

Narumi Sato, Ichiro Kawazoe and Katsumi Aida  
Department of Aquatic Bioscience, Graduate School  
of Agricultural and Life Sciences, The University of Tokyo,  
Bunkyo, Tokyo 113-8657, Japan.  
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## Induction of Ovarian Maturation by Two Distinct Salmon Gonadotropins in Japanese Eel

### Abstract

Salmon gonadotropin (sGTH) I and II were separated from the sGTH fraction and their effects on the induction of ovarian maturation in Japanese eel were investigated. Immature female silver eels received weekly intramuscular injections of sGTH I, sGTH II or sGTH fraction (2 mg/kg body weight). In the fish that completed vitellogenesis, final oocyte maturation and ovulation were induced by a simultaneous injection of sGTH fraction and 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregnen-3-one. In the fish injected with sGTH I, it took longer time to induce the completion of vitellogenesis than in the fish injected with sGTH II or sGTH fraction. Fertilization rate of eggs from the fish injected with sGTH fraction were higher than those from the fish injected with sGTH I or II, and hatched larvae were obtained. In the fish that received 21- 23 times of weekly sGTH I injection followed by weekly sGTH II injections, oocytes showed rapid maturation and finally ovulated. Fertilization rate of ovulated eggs were high and hatched larvae were also obtained. From these results, the effects of sGTH I and II on the induction of ovarian maturation were different. And it was considered that both sGTH I and II were required for the induction of ovarian maturation and obtaining good quality of eggs.

**Key words:** Japanese eel, Salmon gonadotropin I and II, Ovarian maturation, Vitellogenesis, Ovulation

In Japanese eel *Anguilla japonica* oocyte development has not been observed to occur under ordinary rearing conditions. Therefore, hormonal treatment is necessary to induce ovarian maturation artificially. We have previously demonstrated that the weekly injection of salmon gonadotropin fraction (sGTH) induces ovarian maturation (Sato *et al.* 1996). However, it was not clear which specific GTH is effective since sGTH consists of a mixture of sGTH I and sGTH II. In this study, sGTH I and sGTH II were separated by ion exchange chromatography and their

effects on the induction of ovarian maturation were investigated using immature female eels.

### Materials and Methods

sGTH was extracted from chum salmon pituitaries by the methods of Suzuki *et al.* (1) and desalting by sephadex G-25 gel filtration. sGTH I and sGTH II were separated from the sGTH using DE-52 ion exchange chromatography. Immature female silver eels (n=19, BW 350 ~ 1,120 g) were used for the experiment. Fish received weekly intramuscular

injections of sGTH I (n=7), sGTH II (n=4) or sGTH (n=4) at a dose of 2mg/kg BW using an emulsion prepared with lipophilized gelatin<sup>(2)</sup>. Controls (n=4) were injected with vehicle only. In fish which had completed vitellogenesis, final oocyte maturation and

ovulation were induced by a simultaneous injection of sGTH and 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-3-one (DHP). Ovulated eggs of these fish were stripped, and then fertilized by the dry method using sperm collected from mature males injected with HCG.

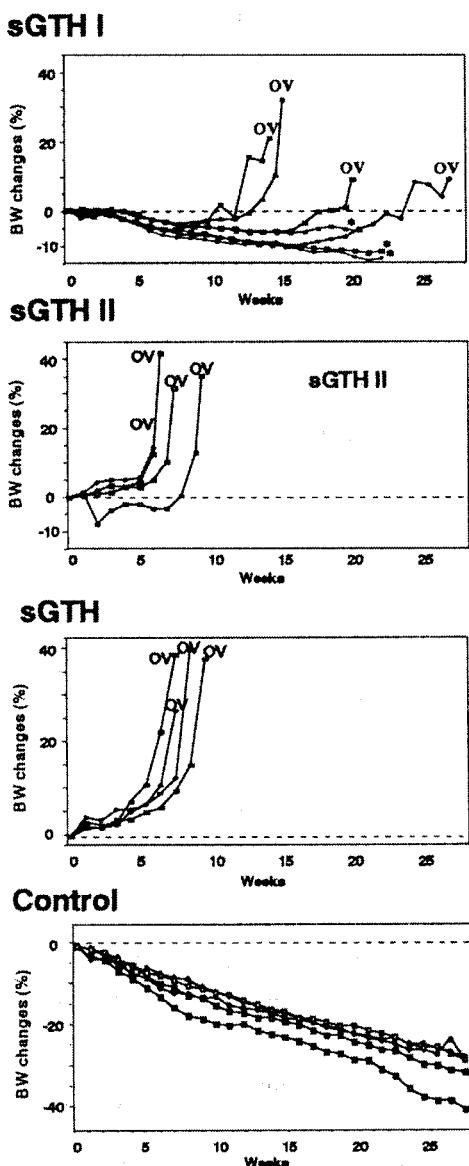


Fig. 1. BW changes in the fish treated with sGTH I, sGTH II or sGTH and controls. OV: ovulation.

## Results

It is well known that rapid increases in BW occur due to hydration of the oocytes during final maturation. For this reason, we utilized rapid BW increase as an indicator of the last phase of ovarian maturation. In all fish injected with sGTH II or sGTH, rapid increases of over 110% in BW occurred after 7-11 occasions of weekly injection. Both groups injected with sGTH II or sGTH showed similar results. In fish injected with sGTH I, 4 individuals showed rapid increases in BW after 12-28 occasions of weekly injection, whereas the other individuals failed to show rapid increases in BW. In these fish, we replaced sGTH I treatment with sGTH II after 21-23 occasions of sGTH I injection; thereafter, rapid increases in BW could finally be induced. Following sGTH and DHP treatment, all fish ovulated.

In fish which matured due to sGTH I and sGTH II treatment, fertilization rates of ovulated eggs were 0% in almost all individuals. In most fish injected with sGTH, fertilization rates of ovulated eggs ranged from 1.9 to 75.4%, and hatched larvae were obtained. In fish which received 21- 23 weekly injections of sGTH I followed by weekly sGTH II injection, oocytes showed rapid maturation culminating in ovulation. Fertilization rate of ovulated eggs were 45-60% in 2 fish and hatched larvae were obtained.

## Discussion

Although both sGTH I and sGTH II stimulated vitellogenesis, with sGTH I treatment, a longer period of time was required to complete vitellogenesis than

with sGTH II treatment. It has been shown that the GTH I gene is expressed during the early stages of vitellogenesis, and that the expression of the GTH II gene rapidly increases with the progression of vitellogenesis, reaching a peak at final oocyte maturation and ovulation<sup>(3)</sup>. Therefore, it is suggested that sGTH I is necessary to initiate vitellogenesis, and that sGTH II is effective in maintaining vitellogenesis and inducing final maturation and ovulation. Fertilization rates in the sGTH group and in fish treated with a combination of sGTH I and II were higher than those in fish injected with sGTH I or II only. From these results, it can be concluded that the effects of sGTH I and II on the induction of vitellogenesis clearly differ. It is considered that sGTH I administration followed by sGTH II administration is adequate for inducing ovarian maturation and obtaining eggs of good quality.

## References

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