Biological Study on Ovulation *in vitro* of Fish—III. The Induction of *in vitro* Ovulation of *Oryzias latipes* Oocytes Using Salmon Pituitary Gonadotropin

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This study was carried out to examine the effects of partially purified salmon (*Oncorhynchus tshawytscha*) gonadotropin (SG-G100) on ovulation *in vitro* in *Oryzias latipes*. Incubation of oocytes with 0.04–400 $\mu\text{g/ml}$ SG-G100 was initiated at 10.00 a.m., 5.00 p.m. and 11.00 p.m. and the percentage of oocytes which had ovulated was determined at 10.00 a.m. on the following day. In the 10.00 a.m. group slight ovulation was observed at the 4 $\mu\text{g/ml}$ dose. In the 5.00 p.m. group, ovulation occurred at all dosages from 0.04 $\mu\text{g/ml}$ to 400 $\mu\text{g/ml}$ gonadotropin, with maximum ovulation (67%) at 0.4 $\mu\text{g/ml}$ SG-G100. Luteinizing hormone (NIH-LH-S16) was less effective than SG-G100. In the 11.00 p.m. group there was 19% ovulation in the control group and the most effective dosage of SG-G100 was 4 $\mu\text{g/ml}$, which induced 60% ovulation.

It is well known that on attempting to examine the effects of pituitary gonadotropin on ovulation in fish the phenomenon of species specificity of the hormone must be taken into account. To date, there have been several attempts to extract and purify gonadotropin from the piscine pituitary gland¹⁻⁷⁾. Recently, salmon gonadotropin has been partially purified using ethanol extraction and gel filtration on Sephadex G100^{8,9)} and its biological activity has been determined using a spermiation bioassay and observations on spermatogenesis, vitellogenesis and *in vivo* ovulation in the hypophysectomized goldfish^{10,11)}.

In this report, one of a series on ovulation *in vitro* in fish, the authors have attempted to examine the effects of this partially purified salmon pituitary gonadotropin (SG-G100) on ovulation *in vitro* in *Oryzias latipes*.

Material and Methods

The fish, the red variety of *Oryzias latipes*, were maintained under conditions similar to those described in a previous report¹²⁾. The methods used for the maintenance *in vitro* of the *Oryzias latipes* oocytes also were described in the previous report¹²⁾. Three experiments were carried out in which incubation of oocytes in various concentrations of gonadotropin was initiated at 10.00 a.m., 5.00 p.m. and 11.00 p.m. At 10.00 a.m. on the following day the oocytes were inspected using a dissecting microscope. The per-

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centage which had ovulated was determined by observation of the villi and attaching filaments around the eggs and by confirmation of their fertilization and subsequent development.

For this study, the partially purified salmon pituitary gonadotropin was prepared by the method of DONALDSON *et al.*⁹⁾, using *Oncorhynchus tshawytscha* pituitary glands. Luteinizing hormone NIH-LH-S16 (ovine) also was used to obtain a comparison with the ovulation inducing potency of salmon gonadotropin.

Results

In the experiment initiated at 10.00 a.m., dosages of 0.04, 4.0 and 400 $\mu\text{g/ml}$ SG-G100 were tested together with a control. At this hour, as shown in a previous report¹³⁾, the partially purified salmon gonadotropin was only slightly effective, some ovulation occurring only at the 4.0 $\mu\text{g/ml}$ dosage level (Table 1).

However, in the incubation started at 5.00 p.m. the salmon gonadotropin was very effective in inducing *in vitro* ovulation in the *Oryzias* oocytes (Table 1). In this experiment the dosage ranged in tenfold increments from 0.04 to 400 $\mu\text{g/ml}$ SG-G100. While all concentrations induced ovulation the most effective dose was 0.4 $\mu\text{g/ml}$, which induced

Table 1. The effects of salmon gonadotropin (SG-G100) on ovulation *in vitro* of *Oryzias* oocytes incubated in 3 ml of the medium.

	No. of oocytes used	No. of the ovulated eggs	Percentage of ovulation
At 10.00 a.m. (0.83-0.95)			
Control	20	0	0
SG-G100 400 $\mu\text{g/ml}$	28	0	0
4	28	2	7
0.04	25	0	0
At 5.00 p.m. (0.95-1.05)			
Control	22	0	0
SG-G100 400 $\mu\text{g/ml}$	22	9	41
40	20	10	50
4	19	12	63
0.4	21	14	67
0.04	27	7	26
LH 4 $\mu\text{g/ml}$	20	9	45
At 11.00 p.m. (0.95-1.15)			
Control	21	4	19
SG-G100 400 $\mu\text{g/ml}$	22	8	36
4	20	12	60
0.04	21	8	38

The oocyte sizes (mm) at initial were shown in parentheses.

The changing sizes of oocytes accompanying to time passed is referred to the previous report.¹³⁾

67% of the oocytes to ovulate, and the maximum mode of the potency was between 0.4 and 4.0 $\mu\text{g/ml}$. Ovine LH, by comparison, caused 45% ovulation at a concentration of 4 $\mu\text{g/ml}$. The salmon gonadotropin used thus had a higher potency than that of ovine LH in both the present and a previous investigation¹³⁾.

In the experiment started at 11.00 p.m., 19% ovulation occurred in control oocytes. This was matched by a higher response in the 0.04 $\mu\text{g/ml}$ group than occurred in the 5.00 p.m. experiment but the response in the 4 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ groups was slightly less than at 5.00 p.m. This result in the 11.00 p.m. experiment may be related to the conditions of tissue culture used¹³⁾.

Discussion

The salmon pituitary gonadotropin preparation SG-G100 was found to induce *in vitro* ovulation in *Oryzias* oocytes. Of the three times tested, the most effective for induction of ovulation using salmon gonadotropin was 5.00 p.m., which is in agreement with previous work¹³⁾. The gonadotropin was only slightly effective in inducing *in vitro* ovulation at 10.00 a.m. This may be due to the fact that the oocytes used in this study were undergoing yolk formation as observed under the electron microscope in a previous investigation¹³⁾.

When the results for salmon gonadotropin in the present paper are compared with the *in vitro* ovulating potency for ovine LH shown in Table 4 in the senior author's first report¹²⁾ on ovulation *in vitro* in fish it can be seen that the salmon gonadotropin preparation is more potent than LH. In fact while the maximum mode of ovulation in the present study was induced by SG-G100 at between 0.4 and 4.0 $\mu\text{g/ml}$, the maximum mode for NIH-LH was between 4.0 and 40.0 $\mu\text{g/ml}$ ¹²⁾.

The higher potency of the salmon gonadotropin compared to the ovine luteinizing hormone suggests a degree of zoological specificity in these hormones and in the receptor mechanism in *Oryzias latipes*. Both the salmon and *Oryzias* are in Division III¹⁴⁾ of the teleostean fishes but while the salmon is a primitive fish in the superorder Protacanthopterygii, *Oryzias* is in the superorder Atherinomorpha and is thus intermediate between the salmonids and the more highly evolved perciform fishes. The ability of the salmon gonadotropin to elicit a significant response in *Oryzias* and several other species^{11, 15, 16)} may reflect the relatively primitive evolutionary status of the salmonids. Several investigators have discussed the single or dual nature of fish gonadotropin¹⁷⁻²¹⁾. Investigation of the effect of various fish gonadotropin preparations on vitellogenesis and ovulation may help to resolve this problem.

In contrast to these findings in *Oryzias latipes*, SG-G100 was capable of inducing *in vivo* ovulation²²⁾ but only marginal *in vitro* ovulation in catfish ovary fragments (Sun-

dararaj *et al.* unpublished). *In vitro* ovulation in the catfish was induced however by deoxycorticosterone or cortisol²³⁾ suggesting that in the catfish, gonadotropin may induce ovulation by stimulating interrenal corticosteroidogenesis while in *Oryzias*, gonadotropin may have a direct effect on the ovary or via steroidogenic processes within the ovary.

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