Induced Ovulation by Injection of 17,20β-Dihydroxy-4-pregnen-3-one in the Artificially Matured Japanese Eel, with Special Reference to Ovulation Time

Hirohiko Kagawa,* 1 Hideki Tanaka,* 1 Hiromi Ohta,* 1 Koichi Okuzawa,* 2 and Norio Iinuma* 3

* 1 National Research Institute of Aquaculture, Nansei, Mie 516-01, Japan
* 2 National Research Institute of Aquaculture, Tamaki, Mie 519-04, Japan
* 3 Faculty of Bioresources, Mie University, Kamihama, Tsu, Mie 514, Japan

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The effects of injection of 17,20β-dihydroxy-4-pregnen-3-one (DHP) on induced ovulation in the artificially matured Japanese eel Anguilla japonica were examined, with special reference to the effect of injection time on the time of ovulation. Ovulation occurred 18 h and 21 h after injection of DHP at 9:00, in 10 of 15 females and in the remaining 5 females, respectively. Injection of DHP at 18:00 induced ovulation 15, 18, and 21 h after injection in 4, 12, and 2 of 18 females, respectively. Fertility and hatching rates were not significantly different between females which ovulated 18 h after injection at 9:00 or 18:00. In the second experiment, eggs from females which ovulated 15 h after DHP injection showed significantly higher fertility (about 63%) and hatching rates (about 54%) than eggs from females which ovulated 18 h after the injection. These results indicate that the time of ovulation depends on the time elapsed after the DHP injection in the eel.

Key words: eel, 17,20β-dihydroxy-4-pregnen-3-one, ovulation time, fertility, hatching rate

Artificial induction of final maturation of oocyte and ovulation in the eel have been examined by many workers. Injection of salmon pituitary extract can often induce final maturation of oocytes and ovulation, but success rates are low and even if ovulated eggs are obtained, the eggs show low fertility and hatching rates. 1,2) Our recent in vitro studies showed that 17,20β-dihydroxy-4-pregnen-3-one (DHP) can induce final maturation of migratory nucleus stage oocytes over 700 μm in diameter. 3) Injection of DHP successfully induced final maturation and ovulation in females which possessed oocytes over 800 μm in diameter. 4) Ovulation occurred in the early morning (3:00 to 6:00) when DHP was injected at 10:00 of the previous day. The former studies also described ovulation or the spawning occurring in the early morning in females treated with gonadotropin-releasing hormone or piscine pituitary homogenates. 5-7) These data suggest that the possibility that the time of ovulation of the eel is entrained by a circadian rhythm. Moreover, our previous study 8) indicates that the fertility and hatching rates fell rapidly after ovulation. These results indicated that ovulation must be checked through the night and eggs must be fertilized soon after ovulation in the early morning. Thus, for practical purposes, regulating the time of ovulation in eels induced by DHP will improve labor-efficiency and increase fertility and hatching rates. Therefore, this study was undertaken to clarify the effects of changing time of DHP injection on ovulation time in the Japanese eel.

Materials and Methods

Two and half year-old eels (500–1,000 g in body weight) which were feminized with estradiol-17β were used in the present study. After acclimation to seawater, they were kept without feeding in 1,000 liter flow-through seawater tanks under a natural photoperiod at a water temperature of 20°C. They were injected intraperitoneally with salmon pituitary extract (20 mg pituitary powder/fish/week). Oocyte diameter and maturity stage was determined by taking oocytes from genital pore with polyethylene cannula in females of which body weight indices (body weight/initial body weight × 100) exceeded 110%. Females which possessed oocytes of over 750 μm in diameter at migratory nucleus stage were processed according to the method described in the previous study. 4) Briefly, females were injected with salmon pituitary extract (20 mg/pituitary powder/fish/week) as a priming dose followed 24 h later by injection of DHP (2 μg/g body weight) intraperitoneally.

Experiment 1 was carried out in the period of September 1994 to February 1995. Injection of salmon pituitary extracts was started in September 1994 for the first experimental group and from November 1994 for the second experimental group. After 8–17 injections, females possessed oocytes of over 750 μm in diameter at the migratory nucleus stage. In the first experimental group, these females were treated with a priming dose of pituitary extract at 9:00 followed 24 h later by DHP injection at 9:00. Fish in the second experimental group received a priming dose of pituitary extract at 18:00 followed 24 h later by DHP injection at 18:00. Ovulation was checked by applying gentle pressure on the abdomen every 3 hours, beginning 15 h after DHP injection for the first experimental group and from 9 h after DHP injection for the second experimental group.
Experiment 2 was performed during the period of September 1995 to March 1996. Injection of salmon pituitary extract was started from September 1995 or December 1995. Each experimental group was treated in the same manner as described above after females possessed oocytes of over 750 μm in diameter. A priming dose of pituitary extract and DHP injection were performed only at 18:00 in the experiment 2.

Fertility and hatching rates were examined in each ovulated female by the methods described previously. Briefly, 2 g of ovulated eggs were inseminated with 1 ml of diluted semen. Approximately 100 eggs were incubated in a plastic Petri dish filled with 20 ml of filtered seawater at 23°C. Mean fertility and hatching rates were estimated from 3 replicates. All data were expressed as a mean ± SEM of these replicate dishes. Comparison of two means was made with Student's t-test.

Results

Experiment 1

Ovulation Time (Table 1)

When DHP was injected at 9:00, ovulation was observed at 3:00 the following day (18 h after DHP injection) in 10 of 15 females. The remaining 5 females ovulated at 6:00 (21 h after DHP injection). However, when the DHP injection was performed at 18:00, ovulation occurred in 4 of 18 females at 9:00 (15 h after DHP injection), in 12 more females at 12:00 (18 h after DHP injection), and in 2 more females at 15:00 (21 h after DHP injection) the following day.

Fertility and Hatching rates (Table 2)

Fertility of fish which ovulated 18 h and 21 h after injection of DHP at 9:00 were 12.8±6.9% and 2.0±1.9%, respectively. In fish injected with DHP at 18:00, fertility at 15 h and at 18 h after injection was 53.8±11.2% and 9.2±4.4%, respectively. Fertility of the eggs ovulated 18 h after injection of DHP at 9:00 was not significantly different from that of eggs ovulated at 18 h after injection of DHP at 18:00.

Hatching rates after injection of DHP at 9:00 were 9.6±5.5 and 0.28±0.28% in fish which ovulated at 18 h and 21 h, respectively. In fish injected with DHP at 18:00, hatching rates were 39.7±13.5% and 7.2±4.4% at 15 h and 18 h after DHP injection, respectively. Hatching rates of eggs obtained at 18 h after injection of DHP at 9:00 or 18:00 were not significantly different. Both fertility (p<0.01) and hatching rates (p<0.05) in fish which ovulated at 15 h after injection of DHP at 18:00 were significantly higher than those that ovulated at 18 h after DHP injection. Fertility and hatching rates were 0% in fish which ovulated at 15:00 (21 h after the injection of DHP at 18:00).

Experiment 2

Ovulation Time (Table 3)

Injection of DHP at 18:00 induced ovulation in 8 of 34 females at 9:00 (15 h after DHP injection). Eighteen more females ovulated at 12:00 (18 h after DHP injection) and the remaining 8 females ovulated at 15:00 (21 h after DHP injection).

Fertility and hatching rates (Table 4)

Fertility of females which ovulated 15, 18, and 21 h after DHP injection were 62.8±12.4%, 28.0±6.3%, and 3.2±0.9%, respectively. Hatching rates were 54.5±11.1%, 18.4±5.4%, and 0.8±0.6% in females which ovulated 15, 18, and 21 h after DHP injection. Both fertility (p<0.05) and hatching rates (p<0.01) of eggs from fish which ovulated 15 h after DHP injection were significantly higher than those ovulating at 18 h after injection.

Discussion

The present study shows that ovulation occurred 18 to 21 h after injection of DHP at 9:00. These results confirm previous observations showing most of females ovulated 17-20 h after the injection of DHP at 10:00. The present study also clearly shows that even if the time of DHP injection was changed from 9:00 to 18:00, a majority of the fish (12 of 18 females in the Experiment 1 and 18 of 34 females in the Experiment 2) ovulated 18 h after DHP injection. These data indicate that approximately 18 h are necessary for induction of ovulation in the eel by DHP injection, and the time of ovulation depends on the time elapsed after the DHP injection, not on a circadian rhythm. Thus, a shift in the time of DHP injection can change the time of

| Table 1. Ovulation time of fish injected with DHP at 9:00 or 18:00 |
|--------------------------|--------------------------|
| Injection time | 0:00 (15 h) | 3:00 | 6:00 | 9:00 | 12:00 | 15:00 |
| 9:00 | 15 | 0 | 10 | 5* | 0 | 4 | 12 | 2 |
| 18:00 | 18 | | | | | | |

* Number of fish ovulated.
ovulation, indicating the possibility that ovulation can be induced at a desired time by correctly timing of DHP injection. Fertility and hatching rates 18 h after DHP injection were not significantly different between two experiment groups. Thus, changing the time of the injection appears not to affect fertility and hatching rates. Progestogens can induce not only final maturation of oocytes but also ovulation in vitro in yellow perch,8) goldeye,9) and eel.10) In the perch, DHP may induce ovulation by stimulating synthesis of a prostaglandin in the ovarian follicle.11) Similar mechanisms may be involved in the action of DHP during induced in vitro and in vivo ovulation in the eel.

In the present study, fertility and hatching rates of females which ovulated first after DHP injection were much higher than those of other females. In particular, fertility and hatching rates in females which ovulated 15 h after DHP injection were over 50% in Experiments 1 and 2. Thus, we can obtain sufficient numbers of high quality ovulated eggs for practical purposes, since one female ovulates over 500,000 egg at one time. Although the reason for high fertility and hatching rates in these females are unclear, further studies are necessary to develop methods for obtaining females which consistently ovulate in the shortest possible time after DHP injection.

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References