EFFECTS OF HYPOPHYSECTOMY ON THE OVULATION, OVIPOSITION AND SEXUAL BEHAVIOR IN THE GOLDFISH, *CARASSIUS AURATUS*[1)]

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The fact that the ovulation in fish is under the control of pituitary gonadotropins has been clearly demonstrated by Vivien (1941) who removed the pituitary gland from female gobies shortly before spawning and thereby prevented ovulation during the period when control fish spawned. This fact has also been supported by many workers showing that injection of pituitary materials brings about ovulation and oviposition in fishes (Pickford & Atz 1957).

On the other hand it is generally accepted that sexual behavior in fish depends directly on gonad hormones (Ikeda 1933, Okada & Yamashita 1944, Tavolga 1941, Jones & King 1952) and indirectly on pituitary hormones through the induced activity of the animal's own gonads (Burger 1941).

However it is also known that there are some actions and processes in sexual behavior controlled directly by the pituitary gland. Pickford (1952) and Wilhelmi *et al.* (1955) demonstrated that neurohypophysial substances induce the rapid occurrence of spawning reflex, when it is injected into immature killifish. Moreover, Egami (1959) found that injection of a mammalian neurohypophysial extract causes not only the spawning reflex in both males and females of *Oryzias latipes*, but also oviposition in fish with ovulated eggs, although the extract does not bring about ovulation.

As above, the ovulation, oviposition and sexual behavior involve complicated problems connected with the secretions of pituitary glands and gonads, so that the present writer has carried out this study aiming to get some light on the problems by means of hypophysectomy.

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Material and Method

Goldfish varying in body length from 7.1 to 11.6 cm were used. The spawning

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of the fish cultured in an aquarium of the Fisheries School, Hokkaido University begins in the middle of May and lasts to the early part of July. The water temperature was about 14°C. Suitable materials for this experiment, i. e., ovulated fish with ripe eggs in the ovarian lumen, and non-ovulated fish without ripe eggs in the ovarian lumen but getting in active pursuit of the male, were obtained as the result of raising the water temperature of the aquarium or putting aquatic plants into it. All the males used in the present study were showing active courtship display.

Hypophysectomy and "mock operation" were carried out by the method of the opercular approach (cf. Yamazaki 1961) during the period from the 5th to 27th of June 1961. After operation the fish were put into 0.2 per cent cooled saline water (about 6°C) and kept in it for 3 hours. Then the fish were transferred into an outdoor aquarium of 130 cm length, 60 cm width and 45 cm depth, which was divided into two compartments with a wire-netting partition and set with a bunch of aquatic plants. The water temperature of the aquarium showed 18.1°C in average. One or two males were placed together with one operated female into each compartment, and observations on oviposition and sexual behavior have been made; then the fish were sacrificed.

After body length, body weight and gonad weight were recorded, pituitary regions and gonads were preserved with Bouin's solution and they were cut at 10 microns by the usual paraffin method. Pituitary preparations stained by the methods of Dawson & Friedgood (1938) were used for proving the success of the operations and gonadal preparations stained with Delafield's hematoxylin—eosin and Heidenhain's iron hematoxylin—light green for the examination of gonad conditions.

Results

(1) *Effects of hypophysectomy on ovulation*

Six non-ovulated females were hypophysectomized and removed to the outdoor aquarium. Experimental results are summarized in Table 1. In the Table the time to spawning represents the time elapsed before the starting of oviposition after the fish had been transferred to the outdoor aquarium, and the duration of spawning is the time from the first oviposition to the last. Observations were continued for 5 days. During these days the hypophysectomized fish showed constant active pursuit by males, but they did not show any sign of spawning. Dissection showed that they had no eggs in the ovarian lumen.

On the other hand, three fish operated by the mock technique spawned almost naturally. The time to spawning in these fish ranged from 22 to 43 hours, dura-

tion of spawning from 40 to 336 minutes, and number of spawning actions from 18 to 443 times. However fish No. 2 and No. 3 had spawned some eggs on the wall of the aquarium before observations were carried out.

Microscopical observations on the ovaries of hypophysectomized fish revealed no empty follicles but some atretic eggs (Fig. 1). All eggs in the ovaries were below the stage of the migratory nucleus (Fig. 1). On the contrary, the ovaries of the mock control contained many empty follicles (Fig. 2).

From these facts it is clear that hypophysectomy inhibits ovulation.

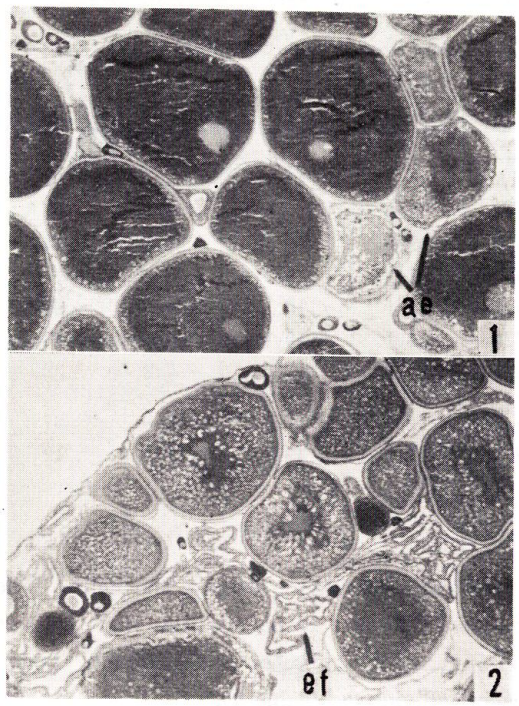

Fig. 1. Ovarian portion from a female hypophysectomized on June 19 and sacrificed on June 24
This fish showed active pursuit by the males before and after operation, but no ovulated eggs were observed. *ae* atretic egg. ×28

Fig. 2. Ovarian portion from a female subjected to "mock operation"
Many empty follicles are found. *ef* empty follicle. ×32

(2) *Effects of hypophysectomy on oviposition*

The pituitary of seven ovulated fish were removed. At the time of the operation, eggs in amount of 0.2–0.5 g were forced out from the genital pore to the

Table 1. Effects of hypophysectomy on the oviposition of experimental fish

	Operation	No. of fish	Body length (cm)	Initial water temperature (C)	Water temperature of experimental aquarium (C)	Variation in water temperature (C)	Time to spawning (minutes)	Duration of spawning (minutes)	No. of spawning actions
Non-ovulated female	H. sectomy	1	8.6	9.0	20.1	11.1	—	—	—
		2	8.4	12.0	17.0	5.0	—	—	—
		3	10.0	11.1	17.0	5.9	—	—	—
		4	8.0	13.0	18.0	5.0	—	—	—
		5	7.6	13.0	18.0	5.0	—	—	—
		6	7.5	17.0	19.1	2.1	—	—	—
	"Mock"	1	9.3	9.5	18.5	9.0	43 (hours)	336	443
		2	7.5	13.0	18.5	5.5	22 //	130	18
		3	9.8	9.8	17.0	7.2	25 //	40	21
Ovulated female	H. sectomy	1	9.5	9.5	18.5	9.0	14	258	196
		2	8.8	12.0	17.0	5.0	35	20	5
		3	10.8	12.0	17.0	5.0	55	75	32
		4	9.0	11.1	17.0	5.9	13	85	53
		5	9.3	11.1	19.1	8.0	27	143	14
		6	7.1	11.1	19.1	8.0	43	125	18
		7	11.5	9.8	17.0	7.2	32	265	30
	"Mock"	1	8.7	12.0	17.0	5.0	37	133	169
		2	8.1	17.0	19.1	2.1	12	58	29

operation board by mechanical pressure against the belly. The time to spawning in the hypophysectomized fish was 14–55 minutes, and the duration was counted as 20 minutes in minimum and 265 minutes in maximum. Spawning actions varied from 5 to 196 times in individuals (Table 1).

The interval of spawning action was about 30 seconds at the minimum and gradually became longer as the spawning drew near the end. They spawned almost all the eggs in the ovarian lumen except one fish (No. 7) which still retained eggs of 0.4 g in weight in the ovarian lumen the next day.

Two ovulated fish which got "mock operation" also spawned in 12 and 37 minutes after being put into the outdoor aquarium (Table 1). No difference was observed in spawning behavior between the mock control and the hypophysectomized fish.

These results make clear that ovulated fish can normally extrude their eggs even after the removal of the pituitary, if other conditions are suitable.

Table 2. Effects of hypophysectomy on the sexual behavior and sperm emission in males

Operation	No. of fish	Body length (cm)	Initial water temperature (C)	Water temperature of experimental aquarium (C)	Variation in water temperature (C)	Time to sexual behavior (minutes)	Sexual behavior		Days that sperm emission was observed after the operation
							after 3 hours	after one day	
H. sectomy	1	8.3	9.5	18.5	9.0	3	+	−	5
	2	10.7	17.0	19.5	2.5	10	+	+	5
	3	9.9	11.0	17.0	6.0	3	+	−	5
	4	9.8	11.0	17.0	6.0	4	+	+	4
	5	11.5	12.0	17.0	5.0	28	+	−	4
	6	11.6	12.0	17.0	5.0	32	+	−	3
	7	10.5	9.5	18.5	9.0	198	−	−	4
"Mock"	1	11.4	12.0	17.9	5.9	10	+	+	50•
	2	7.6	12.0	17.9	5.9	8	+	+	22
	3	7.7	11.1	19.1	8.0	51	+	+	29
	4	11.9	9.8	17.0	7.2	24	+	+	34
	5	10.3	12.2	19.2	7.0	5	+	+	41•
	6	12.3	12.2	19.2	7.0	54	−	−	41•
	7	10.9	12.2	19.2	7.0	80	+	+	41•

(3) *Effects of hypophysectomy on the sexual behavior of males*

Seven experimental fish and the same number of controls were used for this study. The results obtained are summarized in Table 2. Six hypophysectomized fish began to follow the females within 3–32 minutes after being put into the experimental aquarium, and they fertilized eggs. The same behaviors were observed 3 hours later or one day later. An exceptional one (Fish No. 7) showed only weak courtship activity. The mock controls were almost the same in this activity as the experimentals.

On the other hand, the effects of hypophysectomy on sperm emission differed clearly from those of "mock operation." In the hypophysectomized fish, sperm emission was observed for 3–5 days, while in mock control it lasted for 22–50 days after the operation, when the fish were pressed on the abdomen (Table 2). The solid points in Table 2 mean that the fish still continued sperm emission even on the last day of observation. During the periods some fish occasionally followed the females. The eggs spawned by the hypophysectomized fish were fertilized by the hypophysectomized males and they hatched normally.

The above facts show that hypophysectomy has no effect on the sexual behavior of male fish, but clear effect on spermatogenesis.

Discussion

The present finding that hypophysectomy completely inhibits ovulation shows clearly that pituitary hormones play an important role in the ovulation of goldfish. This agrees well with the results of Vivien (1941). In his preceding study the writer demonstrated that pituitary hormones are indispensable in the second-phase oocyte growth in goldfish (Yamazaki 1961). Thus, the two processes, the second-phase oocyte growth and ovulation are controlled by pituitary hormones. However, is there any difference in quality or quantity between pituitary hormone concerned with oocyte-growth and that with ovulation? Witschi's results (1955) suggest that fish pituitary contains FSH and LH as designated in mammalian. Ball (1960) stated an opinion that a qualitative difference is possible to exist between vitellogenesis-inducing hormone and ovulation-inducing hormone. Although no positive evidence supporting his opinion has been obtained in the present study, the writer tends to agree with him, because the second oocyte-growth and ovulation are considered to be distinct phases in oogenesis, as ovulation is naturally induced by stimulation due to male's courtship display (Yamamoto & Yamazaki 1961). On this point it is expected that further studies will be performed in the near future.

The goldfish with ovulated eggs could spawn normally even if the pituitary gland was removed. This finding apparently is contradictory to the observations of Wilhelmi *et al.* (1955) and Egami (1959) who showed that neurohypophysial hormones bring about spawning actions. Of course, this may be explained without contradiction in consideration of the secretion of neurohypophysial hormones from hypothalamus (Pickford & Atz 1957) or the existence of the hormones in blood before operation. However this hormone is not only a factor concerned with spawning activity. In mammalians childbirth takes place normally without pituitary gland (Firor 1933, Nakano, *et al.* 1957) and pituitary hormones play no direct role in sexual behavior (Beach 1948). On the other hand, Noble & Kumpf (1936) demonstrated that the sexual activity of female fish is concerned mainly with the gonads. These facts seem to suggest the possibility that oviposition may be induced without pituitary hormones via some pathway other than that surmised by Egami & Nambu (1961).

In the present study the males also showed typical sexual behavior after the removal of the pituitary gland. This fits in with the results of Vivien (1941) on *Gobius*, but not of Tavolga (1955) on *Bathygobius*. The present writer has on idea as to the reason why such a difference is induced in these species, whether species specific or not. At any rate, it seems true that in goldfish the sexual behavior of the male does not depend upon pituitary hormones as in mammalians.

Summary

1. Hypophysectomy inhibited ovulation, but not oviposition in goldfish.

2. Hypophysectomized males showed normal sexual behavior, but ceased sperm emission within a short time after hypophysectomy.

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