EFFECTS OF HORMONIC STEROIDS ON OVARIAN GROWTH OF ADULT *ORYZIAS LATIPES* IN SEXUALLY INACTIVE SEASONS

NOBUO EGAMI

Zoological Institute, Faculty of Science, Tokyo University

SIX FIGURES

In the previous paper (1954b), the author has demonstrated that the atrophic ovaries of *Oryzias latipes* in winter are caused to develop if the fish are kept at 22~26°C, and that the ovarian development is inhibited by administering estrogens to the fish.

In the present paper, the results of experiments designed to study the effects on the ovarian growth of the implantation of a pellet of estrone, testosterone propionate, progesterone, desoxycorticosterone acetate (DOCA) or cholesterol will be presented. The ovarian development in the fish kept in a solution of estradiol, and in those subjected to constant darkness will also be described.

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EXPERIMENTS

All experiments were carried out with adult females of *Oryzias latipes*, of 30~32mm. in total length. The fish were fed living fresh water oligochaetes during the course of experiments.

1. Implantation of pellet of hormonal steroid

The fish employed for experiment were the red variety specimens, which had been reared in an unheated room until the beginning of experiment. Five groups, each comprising nine females (groups I, II, III, IV and V) received subcutaneous implants of a small pellet of estrone, testosterone propionate, progesterone, desoxycorticosterone acetate or cholesterol, respectively. Two other groups of nine intact females served as controls (groups VI and VII). Each group was kept separately in a vessel containing about two liters of water. All the groups receiving pellet implants and one of the control groups were kept at 23~27°C (groups I, II, III, IV, V and VI), while the other control group (group VII) was placed in a cold room (10°C or lower). All these groups were subjected to natural daylight conditions.

In the second series, five groups of six females (groups VIII, IX, X, XI, and

XII) were given subcutaneous implants of a small pellet of hormonal steroid. Another group of six intact fish served as controls (group XIII). Each group, both the steroid-given and the control, was kept separately in a vessel containing about one liter of water, at 25~26°C. in a dark incubator (see Table 1).

Pellets were given on January 16. Water heating, when performed, was begun on the same day. All surviving fish were sacrificed on February 2. The ovaries were dissected out and weighed (Table 1).

Table 1. Effects of implants of hormonal steroids on ovarian development of *Oryzias*

Group	Implanted steroid	Temperature	Light condition	Weight of ovaries (mg.)	
I	Estrone	23~27°C.	natural daylight	6,7,7,8,9,10,12.	Mean 8.4
II	Testosterone propionate	"	"	6,8,10,10,10,11,13.	9.7
III	Progesterone	"	"	8,11,13,15,16,19*,20.	14.6
IV	DOCA	"	"	5,6,7,7,9,13,16.	9.0
V	Cholesterol	"	"	9,10,13,15,16,18.	13.5
VI	none	"	"	11,14,16,18,18,19,21,23.	17.5
VII	none	10°C. or lower	"	5,6,7,7,8,8,10,14.	8.1
VIII	Estrone	25~26°C.	constant darkness	5,8,8,9,10.	8.0
IX	Testosterone propionate	"	"	6,7,8,10,11,16.	9.7
X	Progesterone	"	"	9,11,13,14,16,19.	13.7
XI	DOCA	"	"	7,9,10,18.	11.0
XII	Cholesterol	"	"	11,13,15,17,19.	15.0
XIII	none	"	"	11,14,16,18,19,23.	16.7

* This individual laid eggs.

Of all the fish receiving a steroid pellet, one individual of the progesterone-group (group III) laid five unfertilized eggs on February 2. Ovaries of the intact fish kept in warm water were markedly heavier than those of the fish kept in cold water. Among the warmed groups, however, the ovarian weights of the fish without a pellet were decidedly heavier than those of the fish receiving a pellet of estrone, testosterone propionate, or desoxycorticosterone acetate. In the fish of progesterone- and cholesterol-groups, inhibition of the ovarian development was less marked than in the groups which had been given the androgen, estrogen or the mineralocorticoid. No significant differences in ovarian weights were observable between the fish placed under natural daylight conditions and those subjected to constant darkness.

2. Breeding of fish in aqueous solution of estradiol

Experiments were carried out with wild specimens of *Oryzias* collected in January. Until the beginning of experiments, the fish were kept in aquaria at room temperature (10°C. or lower). On February 2, 50 females were divided into ten groups. Four groups were separately kept in different vessels, each containing one liter of tapwater in which 0.2, 0.06, 0.02 or 0.004mg., respectively, of estradiol was dissolved. The fifth group was kept in normal tapwater. The vessels were placed under natural daylight conditions at 23~26°C. Five other groups, similarly treated, were placed in constant darkness at 24~25°C. (Table 2).

Figs. 1 and 2. Ovary with a large cavity of specimen kept in estradiol-water at 23~26°C (Ovary became shrunk while having been placed in Bouin.) (Table 2, group II). Figs. 3 and 4. Ovary of specimen kept in estradiol-water at 23~26°C (Table 2, group I). Figs. 5 and 6. Ovary of specimen kept in tapwater at 23~26°C (Table 2, group V). Figs. 1, 3 and 5, $\times 16$; Figs. 2, 4 and 6, $\times 60$.

Table 2. Effects of breeding in aqueous solution of estradiol on ovarian weight

Group	Concentration of estradiol (mg./l.)	Temperature	Light condition	Weight of ovaries (mg.)	
I	0.2	23~26°C.	natural daylight	4,5,5,5,8.	Mean 5.4
II	0.06	//	//	7,12.(19.)*	9.5
III	0.02	//	//	5,8,9,14.	9.0
IV	0.004	//	//	16.	16.0
V	0	//	//	11,13,17,22,33.	19.2
VI	0.2	24~25°C.	constant darkness	5,5,7,7.(17.)*	6.0
VII	0.06	//	//	5,6,6,12.	7.3
VIII	0.02	//	//	6,15.	10.5
IX	0.004	//	//	8,13,16,16,21.	14.8
X	0	//	//	13,15,18,21,23.	18.0

* Ovaries containing a large cavity.

Water was not renewed during the course of experiment. On February 18, autopsies were performed. The ovaries of surviving fish were weighed (Table 2), fixed in Bouin's fluid and stained *in toto* with borax carmine for histological study.

In two individuals of each estradiol-group (groups II and VI), the ovaries contained large cavities, irregular in shape and filled with fluid (Figs. 1 and 2). These ovaries were unusually heavy, but the development of oocytes in them was never more marked than in the ovaries of other fish of the respective group. Table 2 illustrates that, in both the lighted and the dark-confined groups, the weights of ovaries at autopsy in the estradiol-fish were lighter than those in the intact fish. The differences were clearly recognizable when 0.2, 0.06 or 0.02mg. of estradiol was dissolved in one liter of water. At 0.004 mg. per liter concentration, however, estradiol failed to exert any noticeable effect on ovarian development. The ovarian weight in each concentration group kept in darkness was not significantly different from those of the corresponding group placed under natural daylight conditions. Histological studies revealed that the increase in ovarian weight was due to the accumulation of yolk in the oocytes (Figs. 3, 4, 5 and 6).

DISCUSSION AND CONCLUSIONS

The results of the experiments presented in this paper seem to show that the implants of pellets of some hormonal steroids inhibit the development of winter ovaries of adult *Oryzias* which usually takes place when the fish are subjected to high temperature (23~27°C.). Estrone is the most potent in suppressing the ovarian development. Testosterone propionate and desoxycorticosterone acetate are less effective, and progesterone and cholesterol are almost inactive in this respect.

The development of ovaries of warmed winter fish is also inhibited when the fish are reared in water containing estradiol at concentrations of 0.2, 0.06 or 0.02mg. per liter. Concentration of 0.004mg. per liter is ineffective. Kawamoto (1950) reported that the ovaries of *Ctenopharyngodon idellus* were stimulated to grow by the oral administration of estrone. However, no evidence for the promotion of

ovarian growth by hormonal steroids has been obtained in *Oryzias*, at least under the experimental conditions described in this paper. In the loach, inhibitory effect of estrogen on ovarian development has already been reported by the present author (1954a).

Matthews (1939) and Burger (1939) have reported that light has no significant effect on the annual gametogenetic cycle in *Fundulus* testis. On the other hand, it has been demonstrated in *Oryzias* that light is an important factor for determining the time of ovulation and oviposition in the breeding season (Robinson and Rugh, 1943; Egami, 1954c). The present experiments seem to indicate that the hormonal steroids are capable of inhibiting ovarian growth in winter specimens of *Oryzias*, in light as well as in darkness.

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