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In Vitro Induction of Final Maturation of Oocytes from Coho Salmon[1]

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Abstract

The most effective compounds tested in vitro for inducing maturation of oocytes from one coho salmon *Oncorhynchus kisutch* in fresh water were partly purified salmon gonadotropin and 17α-hydroxy-20-β-dihydroprogesterone (17α,20β-dihydroxy-4-pregnen-3-one). The steroid, 20β-hydroxyprogesterone (20β-hydroxy-4-pregnen-3-one), was less effective, and progesterone, cortisol, and cortisone were relatively ineffective. With one fish retained in seawater during final maturation, 17α-hydroxyprogesterone was only slightly effective in inducing germinal vesicle breakdown in oocytes. Oocyte maturation was not induced by any other treatment of the other fish tested from seawater.

Steroids and gonadotropin have been effective in vitro inducers of oocyte maturation and ovulation in different species of fish. The regulation and role of hormones at final maturation, however, are still unknown. This is particularly true with Pacific salmon, in part due to the major fraction of their life cycles spent in the ocean, where they are inaccessible for controlled studies.

A hormone, 17α-hydroxy-20β-dihydroprogesterone (17α,20β-dihydroxy-4-pregnen-3-one), has been implicated as the most potent mediator of final maturation (migration of the germinal vesicle and subsequent breakdown) in vitro and in vivo in rainbow trout *Salmo gairdneri* (Jalabert 1976; Jalabert et al. 1978). This steroid occurs at high concentrations in rainbow trout during sexual maturation (Campbell et al. 1980). Jalabert (1976) considered ovulation (the release of the matured oocyte from the follicle) to be separated from maturation as a distinct process that is mediated indirectly by gonadotropin and triggered by epinephrine or prostaglandin(s).

No in vitro studies have been reported previously for oocyte maturation or ovulation for coho salmon. The coho salmon that were tested in this study were held in fresh water (their natural environment) or in seawater (an unnatural environment) throughout the spawning season. Salmon retained in seawater during final maturation and ovulation have osmoregulatory difficulties, ion imbalance, dehydrated eggs, and high adult mortality (Sower 1980). An attempt was made to determine if, in vitro, ova from coho salmon retained in seawater and ova from fish held in fresh water differed in their responses to hormone treatments. Consequently, the main objective of the present study was to determine the effects of steroids and gonadotropin on oocyte maturation in coho salmon.

Methods

Seven adult female coho salmon, obtained from Ore-Aqua Foods (Weyerhaeuser, Incorporated), were killed by suffocation or by a blow to the head to obtain oocytes for incubation by Goetz's (1976) procedures. Two fish were from fresh water at Jefferson, Oregon, and five were from seawater at Newport, Oregon. The fish at Newport remained in seawater throughout final maturation and ovulation.

After the fish were killed, the ovaries were removed and held in ice-cooled Cortland's salt solution (Wolf 1963; pH 7.2). The ovaries were then transported to Oregon State University (99 km east of Newport), cut into groups of about 10 oocytes each, and immediately placed into 25-ml Erlenmeyer flasks containing 10 ml of Cortland's saline solution. The flasks were either control flasks or contained the Cortland's saline solution with the appropriate dosage of hormone. Control flasks received either propylene glycol : ethanol (1:1), or no additive. The steroid hormones were initially suspended in either propylene glycol or propylene glycol–ethanol; SG-G100 was initially dissolved in Cortland's saline solution. Aliquots of these concentrated solutions were dissolved directly in the salt solution, never exceeding 1 μl/ml of medium. Each treatment was duplicated. The flasks were held in the Seecubator in a constant-temperature room at 15.6 C. The Seecubator was flushed with a mixture of 60% O_2 : 40% N_2 and shaken pe-

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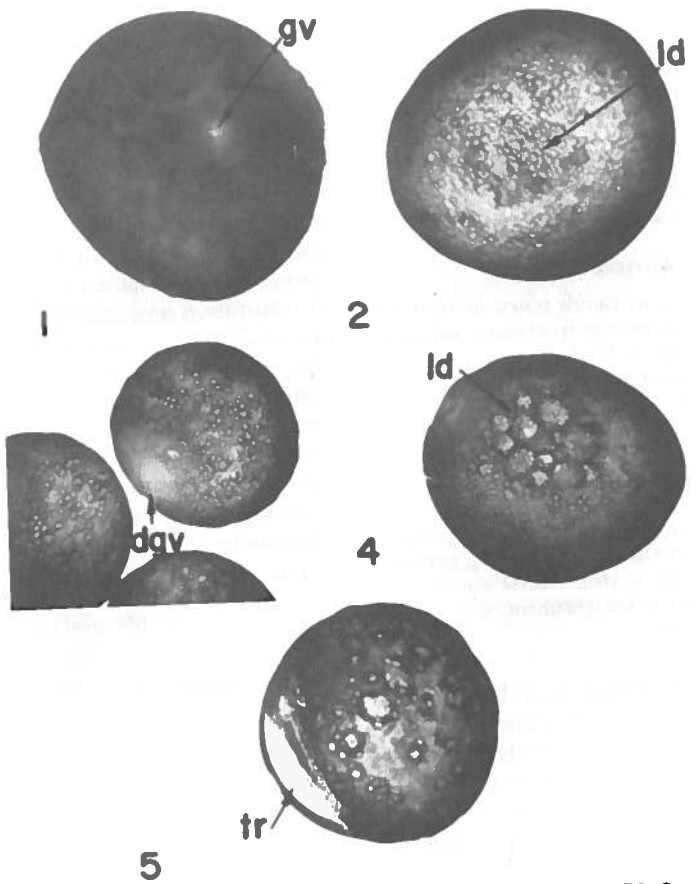

FIGURE 1.—*Oocytes from coho salmon undergoing maturation and ovulation in fall 1979. Stages of oocyte maturation include: (1) migrating germinal vesicle, (2) peripheral germinal vesicle, and (3) and (4) germinal vesicle breakdown; also shown is an overripe egg (5) which is characterized by partial transparency (gv = germinal vesicle; ld = lipid droplets; dgv = diffused germinal vesicle; and tr = transparency).*

riodically until the end of the test period. At the end of incubation, oocytes were removed from the flasks and placed into Stockard's solution for fixation and clearing. After treatment in the fixing agent, the oocytes were carefully separated from each other and evaluated under a dissection microscope. Stages of oocyte maturation as described by Jalabert et al. (1976) for rainbow trout were evaluated for coho salmon as follows: (1) premigrating germinal vesicle; (2) migration of germinal vesicle and migration and segregation of lipid drops; (3) germinal vesicle against chorion with distinct contour; and (4) vitelline maturation, coalescence of lipid drops, and germinal vesicle breakdown.

Oocytes from three fish from seawater and one fish from fresh water were treated with 17α-hydroxyprogesterone, 20β-hydroxyprogesterone, progesterone, or deoxycorticosterone and incubated for 8, 19, 26, 49, 51, 68, or 87 hours, to determine the effects of the duration of the treatments on oocyte maturation. Oocytes from one fish from fresh water and from two fish from seawater were treated with 17α-hydroxy-20β-dihydroprogesterone, 17α-hydroxyprogesterone, 20β-hydroxyprogesterone, progesterone, cortisol, cortisone, or SG-G100 to determine the effects of these hormones on germinal vesicle breakdown at various concentrations.

Results

The maturation of oocytes in coho salmon appeared to be slightly different from that re-

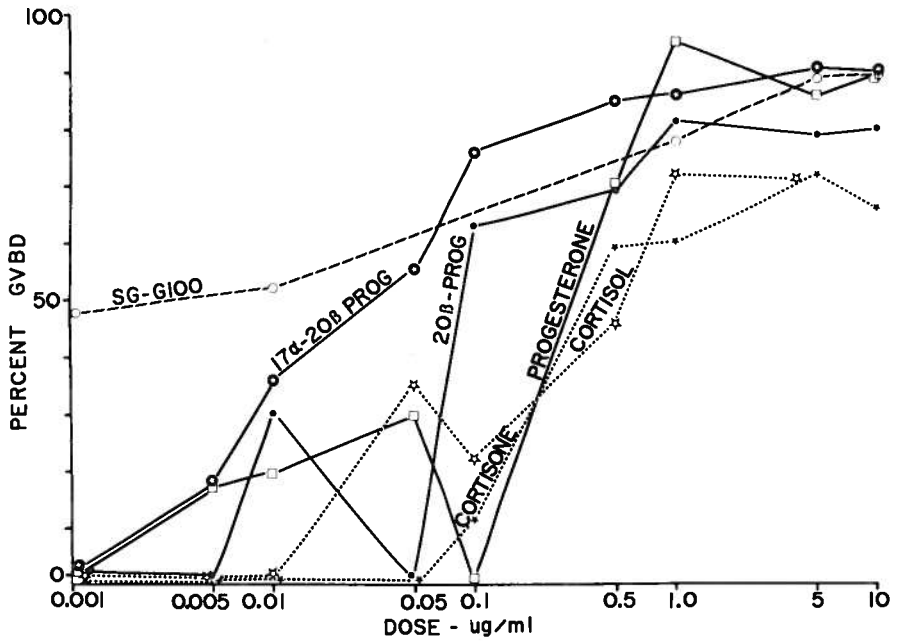

FIGURE 2.—*Percentage of germinal vesicle breakdown (GVBD) during oocyte maturation of coho salmon as a function of the dose of six hormones: partly purified coho salmon gonadotropin (SG-G100), 17α-hydroxy-20β-dihydroprogesterone (17α-20β-PROG), 20β-hydroxyprogestrone (20β-PROG), progesterone, cortisone, and cortisol. Each percentage was calculated from a lot of 20 to 30 oocytes. All data represent oocytes from one female from fresh water in 1979. In the controls (not shown), GVBD was less than 6.5%.*

ported by Jalabert et al. (1976) for rainbow trout and by Osanai et al. (1972) for pink salmon (*Oncorhynchus gorbuscha*). We therefore modified the four stages described by these investigators as follows (Fig. 1): (1) premigrating germinal vesicle; (2) migration of germinal vesicle, migration and segregation of lipid drops, development of cortical cytoplasm; (3) germinal vesicle against chorion with distinct contour; and (4) vitelline maturation, coalescence of lipid drops, and germinal vesicle diffused against the chorion.

Oocytes from four (of five) seawater coho salmon and from one (of two) freshwater fish either had high mortalities or did not respond to treatments. These included all oocytes from the time-course study.

For oocytes from the other freshwater coho salmon, SG-G100 and 17α-hydroxy-20β-dihydroprogesterone were the most potent hormones in inducing maturation after 67 hours. The median effective doses for SG-G100 and 17α-hydroxy-20β-dihydroprogesterone were near 0.005 and 0.03 μg/ml medium, respectively (Fig. 2). The other compounds—20β-hydroxyprogesterone, progesterone, cortisone, and cortisol—were less effective in inducing oocyte maturation; the median effective doses were about 0.07, 0.25, 0.35, and 0.5 μg/ml medium, respectively. Less than 6.5% germinal vesicle breakdown occurred in controls.

Oocytes of the female from seawater responded to 17α-hydroxyprogesterone, but the effect was small: 35% germinal vesicle breakdown (versus 0% for controls) and no ovulation. Oocytes that appeared to have undergone germinal vesicle breakdown also were collapsed—a condition probably indicating dehydration and ion imbalance. A few of the eggs from other treatments ovulated, but most of the oocytes appeared overripe. In live fish, overripe eggs usually are retained in the body cavity for an extended period after ovulation and are characterized by their semitransparency (Fig. 1).

Discussion

For oocytes from the one female coho salmon held in fresh water, SG-G100 was the most ef-

fective inducer of germinal vesicle breakdown, followed by the progestogens and the 11-oxygenated corticosteroids. At low dosages, SG-G100 was capable of stimulating germinal vesicle breakdown in coho salmon oocytes. Jalabert et al. (1974) have shown gonadotropin to be effective in rainbow trout oocyte maturation. Goetz (1976) demonstrated that crude and partly purified pituitary preparations from the common carp *Cyprinus carpio* are very effective in inducing germinal vesicle breakdown in oocytes of brook trout *Salvelinus fontinalis,* and that these preparations are more effective than non-pituitary mammalian gonadotropins such as human chorionic gonadotropin.

The three steroids effective in inducing maturation of oocytes from the one coho salmon were 17α-hydroxyprogesterone, particularly 17α-hydroxy-20β-dihydroprogesterone, and to a lesser extent 20β-hydroxyprogesterone. Progesterone was effective at a much higher concentration. Progesterone concentrations measured in rainbow trout (Campbell et al. 1980) and coho salmon (Sower and Schreck 1982a) were low compared with the progesterone-derivative concentrations of 17α-hydroxy-20β-dihydroprogesterone and 17α-hydroxyprogesterone during final maturation and ovulation.

In rainbow trout, 11-oxygenated corticosteroids have not been shown to be effective inducers of oocyte maturation; however, cortisol and cortisone increased follicular sensitivity to gonadotropin and thus increased the percentage of oocyte maturation (Jalabert 1976). Cortisol and cortisone in our study were relatively ineffective in inducing maturation, except at high concentrations (exceeding 0.3 μg/ml medium).

The steroid 17α-hydroxyprogesterone at 2 ng/ml medium was relatively ineffective in inducing maturation—perhaps because the dosage used was inadequate or because the ova were from fish held in seawater. This steroid has been shown to be effective in inducing germinal vesicle breakdown in northern pike *Esox lucius* (Jalabert 1976), and in yellow perch *Perca flavescens* and brook trout (Goetz and Bergman 1978). It is difficult to generalize from this one experiment about the effects of hormones on fish that were retained in seawater during maturation. However, most of the ova involving fish in seawater did not respond to treatments, partly due to high mortality and unusual clearing, generally associated with overripe eggs. Coho salmon retained in seawater during the spawning season experience osmoregulatory and reproductive difficulties (Sower 1980). Furthermore, in vivo studies of coho salmon showed hormonal treatments that were effective in accelerating maturation and ovulation in females in fresh water were either less effective or ineffective in fish retained in seawater (Sower and Schreck 1982b).

We suggest that coho salmon gonadotropin and 17α-hydroxy-20β-dihydroprogesterone may be two of the more important natural mediators involved in germinal vesicle breakdown in coho salmon, as others have proposed for rainbow trout. This statement is further supported by in vivo studies that demonstrated coho salmon gonadotropin and 17α-hydroxy-20β-dihydroprogesterone to be almost equally effective in accelerating maturation and ovulation in coho salmon (Sower and Schreck 1982b). It should be noted, however, that other hormones, such as androgens and estrogens, certainly are implicated either directly or indirectly in some known yet undefined role (Sower and Schreck 1981a); further studies are recommended to elucidate the involvement of such hormones in the final maturational processes in coho salmon.

Acknowledgments

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