Induction of Ovulation in Ayu, *Plecoglossus altivelis*, with Salmon Pituitary Gonadotropin*

Rikizo ISHIDA,** Keiji HIROSE*** and Edward M. DONALDSON****

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This study was carried out to determine the practical effective dose of partially purified salmon gonadotropin required for inducing ovulation in intact ayu, *Plecoglossus altivelis*. Salmon gonadotropin administered as a single injection of 10 μg/g was very effective in inducing ovulation of ayu. Based on the previous tests in ayu using human chorionic gonadotropin (HCG), 10 μg salmon gonadotropin (SG-G100) was equivalent to 1,250 IU of HCG.

An increase in body weight was shown in the gonadotropin-treated fish. This increment was dose-dependent and may have been due to an increase in ovarian weight resulting from water uptake by the oocytes.

In a previous report1) by ISHIDA *et al*, commercial mammalian gonadotropin (HCG) was shown to be effective for inducing ovulation in ayu cultured commercially in Japan and the optimum dose was estimated. Studies of teleost gonadotropin2–8) have recently culminated in the purification of gonadotropin from carp9) and salmon10,11) The biological activity of the partially purified salmon gonadotropin (SG-G100) has been examined using a spermiation bioassay and observations on spermatogenesis, vitellogenesis and ovulation in the hypophysectomized goldfish.12,13) More recently, HIROSE and DONALDSON14) have shown that this gonadotropin is more effective in inducing ovulation *in vitro* in *Oryzias latipes* than mammalian gonadotropins such as luteinizing hormone and HCG. Thus while it appears that fish gonadotropin is more effective than mammalian gonadotropin for inducing ovulation in fish, it is not known under what conditions salmon gonadotropin could be used on a practical basis in the culture of ayu.

This investigation was carried out to determine the effective dosage of salmon gonadotropin required for induction of ovulation in ayu, *Plecoglossus altivelis*, and also to obtain further information on the mechanism by which gonadotropin induces ovulation in this fish. A concomitant study has implicated a change in water metabolism which occurs prior to ovulation (HIROSE *et al.*, in preparation).

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Materials and Methods

Mature female ayu, *Plecoglossus altivelis*, weighing 15 to 30 g were used in experiment 1 (October 1971). The fish were kept in a freshwater pond (16±1°C) at the Freshwater Fisheries Research Laboratory. The fish were not fed for several days prior to use. In each experimental group 4 fish were used. The groups received, intraperitoneally, 0, 2, 10 and 20 μg/g body weight of the partially purified salmon gonadotropin (SG-G100). Injection volume was adjusted to 1% of the body weight. Three days after the first injection, the fish were weighed and the presence of ovulation was determined by gently pressing the abdomen of the fish. Those fish, which were induced to ovulate after the first injection, were killed on this day. The other non-ovulated fish received a second injection. Two days after the second injection the fish were anesthetized, and ovarian and body weights were measured.

Experiment 2 was undertaken using the same method as experiment 1. These fish were exposed to long daylength from August to October in order to delay their ovarian maturation and weighed 40-70 g. This experiment was carried out in November 1971. In each experimental group 10 fish were prepared. The groups received two injections of 0, 1, 2, and 10 μg/g salmon gonadotropin. The interval between injections was 2 days, and after each injection the fish were sampled as described for experiment 1.

In both experiments the ovaries were fixed in 10% neutral formalin or BOUIN. The tissues were embedded in paraffin and the sections were stained with the Mayer's hematoxylin-eosin or PAS procedure. One hundred eggs, fixed in 10% formalin, were used to determine the mean egg diameter using a dissecting microscope.

| Table 1. Effect of salmon gonadotropin on ovulation, body weight, GSI and egg diameter in Ayu (experiment 1). |
|---|---|---|---|---|---|
| Salmon gonadotropin (μg) | No. | Body weight initial (g) | Body weight change (%) | No. of fish ovulated | GSI | Egg diameter (mm) |
| 0 | 3 | 20.9 ± 3.12 | 1st -3.06 ± 1.10 | 0 | 34.03 ± 1.71 | 0.81 ± 0.013 |
| 2 | 4 | 25.1 ± 2.28 | 1st 1.95 ± 1.46 | 1 | 44.76 ± 2.02 | 0.85 ± 0.015 |
| 10 | 4 | 24.7 ± 3.02 | 1st 3.23 ± 1.16 | 4 | 39.66 ± 1.39 | 0.83 ± 0.004 |
| 20 | 3 | 25.4 ± 2.17 | 1st 4.73 ± 2.05 | 3 | 44.71 ± 2.20 | 0.84 ± 0.004 |

1) In two groups, one fish containing atretic oocytes was excluded.
2) Mean and standard error
3) The fish, which were not induced to ovulate after the first injection, were again weighed after the second injection. Only fish which received a second injection were used to calculate this mean value.
4) Only values from the ovulated fish were used to calculate GSI and egg diameter.
5) Significant compared with the control.
Results

The effects of salmon gonadotropin on ovulation, body weight, GSI (gonadosomatic index, gonad weight $\times$ 100/body weight) and egg diameter of ayu, *Plecoglossus altivelis*, are shown in Table 1 (experiment 1). The ovary at the initiation of this investigation was mostly composed of oocytes in the last phase of the yolk stage. Some migratory

![Fig. 1. Oocytes at the last phase of the yolk stage in ayu. $\times$800.](image1)

![Fig. 2. The migratory nucleus stage oocyte (arrow) in ayu. $\times$700.](image2)

nucleus stage oocytes were observed (Figs. 1 and 2). Ovulation was observed in all the fish treated with 10 or 20 $\mu$g salmon gonadotropin within 3 days after the first injection. In the fish which received the minimum dose of 2 $\mu$g, ovulation was confirmed within 2 days after the second injection. GSI and egg diameter were greater in the gonadotropin-treated fish than in the control. In the ovulated fish, there was a positive cor-
Table 2. Effect of salmon gonadotropin on ovulation, body weight, GSI and egg diameter in Ayu (experiment 2).

<table>
<thead>
<tr>
<th>Salmon gonadotropin (µg)</th>
<th>No.</th>
<th>initial (g)</th>
<th>Body weight change (%)</th>
<th>No. of fish ovulated</th>
<th>GSI</th>
<th>Egg diameter (mm)</th>
</tr>
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<tr>
<td>0</td>
<td>10</td>
<td>59.6±2.29</td>
<td>1st -2.28±0.02</td>
<td>0</td>
<td>25.37±0.97</td>
<td>0.77±0.008</td>
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<td></td>
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<td></td>
<td>2nd -4.27±0.37</td>
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<tr>
<td>1</td>
<td>10</td>
<td>53.3±1.28</td>
<td>1st -1.07±0.02</td>
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<td></td>
<td></td>
<td>2nd -0.16±0.91</td>
<td>5</td>
<td>30.65±1.70</td>
<td>(p&lt;0.05)</td>
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<td></td>
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<tr>
<td>2</td>
<td>91</td>
<td>55.7±2.72</td>
<td>1st 0.02±0.46</td>
<td>3</td>
<td>30.46±1.21</td>
<td>(p&lt;0.01)</td>
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<td></td>
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<td></td>
<td>2nd 2.42±0.70</td>
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<td>(p&lt;0.01)</td>
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<tr>
<td>10</td>
<td>10</td>
<td>57.2±2.41</td>
<td>1st 0.86±0.27</td>
<td>8</td>
<td>30.72±0.87</td>
<td>0.81±0.009</td>
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<td></td>
<td>2nd 3.41±0.79</td>
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<td></td>
<td>(p&lt;0.01)</td>
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</tbody>
</table>

1) One fish was lost during the experiment.
Refer to the footnotes in Table1.

Fig. 3. Relationship between GSI and egg diameter in the gonadotropin-treated fish. Open circle (exp. 1); Closed circle (exp. 2)

Fig. 4. Relationship between percent change in body weight and GSI in the gonadotropin-treated fish (exp.2).

The relation between GSI and egg diameter (\( r > 0.89 \), Fig. 3). Interestingly, in the gonadotropin-treated fish the body weight increased by a few percents after the injections, while it decreased by about 3% in the controls. The increase in body weight was significant when compared with the control (\( p < 0.05 \) or \( p < 0.01 \)).

In experiment 2, the response of the fish to salmon gonadotropin at a dosage of
less than 10 µg/g was examined (Table 2). Ovulation was not observed in fish treated with 1 µg of gonadotropin within 2 days after the first injection, but ovulation was induced in half of the fish after the second injection. Injection of 10 µg gonadotropin induced ovulation in 80% of the fish after the first injection and in the remaining 20% after the second injection. In the group receiving 1 µg gonadotropin an increase in body weight similar to that in experiment 1 did not occur, but an increase in body weight was observed in the 10 µg group. GSI and egg diameter were also greater in the gonadotropin-treated fish than in the control. The differences were highly significant when compared with the control. Furthermore, the positive correlation between the percent change in body weight and GSI is shown in Fig. 4 (α>0.76).

Discussion

In this investigation, salmon gonadotropin partially purified using ethanol extraction and gel filtration on Sephadex G-100 was very effective in inducing ovulation in intact ayu, Plecoglossus altivelis, which is one of the important fish used for aquiculture in Japan. Although there were differences in the body weight and GSI of the fish between the two experiments, in the salmon gonadotropin (10 µg or 2 µg)-treated fish, similar occurrence of ovulation was observed in both cases. Injection of 1 µg gonadotropin was partially effective in inducing ovulation in the fish, while that of 10 µg was 100% effective. Therefore the practical effective dosage of gonadotropin shall be 10 µg/g. This effective dosage was higher than that in the intact catfish suggesting that there was species difference in inducing ovulation of teleost. When compared with the previous data using commercial mammalian gonadotropin HCG (Puberogen) for inducing ovulation in ayu, 10 µg purified salmon gonadotropin corresponded to 1,250 IU of HCG. It was reported that bioassay of HCG purified by Sephadex G-100 gave potency values of 10,000–20,000 IU/mg. Thus the salmon gonadotropin was more effective in inducing ovulation in ayu than the commercial mammalian gonadotropin. This higher potency of the salmon gonadotropin indicates a degree of species specificity.

An increase in body weight was clearly shown in the gonadotropin-treated fish, while the body weight in the control fish decreased by about 3% during the period of experiment. This increment was dose-dependent and, furthermore, parallel to the increasing rate of ovulation. Moreover, in the gonadotropin-treated fish the positive correlation between GSI and the increase in body weight was clearly shown (see Fig. 4). This increase in body weight seemed to be mostly due to an increase in ovarian weight in the gonadotropin-treated fish. The ovaries in this study were composed of the oocytes in the last phase of the yolk stage and in the migratory nucleus stage. Electron microscopy of the Oryzias oocytes during maturation and ovulation indicates that these
oocytes were not undergoing yolk formation. Therefore, the increase in egg diameter observed in the gonadotropin-treated ayu may be due to water uptake by the oocyte. Consequently, since the fish were not fed during the period of experiment, it can be suggested that the increase in body weight may be ascribed to the ingestion of water and its subsequent movement into the ovary. This suggestion is supported by the recent report of Hirose et al. (in preparation), describing the relationship between the drinking rate of water and ovulation in ayu treated with salmon gonadotropin.

Acknowledgements

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References