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Trials on Induced Ovulation of *Fugu niphobles* (Jordan and Snyder) with Human Chorionic Gonadotropin

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ABSTRAK

Kesan penggunaan HCG (Human Chorionic Gonadotropin) untuk ovulasi Fugu niphobles *telah dikaji. Ikan betina yang buncit abdomennya dibahagikan kepada empat kumpulan 1, 2, 3 dan 4, dan dirawat dengan hormon HCG pada takaran masing-masing, 0, 100, 200 dan 300 I.U.. Selepas ikan betina dikumpulkan mengikut kumpulan, ikan-ikan disuntik dan ikan yang didapati tidak berovulasi disuntik semula selepas 72 jam. Takaran suntikan dikira selepas berat badan betina diambil kira dan takaran diberikan sebagai I.U./g berat badan. Data kejayaan ovulasi untuk rawatan 0, 1, 2, 3 dan 4 I.U./g berat badan masing-masing, 40%, 83%, 70%, 90% dan 80%. Ini menunjukkan bahawa ikan dalam kumpulan kawalan iaitu kumpulan yang tidak menerima suntikan hormon dapat berovulasi dalam keadaan kurungan dan penggunaan HCG dapat meninggikan kadar kejayaan ovulasi.*

ABSTRACT

The effect of administration of HCG (Human Chorionic Gonadotropin) on ovulation of Fugu niphobles *was studied. The female fish which exhibited distended abdomen were randomly assigned to four groups and the treatments for groups 1, 2, 3 and 4 were 0, 100, 200 and 300 I.U. of HCG per fish respectively. The fish were injected immediately after the groups were established and unovulated females were reinjected after 72 hours. The dosage for each fish was calculated based on the body weight and expressed as I.U./g body weight. The ovulatory success in fish administered with 0, 1, 2, 3 and 4 I.U. of HCG/g body weight was 40%, 83%, 70%, 90% and 80% respectively. This indicated that fish in the control group could also ovulate in captivity and that administration of HCG would enable a higher degree of ovulatory success.*

INTRODUCTION

Fugu niphobles locally known as Kusafugu in Japan is toxic and the poison Tetrodotoxin is mainly found in the ovary, liver, blood and skin. In recent years, research on the origins and metabolism of the poison has been actively pursued in Japan. The changes in the amount of the poison during egg development, growth and maturation are important. The fry of this fish could be used as food for the more economically valuable Torafugu (*Fugu rubripes*) to reduce cannabalism during fry and fingerling rearing.

The earliest reports on breeding of this fish were those by Uno (1955) and Fujita *et al.* (1966). The natural breeding season for this fish in Japan ranges from May to early July. It ordinarily spawns only 1-5 days following each

full or new moon, at rising tide. The males shed milt everyday during one or three series of high tides in succession whereas the females appear to discharge sticky eggs completely in one spawning period (Uno, 1955).

Fujita *et al.* (1966) successfully ovulated *F. niphobles* using Synahorin at a dose of 10 RU per fish and it was also reported that ovulation occurred 24 to 48 hours after the administration of Synahorin.

As it is difficult to collect naturally spawned eggs or fry from the sea, there is a need to be able to spawn this fish in captivity and also be able to culture the fry and fingerlings. In order to pursue the problem of establishing a consistent technique for breeding *F. niphobles*, an experiment was conducted to determine the effect of administration of HCG (Human Chorionic Gonadotropin, commercial name-Puberogen) at different dosages on ovulation. The results of the ovulatory response and egg incubation experiments are discussed.

MATERIALS AND METHODS

Fugu niphobles (Jordan and Snyder) that were collected from fishermen at Ehima, Atsumi Peninsula, Aichi Prefecture, Japan in the months of June and July 1985, were packed in 30 litre plastic bags under oxygen and transported back to the University of Tokyo hatchery at Bentenjima, Shizuoka Prefecture. At the hatchery, the broodfish were immediately separated by sex and the females that had ovulated before the start of the experiment were separately grouped, then anaesthetized with 1% urethane, weighed, stripped and their eggs fertilized followed by egg incubation.

The unovulated females which exhibited distended abdomen were randomly divided into four groups (1, 2, 3 and 4) and immediately anaesthetized, weighed and then injected with HCG (Puberogen) intramuscularly at the dosages of 0, 100, 200 and 300 I.U. per fish respectively. The amount of HCG administered per fish was then computed based on the body weight of the fish and then expressed in terms of I.U. of HCG/g body weight of fish. The fish were then released into 2.0 ton tanks containing 1.5 m^3 of water. The tanks had a flow through system and fish within the same treatment were kept together. Fish that did not ovulate in the hormone treated groups after three days were reinjected with a second and final dose of the hormone according to the specific treatment dosages.

In the first trial, the fish were checked for ovulatory response once a day at 1500 hours but in the subsequent trials, the fish were checked twice a day at 1000 hours and 1500 hours. When a gentle pressure on the abdomen resulted in the release of eggs, the females were anaesthetized and weighed. The weight of eggs that were stripped from each female was also determined. The data for ovulatory success and failure were subjected to the Kolmogorov-Smirnov test.

A small fraction of about 100 to 200 stripped eggs were then put into three 1 l beakers containing seawater. A semen-water mixture was then poured into the beakers to fertilize the eggs. The beakers were given a fresh change of seawater after 15 minutes and all the labelled beakers were put into a water bath which had a water temperature of 24°C. The water in the beakers were given an 80% change every day. In good eggs, hatching occurred about 5-6 days after fertilization. The hatching rate was determined by counting the number of larvae and number of unhatched eggs.

RESULTS AND DISCUSSION

The ovulatory response of non hormone and hormone administered fish is summarised and presented in Table 1. The results showed that 40% of fish in the control group (non hormone treated) ovulated under tank conditions and that ovulation occurred about 48 hours after the commencement of the experiments. Ovulation and oviposition had already occurred in fish number 1 and as such the weight of eggs collected from it was only 1.2 g.

The ovulatory success in fish that received 1 I.U. of HCG/g body weight was 83%. The number of fish that ovulated 24 and 72 hours after the first injection were 2 and 1 respectively while 2 fish ovulated 24 hours after the second injection. In the study reported by Fujita *et al.* (1966) on induced spawning of this fish, 10 Rabbit Units (RU) of Synahorin were injected per fish in the first trial and the fish

TABLE 1
The ovulatory response of *Fugu niphobles* after administration of Human Chorionic Gonadotropin (HCG).

HCG Hormone (I.U./g)	Fish Number	Total Length (cm)	Body Weight (g)	Ovulatory response	Response Time (Hours)	Weight of eggs (g)
0	1	11.5	63.0	S	48	1.2
	2	11.0	51.5	S	48	13.9
	3	11.8	64.9	S	48	14.9
	4	13.0	84.8	S	48	15.9
	5	11.2	54.5	F		
	6	14.0	90.3	F		
	7	11.4	46.1	F		
	8	12.6	62.9	F		
	9	14.2	94.9	F		
	10	14.0	84.0	F		
1	11	11.8	78.4	S1	24	19.2
	12	12.2	71.7	S1	24	17.5
	13	13.1	82.5	S1	72	23.5
	14	13.4	89.8	S2	24	26.9
	15	12.6	75.6	S2	24	20.0
	16	13.0	77.7	F		
2	17	10.5	41.7	S1	24	0.3
	18	11.8	58.1	S1	48	18.0
	19	13.4	84.6	S1	72	24.2
	20	12.2	61.9	S2	24	12.1
	21	11.6	53.7	S2	24	1.2
	22	14.9	117.5	S2	48	32.1
	23	14.4	96.0	S2	48	17.3
	24	11.3	54.9	F		
	25	11.2	49.0	F		
	26	13.3	83.8	F		
3	27	13.3	96.4	S1	24	21.1
	28	12.7	77.0	S1	24	18.3
	29	11.8	69.3	S1	24	19.3
	30	13.3	96.4	S1	24	21.1
	31	11.8	60.4	S1	24	10.7
	32	13.9	116.9	S1	48	30.5
	33	13.2	75.2	S1	48	24.8
	34	12.3	64.1	S2	24	12.0
	35	13.3	78.4	S2	48	19.2
	36	14.5	89.0	S2	48	20.5
	37	12.2	62.5	F		
4	38	13.0	81.5	S1	24	15.4
	39	12.1	71.4	S1	24	11.4
	40	13.0	73.5	S1	48	14.4
	41	13.3	74.5	S2	48	19.8
	42	12.7	83.8	F		

Note : S – Ovulatory success without hormone administration
S1 – Ovulatory success after the first injection
S2 – Ovulatory success after the second injection
F – Ovulatory failure

ovulated after 24 hours. However, in their second trial, some fish that did not have well enlarged abdomens 24 hours after the first injection required a second injection for successful ovulation.

The ovulatory success in fish which were administered with 2 I.U. of HCG/g body weight was 70%. Three fish ovulated between 24 – 72 hours after the first injection while four fish ovulated between 24 and 48 hours after the second injection.

The highest percentage of ovulatory success i.e. 90% was recorded from fish that had been administered with 3 I.U. of HCG/g body weight. Seventy percent of the fish that ovulated did so after a single injection of HCG (Puberogen). However, three fish could only ovulate after the administration of the second injection. When the ovulatory success and failure rates were subjected to the Kolmogorov-Smirnov test, there were no significant differences in the success rates for the different dosage rates ($P > 0.05$).

In *Limanda yokohamae*, ovulation was successful when the females were injected with HCG (Puberogen) at the rate of 3.4 to 8.6 I.U. /g body weight (Hirose *et al.* 1979 in Lam 1982) but in the case of *Plecoglossus altivelis* higher dosages ranging from 12.7 to 25.0 I.U./ g body weight were required for successful ovulation (Hirose *et al.* 1977 in Lam 1982).

The ovulatory success in fish that were administered with 4 I.U. of HCG/g body weight was 80%. As the sample size was rather small, this result should be viewed with caution.

The effect of the dosage of HCG administered to *Fugu niphobles* on hatching rates is presented in Table 2. In this study it was observed that normal developing eggs turned grey usually after 3 days of incubation and hatched out between 5 to 7 days at 24°C. Generally this study was unable to demonstrate any correlation between the dosage of hormone administered and the hatching rate. Egg samples from fish numbers 14, 20, 35 and 40 had high hatching rates indicating that hatching failure was not due to the stripping, fertilization nor incubation techniques but was probably due to the timing of egg stripping. In this study, the fish were checked for ovulation only twice a day and it was possible that overripening of eggs had occurred prior to stripping and fertilization thus causing zero hatching of egg samples collected from fish numbers 1, 18, 19, 23, 34, 39, and 41. In future studies, the fish should be checked at more frequent intervals especially in the evening and at night. There was also zero or low hatching rates in fish numbers 3, 4, 12, 27, 31, and 38. The plastic bag that was used to pack them under oxygen leaked very badly and as such the stressful conditions in the low oxygenated water may have caused some damage to the eggs.

When *Fugu niphobles* were injected with Synahorin at a dosage of 10 RU per fish, the hatching rates ranged from 91.3 to 98.6% whereas those in the control fish were 94.6% (Fujita *et al.* 1966). As there was a time lapse of at least 1 day from the time the broodstock was caught till the time of hormone administration in the present study, the onset of atresia may have commenced and as such the time interval between capture and hormone administration should be preferably reduced. A comparative study on the use of Synahorin (pituitary gland of horse) versus HCG would be beneficial considering the high hatching rates obtained when Synahorin was administered.

In *Macquaria ambigua*, all females injected with a spawning threshold dosage of 500 I.U. per kg of HCG or dosages of 1000 and 2000 I.U. per kg of HCG spawned completely. However the mean hatching rate of 87.4% of eggs spawned by females injected with 500 I.U. per kg (0.5 I.U./g) of HCG was significantly higher ($P < 0.01$) than the mean hatching rate of 44.2% in fish that were injected with 2000 I.U. per kg of HCG. Thus the use of HCG at the two dosages above the spawning threshold level resulted in apparent deterioration of egg quality in *M. ambigua* (Rowland 1983).

During the preparation of the fish groups for HCG administration, some females were observed to have ovulated. The eggs were then stripped, fertilized and incubated. However the eggs became opaque, white or light brown and did not develop the characteristic grey colour of developing eggs by day 3 at 24°C. Generally there is a certain length of time after ovulation when the eggs remain viable in the ovarian cavity. In this case, it was inferred that the eggs that were collected from females which had

TABLE 2
The relationship between the dosage of HCG administered and hatching rate of *Fugu niphobles* eggs

HCG Hormone Dosage (I.U./g)	Fish number	Hatching rate of eggs (%) in replicate			Mean hatching rate (%)
		1	2	3	
0	1	0	0	0	0
	2	34.4	26.7	32.0	31.0
	3	0	0	0	0
	4	0	0	0	0
1	11	14.3	3.2	6.4	8.0
	12	0	21.3	3.9	8.4
	13	11.8	8.6	14.5	11.6
	14	82.8	88.5	84.7	85.3
	15	62.8	60.4	65.9	63.0
2	17	0	2.4	1.6	1.3
	18	0	0	0	0
	19	0	0	0	0
	20	91.3	89.8	82.5	87.9
	22	73.7	74.9	58.7	69.1
	23	0	0	0	0
3	27	4.2	2.1	7.4	4.6
	28	1.3	1.6	1.9	1.6
	29	47.2	39.0	49.1	45.1
	30	4.2	2.1	7.4	4.6
	31	0	0	0	0
	32	41.9	42.4	23.0	35.8
	33	77.1	66.7	78.9	74.2
	34	0	0	0	0
	35	79.2	91.7	95.9	88.9
	36	80.2	47.1	36.6	54.6
4	38	0	0	0	0
	39	0	0	0	0
	40	60.9	71.1	81.2	71.1
	41	0	0	0	0

ovulated prior to the commencement of the trials had exceeded this critical period of time for successful fertilization and as a result egg samples from 17 fish had zero hatching with the exception of egg samples from 1 fish.

CONCLUSION

Even though administration of Human Chorionic Gonadotropin (HCG) to female *Fugu niphobles* increased the ovulatory success rates, there were no significant differences in the success rates which were the result of administering different dosage rates ($P > 0.05$).

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REFERENCES

FUJITA, Y., T. KOMIYAMA and N. YOKATA. 1966. Artificial Egg Taking and High Density Hatching Method of *Fugu niphobles*. *The Aquiculture* **14(1) :** 31-36. (In Japanese).

LAM, T.J. 1982. Applications of Endocrinology to Fish Culture. *Can. J. Fish. Aquat. Sci.* **39 :** 111-137.

ROWLAND, S.J. 1983. The hormone-induced Ovulation and Spawning of the Australian Freshwater Fish Golden Perch, *Macquaria ambiqua* (Richardson) (Percichthyidae). *Aquaculture* **35 :** 221-238.

UNO, Y. 1955. Spawning Habit and Early Development of a Puffer, *Fugu (Torafugu) nipholes* (Jordan et Snyder). *J. Tokyo Univ. Fish.* **41(2) :** 169-183.

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