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Effects of mesterolone (a synthetic testosterone derivative) treated food on the sex reversal of young guppy, *Poecilia reticulata* (PETERS)

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Abstract

The application of sex control techniques using different synthetic steroids is of particular interest in aquaculture. Guppy, *Poecilia reticulata,* is one of the most popular freshwater fish species which makes a significant contribution to the ornamental fish industry in Sri Lanka. Due to the attractive colour patterns and finnage varieties, male guppies have a higher demand compared to females and therefore, androghenesis is important to produce more male guppy in the aquarium industry. Mesterolone is a synthetic testosterone derivative used in oral androgen therapy and its effects on sex reversal of guppy was studied. 10-day old guppy (1.01±0.74 mm in total length and 4.27mg in weight) from the same brood stock and strain were used. They were fed twice daily with mesterolone treated dry feed (25 mg kg^{-1}) and compared with fish fed with untreated control feed for 49 days. Water temperature and pH were measured daily and dissolved oxygen concentration was measured twice a week in each tank. Sex of the fish and the effect of mesterolone on the gonadal development, was determined by histological studies. "Chi Square Test" was performed to compare the sex ratio between control and treated fish. Results indicated that the oral administration of mesterolone has not induced the masculinization, but caused atresia in most of the vitellogenic oocytes in treated fish. Asynchronization of treatment period with the period of sex differentiation of guppy and insufficient steroid dosage and the treatment period were suggested to be possible reasons for the failure to obtain complete masculinization. There was no significant difference of amount of feed consumed by fish in treated and control groups, enabling to conclude that mesterolone has not affected the preference of fish for treated feed. *Oral administration of mesterolone has retarded the growth of vitellogenic oocytes, which is evident from the presence of atretic oocytes in the ovaries of treated fish.*

Keywords: Precifia reticulata, sex renesal

Introduction

Development of fish culture depends on the success in controlling different aspects of the reproductive cycle of fish concerned. During fish farming high preference for either females or males of different species is a major problem, because sex determination or sex differentiation is a naturally occurring phenomenon.

In many cultured aquatic species, production traits such as growth rate, time or age at maturation, dress out percentage or coloration and finnage differ significantly between sexes (Lutz, 2001). It is therefore, often more profitable to culture and market only the more productive or attractive sex. For many years these goals have been approached by manipulation of either of the phenotypic sex of the fish (Gynogenesis & androgenesis) by treatment with sex steroids or of the genotypic sex by interspecific hybridization. Recently, the third approach, which is the control of genotypic sex, by direct manipulation of the chromosomes at the fertilization (feminization and masculinization) has been introduced (Zohar, 1989). However, the technique that is currently most commonly employed is the control of gender by the administration of synthetic sex steroids to sexually undifferentiated fish (Bye & Lincoln, 1986).

Guppy, (*Poecilia reticulata)* is one of the most popular ornamental fish species, due to their attractive colour patterns and finnage varieties. They exhibit marked sexual dimorphism. Males have bright diverse colours as mentioned above; while females are uniformly grey (Phang and Fernando, 1994). Productions of more attractive and colourful male guppies therefore are very important in ornamental fish culture. Masculinization (the reversal of phenotypic expression of a genetic female into a male), by application of suitable androgen, could be the best methods to produce all male cultures of guppy. However guppy is a viviparous

fish (live-bearer) and therefore present study is designed to explore whether the oral administration of androgens would help androgenesis in new born guppy fry.

Materials and methods

Commercially available aquarium feed (Jayson Aqua Feed) was used for the experiment. One kg of ground feed was sprayed with the alcohol-hormone solution, which was prepared by dissolving one powdered tablet of Proviron (25 mg of Mesterolone) in 200 mL of ethyl alcohol (96% Analar), to prepare hormone treated feed. This method was adopted from that of Crim and Glebe (1990).

3 days old guppy belonged to the variety 'blue neon' measuring 10.1±0.738 mm in total length and 4.27 mg in body weight were collected from the Orna Fish (Pvt.) Ltd., Horana, Sri Lanka. They were obtained from the same brood stock to avoid the impact of differences in strain on gonadal development. Fry were acclimatized to experimental conditions for one week inside the aquarium. Each of the six aerated glass tanks (30×22 ×22 cm^3) were randomly stocked with ten fry of *Poecilia reticulata.*

Three batches (T_1, T_2 & T_3) were handfed *ad libitum* twice daily with hormone treated feed while the other three batches (C_1, C_2 & C_3) were fed with untreated feed. Each feeding occasion was lasted for a period of 20min in order to make sure that all the fish feed to satiety. Feeding operations were carried out for 49 days.

Water temperature and the pH of each tank were monitored daily and dissolved oxygen content in each tank was measured twice a week using Winkler's titrimetric method.

To determine the sex type of fish at the end of experimental period, histological examinations were carried out on the gonads, as described by Cumaranatunga (1985) and Hinton, D.E. (1990). Histological sections with a thickness of 1-2 μm were mounted on clean glass slides. They were stained by using haemotoxylene and eosin and Lee's methylene blue–basic fuchsin staining techniques. Stained sections were mounted under DPX and examined microscopically to identify the histological structure of gonads and photographed using a photo microscope (CARL ZEISS, Germany). "Chi Square Test" was used to compare the sex ratio of treated and control fish samples.

Results

Physico - chemical parameters

Water temperature of all the tanks during experimental period was ranging from 27.9 °C to 28.0 °C and the pH was between 7.26 and 7.28. Dissolved oxygen concentration of the tanks ranged from 9.62 ppm to 10.01 ppm (Table 1).

Table 1. Physico-chemical parameters (Temperature, pH and Dissolved Oxygen Content) in water in each tank during experimental period. Values given are the mean ± standard deviation (SD) for n number of observations.

Tank No.	Temperature/0C	pH	DO/ppm
	Mean±SD (n)	Mean±SD (n)	Mean±SD (n)
T1	27.9±0.21(49)	7.26±0.10(49)	9.93±0.35(15)
T2	27.9±0.21(49)	7.26±0.06(49)	10.01±0.28(15)
T3	27.9±0.18(49	7.28±0.06(49)	9.86±0.24(15)
C1	27.9±0.18(49)	7.26±0.06(49)	9.63±0.59(15)
C2	27.9±0.17(49)	7.26±0.08(49)	9.67±0.46(15)
C3	28.0±0.18(49)	7.27±0.05(49)	9.62±0.57(15)

Percent % Survival:

The survival was 100% in all the tanks except in one control tank where there was 90% survival.

Gonadal development and sex types

Male:female sex ratio of treated & control groups were respectively 30%:70% and 60%:40% and they were not significantly different (Pearson Chi square value; 1.8118, df; 1 sig.0.178) (Table 2). Histological examination of ovaries revealed that *Poecilia reticulata* has a single median ovary, as in most of other viviparous teleosts, and also it has an asynchronous type oocyte development where ovary contains oocytes at all stages of development. Only few matured oocytes were observed in all the ovaris. In addition to that many oogonial cells, which would develop in to oocytes at a later period could be observed. High level of atresia was observed in the

ovaries of fish, which fed with mesterolone treated feed (Plate 1). No atretic oocytes were observed in ovaries of fish which were fed with control feed (Plate 2). The cross sections of testes of treated and control fish are respectively shown in Plates 3 and 4, where no considerable difference in development was evident.

Table 2. Gonadal sex of *Poecilia reticulata* fed with mesterolone treated feed (25mgkg^{-1}) and control feed for 49 days and fed with normal diets after that until the fish are 3 months.

Treatment	%Male	%Female	% Female contain gonads with atretic oocytes
Control	60	40	0
Treated (25mg kg^{-1})	30	70	57.14

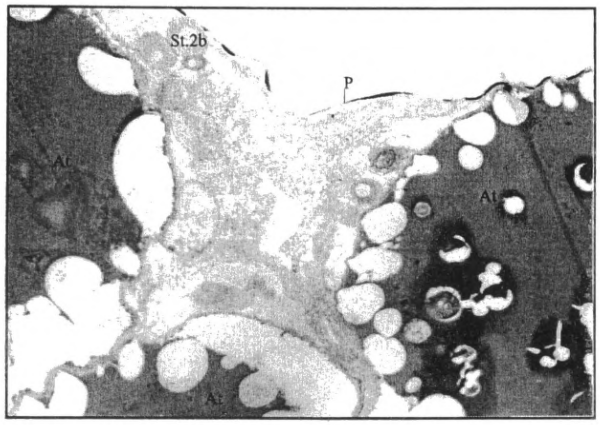

Plate 1. Section of an ovary of 3-month old *Poecilia reticulata*, showing atretic oocytes, fed with mesterolone treated diet. Stained with haematoxylin and eosin. Magnification ×150. P- Peritoneum, St.2b-Stage 2b oocyte, At- Atretic oocyte.

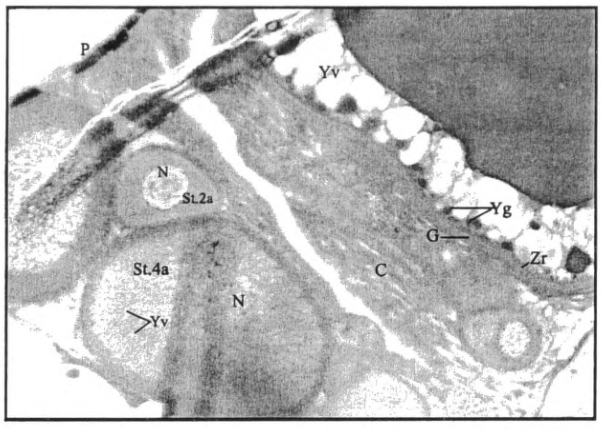

Plate 2. Section of an ovary of 3-month old *Poecilia reticulata* fed with controlled diet. Stained with haematoxylin and eosin. Magnification ×300. P-Peritoneum, C-Connective tissue, N-Nucleus, St.2a-Stage 2a oocyte, St.4a-Stage 4a oocyte, Yv-Yolk vesicles, Yg- Yolk globules, Zr-Zona radiata.

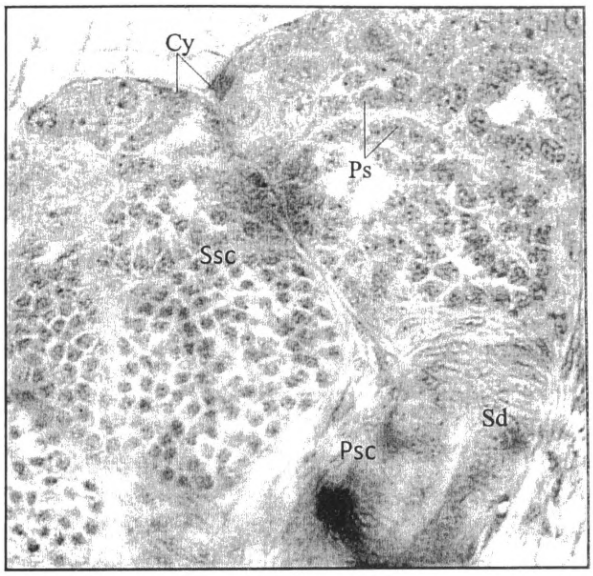

Plate 3. Section of a testis of 3-month old *Poecilia reticulata* fed with Mesterolone treated diet. Stained with haematoxylin and eosin. Magnification ×1000. Cysts, Ps-Primary spermatogonia and Sd-Sperm duct.

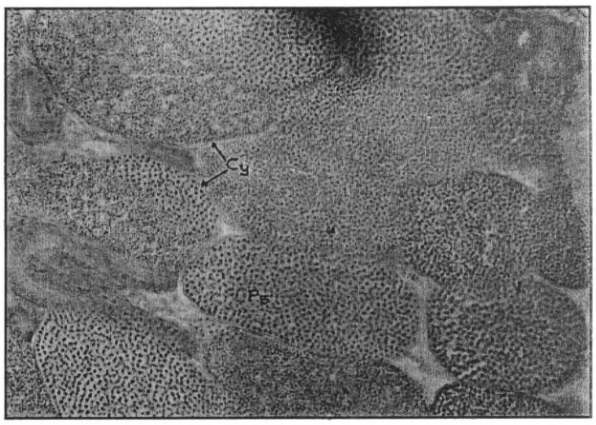

Plate 4. Section of a testis of 3-month old *Poecilia reticulata* fed with controlled diet. Stained with haematoxylin and eosin. Magnification ×200 Cy-Cysts,Psc-Primary spermatogonia, Ssc-Secondary Spermatocytes.

Discussion

Survival

Survival of *Poecilia reticulata* used in this experiment was 90% to 100%, which is very high compared to typical sex reversal treatments (Meriwether and Torrans, 1986). Also no death of fish was observed during experimental period. Therefore it can be concluded that there are no distinct adverse effects of mesterolone on the survival of guppy.

Gonadal development and sex reversal

With the aid of histological studies, it could be determined that *Poecilia reticulata* has a single median

ovary as in most of other viviparous teleosts. This structure has already been described by Nagahama (1983). Male:female ratio of fish was not significantly different between control and treated groups (Table 2). Therefore it can be assumed that the ratio at which they were initially stocked has not been changed during experimental period and there was no considerable effect of mesterolone on sex ratio of fish. Observation of no effect on sex ratios in treated and untreated fish, indicates that during the experimental period of 49 days, concentration of mesterolone administered (25 mg kg^{-1}) to experimental fish has not resulted in reversal of sex and hence any effect on sex ratio. This is confirmed by the absence of cells corresponding to spermatogonia, spermatozoa, etc, in histological sections of ovaries of mesterolone treated fish (Plate 1). Reason for this could be that, in 10 day old fry sex determination has already occurred and the concentration and duration of hormonal treatment was not sufficient to alter the sex but it can affect the growth/maturation of oocytes in females, which is evident from the presence of atretic oocytes at vitellogenic phase in the ovaries of treated fish (Plate 1). This study has confirmed the generally accepted Yamamoto's criterion which says for effective treatment, the species-specific optimal dosage of a particular steroid should be administered from the stage of the undifferentiated gonad through the time of morphological differentiation (Yamamoto, 1969).

Studies by Ball (1960 cited by Nagahama 1983), Lofts and Bern (1972 cited Nagahama 1983), Browning (1973 cited by Nagahama 1983), Guraya (1976 cited by Nagahama 1983) and Saidapur (1978 cited by Nagahama 1983) have shown that atretic oocytes are a very common feature of the teleost ovary which is induced by several factors such as environmental stress, steroid imbalance during vitellogenesis and reductions in food intake (Cumaranatunnga, 1986). However in this study no atretic oocytes could be observed in the ovaries of fish, which received a hormone free controlled diet, while they appeared in the ovaries of treated fish. Therefore it can be concluded that the administration of mesterolone is the only possible reason for atresia as all the other experimental conditions are same for treated and control fish. Takahashi (1975) was able to demonstrate that oral administration of methyltestosterone at 400mg/kg diet to gravid guppies 13-15 days after the preceding parturition resulted in the development of stromal aggregations in gonadal hilus of treated females, which was followed by the degeneration of oocytes and initiation of spermatogenesis. Within 20 days of birth, those fish developed testis. Therefore it could be suggested that the high level of atresia observed in this study may be an indication of sex reversal and perhaps the treated fish were in an intermediate stage of sex reversal. However to confirm this, similar studies should be carried out, using fry at different ages (from the date of birth) gravid females, under treatment with different concentrations of mesterolone.

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