the length of genital papilla and caudal thickening in this fish.

Fourth week experimental

There is significant ($p<0.001$) decline in the thickness (5.20±0.45 μm average) of interlobular septa having a very high percent

number of interstitial Leydig's cells (14.0± 0.33%). A highly significant ($p<0.001$) increase in the size of the Leydig's cells (3.20±0.04 μm average in diameter) has been recorded (Table 1). The granulation of the cytoplasm and size of their nucleus increase accordingly. Marked hypertrophy and hyperplasia is therefore, evident in these cells.

The average length of genital papilla and caudal thickening is highly significant ($p<$ 0.001) and increases 0.66±0.01 cm and 0.61± 0.01 cm respectively (Table 2).

Control

There is a slight change in the average thickness of interlobular septa (8.60±0.39 μm). Also an increase in the number of size of interstitial Leydig's cells has been recorded (8.9±0.29% and 2.81±0.07 μm average in diameter respectively) (Table 1). The granulation of the cytoplasm and position of the nucleus in the Leydig's cells do not show any remarkable difference from that of the previous week.

The average length of the genital papilla and caudal thickening has been recorded 0.56± 0.01 cm and 0.54±0.01 cm respectively (Table 2).

Considering the high percentage and size of interstitial Leydig's cells developed and well SSC it may be inferred that testosterone propionate in the *M. (M) vittatus* has activated the Leydig's cells which in turn have performed their aforesaid function.

DISCUSSION

The present state of our knowledge, regarding the relation of male sex hormone with the testicular activity has been acquired after a number of reversal of opinion. Exogenous androgens are known to stimulate spermatogenesis in both the hypophysectomized and intact mammals (Albert, 1961; Boccabella, 1963 and Harvey, 1963). However, the results with androgen treatment on intact male fish are conflicting (Eversole, 1941; Pickford and Atz, 1957 and Dodd, 1960). Restoration of spermatogenesis in the hypophysectomized teleosts have been demonstrated by androgen treatment (methyl testosterone, testosterone propionate, dehydroepiandrosterone) (Sundararaj *et al.*, 1971; Yamazaki and Donaldson, 1968 and Billard, 1974). Besides the effect of exogenous androgen on the spermatogenesis in *M. (M) vittatus*, its role on the steroidogenesis has also been observed. The testicular cytology with a view to study the steroidogenesis and spermatogenesis was for the first time recorded by Lofts *et al.* (1966) and that remains the sole study of its kind. In the *M. (M) vittatus*, Tables 1 and 2, summarizes a functional parallelism between the steroidogenesis, spermatogenesis and secondary sexual characters. These cytological and numerical variations to a certain extent differ from those studies by the above workers and also studied in hypophysectomized mammal by Boccabella (1963). Eversole (1941) studied the effect of various steroids on the sexual development of Lebistes, and suggested that testosterone propionate hastens the germ cells maturation without stimulating replacement by new cells. His findings were later on supported from the more recent work of Dodd (1960), Lofts *et al.* (1966) who have shown that exogenous androgen can produce initial elevation of the spermatogenetic activity and also of the interstitial cells which appear to be in an inactive state in the hypophysectomized *F. heteroclitus*. The present study shows a significant increase in the diameter and number of these cells from the second and third week of treatment (Figs. 1 and 2) in intact specimens and thus differ from the observations made by above workers and Pandey (1969) in addition.

The nuptial colouration (SSC) in minnow *Phoxinus laevis* was of the first time found dependent to testicular hormone by Kopec (1918, 1928). This was further confirmed in stickle back (*Gasterosteus pungitius* and *Gasterosteus aculeatus* by Van Oordt (1923, 1924), Van Oordt and Vander Mass (1927), Bock (1928), Craig-Bennett (1931) and Ikeda (1933). Burger (1942) and Lofts *et al.* (1966) noted

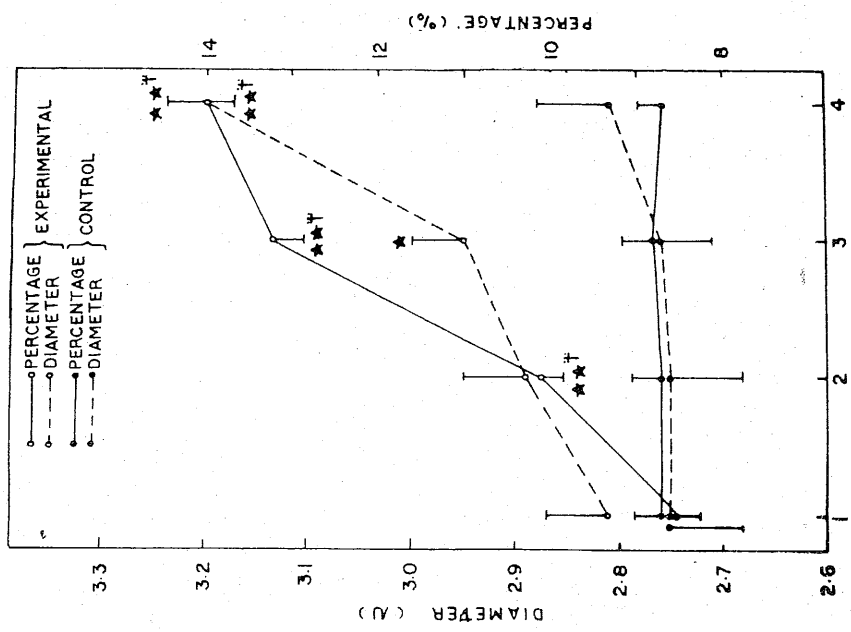

Fig. 1. Graph showing weekly changes in the diameter and percent number of interstitial Leydig's cells in the testes of *Mystus (M) vittatus* following testosterone propionate (3 mg./100 g of body weight) administration.
* $p<0.01$ and **† $p<0.001$ significantly different from corresponding control group when '*t*' test was applied.

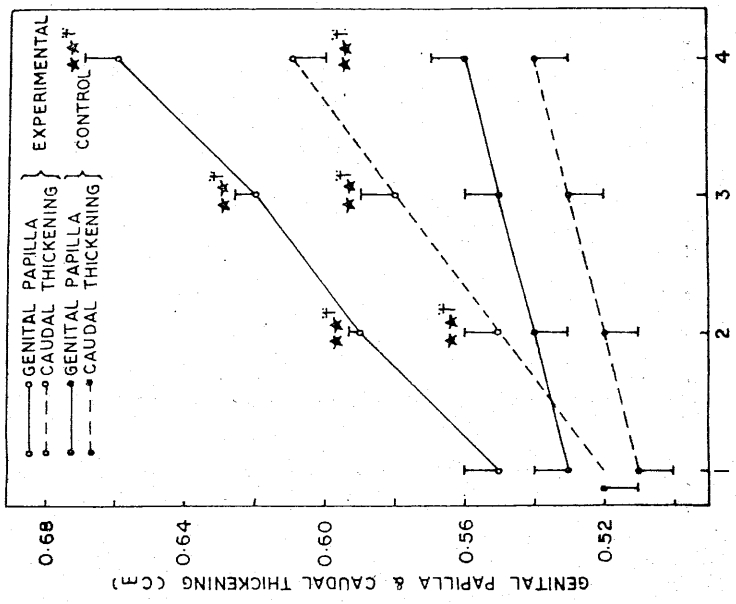

Fig. 2. Graph showing weekly changes in secondary sexual characters of *Mystus (M) vittatus* following testosterone propionate (3 mg./100g of body weight) administration.
**† $p<0.001$ significantly different from corresponding control when '*t*' test was applied.

that the nuptial colouration (regarded as SSC) reappears in hypophysectomized *Fundulus* after testosterone treatment, implicating possible steroid control.

Egami (1959) has recorded the growth of first dorsal spine in intact Gobid fish *Pterogobius zonoleucus* following treatment with testosterone, while Pandey (1969) in the hypophysectomized guppy reported a moderate recovery in the contents of lipophores after testosterone treatment. The enhancement in the size of genital papilla and the caudal thickening in the *M. (M) vittatus* is rather more appreciable and becomes significant ($p<0.001$) even from second week of experimentation (Fig. 2) pointing possible involvement of exogenous androgen.

Under alike conditions of experimentation with equal dose and duration, the above results are though fascinating yet it may be attributed to the specific species differences and also the titre of endogenous androgen present at the time of the experiments in the respective fishes. The correlative changes in the increased number and size of the interstitial cells (Fig. 1) should also be implicated in the advancement of the different secondary sexual characters in *M. (M) vittatus*.

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睪固酮處理熱帶淡水魚 *Mystus vittatus*：睪丸類固醇激素生成與第二性徵

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以睪固丙酮注射至 Mystus vitatus，一週後不引起顯著之睪丸組織與第二性徵之變化。但處理二週後，睪丸萊氏細胞增大與數目增加。處理第三週後，第二性徵表現繼續顯著增進。第四週後生精細管中精子細胞數目比例增高，第二性徵表現顯著，顯示注射之雄性素活化萊氏細胞，繼而加速類固醇激素生成與第二性徵之表現。