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The Role of Steroids in the Control of Oocyte Final Maturation and Ovulation in Yellow Perch (*Perca flavescens*)

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Research has been conducted on the endocrine control of oocyte final maturation and ovulation in various fishs pecies; however, little is known about the control of these processes in members of the extensive and highly evolved teleost order, the perciformes. For this reason, we investigated the hormonal control of oocyte final maturation and ovulation in yellow perch (*Perca flavescens* : Perciformes, Percidae). Using *in vitro* incubation techniques we found that progestogens and 11-deoxycorticosteroids induced germinal vesicle maturation (GVM-germinal vesicle migration and breakdown) and ovulation of perch oocytes. The most potent steroid was 17α-hydroxy-20β-dihydroprogesterone (17α-20β-PROG). This steroid induced significant GVM at concentrations as low as 0.12 ng/ml and ovulation at 0.24 ng/ml. At higher 17α-20β-PROG concentrations ovulation was apparently independent of GVM since oocytes were frequently ovulated prior to germinal vesicle breakdown. The number of oocytes ovulating with intact germinal vesicles was related to the degree of germinal vesicle migration prior to incubation. Therefore, in early oocytes in which the germinal vesicle was in the center prior to incubation the percentage of oocytes ovulating with intact germinal vesicles was higher than in oocytes in which the GV had already begun migration towards the surface prior to incubation. The concentration of 17α-20β-PROG required for GVM was always lower than that required for ovulation within the 48 hr incubation period.

In vitro 17α-20β-PROG-induced ovulation of yellow perch oocytes was inhibited by indomethacin but occurred normally in indomethacin-blocked follicles following the addition of prostaglandins $F_{2\alpha}$, E_1 or E_2 to the incubation medium. Of the three prostaglandins E_2 was the most potent.

Introduction

In fish the final stages of oocyte maturation include the peripheral migration and subsequent breakdown of the germinal vesicle (GV), lipid droplet coalescence and an increase in oocyte transparency.

Certain C_{21} steroids are capable of inducing germinal vesicle breakdown (GVBD) *in vitro* in oocytes of various fish species representing several teleost orders (Goetz & Bergman 1978, Goswami & Sundararaj 1971, 1974; Hirose 1976, Iwamatsu 1978, Iwamatsu & Katoh 1978, Jalabert 1976, Van Ree et al. 1977, Wallace & Selman 1978). In the majority of the species studied, progestational steroids are the most effective in

inducing GVBD, though in brook trout (*Salvelinus fontinalis*), northern pike (*Esox lucius*) and goldfish (*Carassius auratus*) the 11-deoxygenated corticosteroids are also effective (Goetz & Bergman 1978, Jalabert 1976). Apparently, oocytes of these species are relatively unresponsive to 11-oxygenated corticosteroids. In contrast, in the Indian catfish(*Heteropneustes fossilis*)both 11-deoxygenated and 11-oxygenated corticosteroids effectively induce GVBD, whereas progestational steroids are relatively less effective (Goswami & Sundararaj 1974). And, in the zebrafish (*Brachydanio rerio*), deoxycorticosterone is the most potent of the steroids tested (Van Ree et al. 1977).

No *in vitro* investigations have been attempted on oocytes of any member of the extensive and highly evolved perciformes. For this reason, we began a study several years ago on oocyte final maturation in percid fish, especially the yellow perch (*Perca flavescens:* Perciformes, Percidae).

Materials and Methods

Throughout these studies we have used the same basic *in vitro* incubation techniques (Goetz & Bergman 1978, Goetz & Theofan 1979). Mature perch oocytes are removed from the gravid female during the reproductive season and are incubated at 15°C in Cortland's balanced salt solution (Wolf & Quimby 1969). Twenty-five to thirty intrafollicular oocytes are incubated in 5 ml of the incubation medium containing the steroid compound. Incubates were originally held in a constant shaking waterbath (Goetz & Bergman 1978), however, we now hold incubates in a plexiglass gassing chamber mounted on a shaking platform inside a constant temperature incubator (Goetz & Theofan 1979). The gassing chamber is purged with a 50% O_2: 50%N_2 gas mixture. Following incubation oocytes are evaluated for GVBD and ovulation. The determination of GVBD is facilitated by the use of a fixative that increases cytoplasmic transparency (Goetz & Bergman 1978).

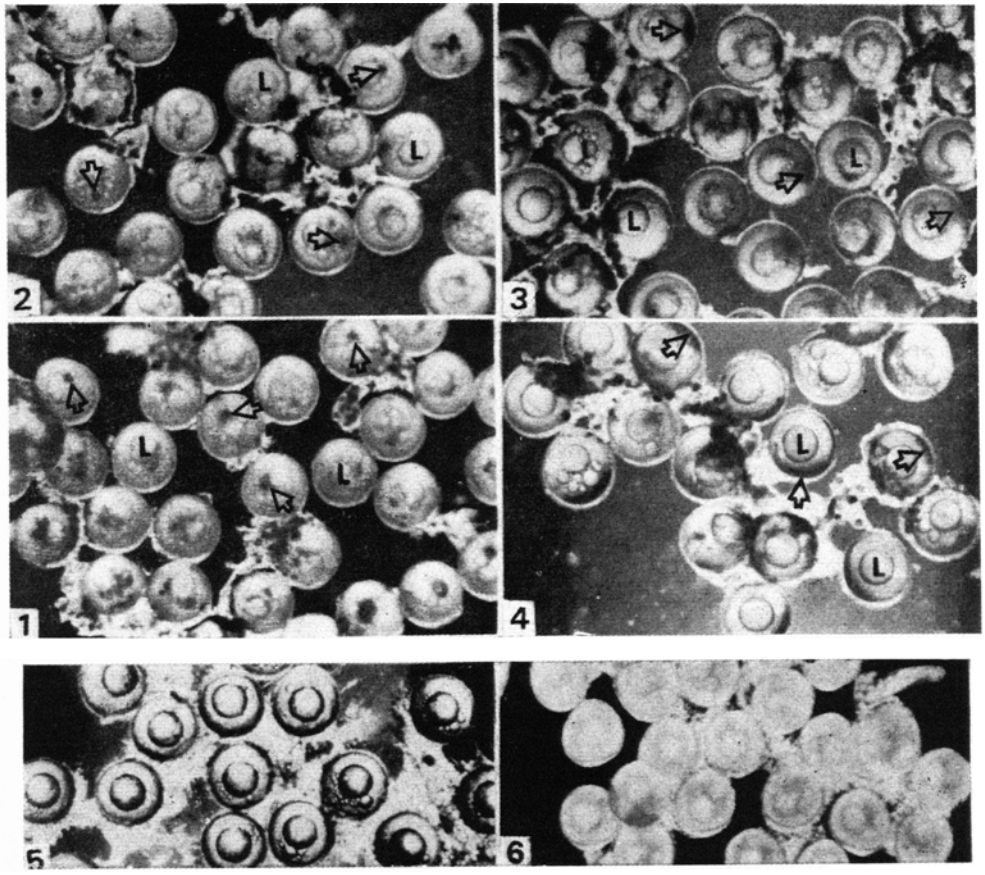
Results and Discussion

Final maturation of percid oocytes

Photographs of yellow perch oocytes undergoing final maturation are not available; however, figures 1-6 illustrate these changes in oocytes of the walleye (*Stizostedion vitreum*), a very closely related species of the same family. The same cytological changes are also observed in yellow perch oocytes and similar changes have been reported in oocytes of the striped bass (*Morone saxatilis*) (Stevens 1966). However, in yellow perch the mature ovulated oocyte observed after spawning (prior to fertilization) is somewhat different since there is also an elevated animal pole, a space between the chorion and the oocyte and an extensive chorion layer that connects one oocyte to another and allows the eggs to be spawned in a large mass (see figure 7).

Steroidal effects on GVBD and ovulation of yellow perch oocytes

In vitro, we have been able to stimulate GV migration, GVBD, complete lipid droplet coalescence, an increase in cytoplasmic transparency and ovulation with certain steroid treatments. Earlier studies revealed that only C_{21} steroids effectively induced GVBD and ovulation of yellow perch oocytes and of these the progestogens and 11-deoxygenated corticosteroids were the most effective (Goetz & Bergman 1978). It was also observed that at certain steroid concentrations a significant percentage of oocytes with intact GVs were also ovulated (figure 8). We hypothesized that this might be a result of the degree of GV migration prior to incubation. Therefore, we conducted several experiments with a wider range of steroid concentrations on oocytes in which the GV was in different stages of migration prior to incubation. We



Figures 1-6 GV migration and GVBD in walleye (*Stizostedion vitreum*) oocytes taken from *in vitro* incubations: **1-4**, oocytes treated with fixative (see Materials and Methods); Arrows indicate GV or animal pole and "L" indicates coalescing lipid droplet. **1**, Early GV migration and lipid droplet coalescence; **2**, Later GV migration and lipid droplet coalescence; **3**, Beginning of GVBD with GV just beneath oocyte surface; **4**, GVBD complete. Lipid droplet coalescence complete but droplet still located in oocyte center **5 & 6**, Untreated oocytes: **5**, Mature,unfixed, preovulatory oocytes with lipid droplet beneath oocyte surface; **6**, Unfixed oocytes in approximately the same stage of GV migration as these shown in figure 2

also tested the effects of 17α-hydroxy-20β-dihydroprogesterone (17α-20β-PROG) since it was the most effective steroid in inducing GVBD in goldfish, northern pike and rainbow trout (*Salmo gairdneri*) (Jalabert 1976). In every experiment, 17α-20β-PROG was the most effective steroid in inducing GVBD or ovulation of yellow perch oocytes (figures 9 and 10, and Goetz & Theofan 1979). 20β-dihydroprogesterone was the next most potent steroid followed by 11-deoxycortisol.

Results of incubations of yellow perch oocytes in which the GV was in the center of oocyte prior to incubation revealed that in most cases higher levels of 17α-20β-PROG

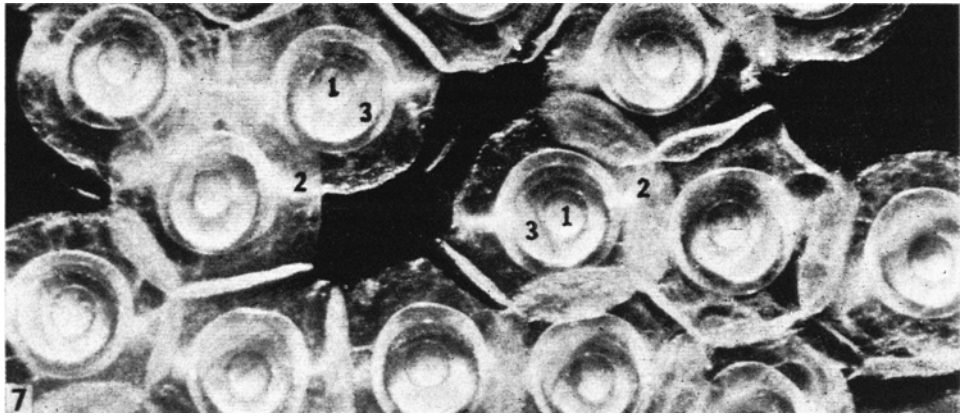


Figure 7 Fully mature, ovulated and spawned yellow perch (*Perca flavescens*) oocytes. **1**, lipid droplet **2**, chorion; 3, animal pole

induced a very large percentage of dead oocytes (figure 11 and Goetz & Theofan 1979). However, these oocytes had already ovulated and it is likely that they had ovulated prior to GVBD. Lower concentrations appeared to induce a greater degree of GV maturation but in certain donors oocytes were still ovulated prior to GVBD (figure 11). In contrast, ovulated oocytes with intact GVs were never observed in incubations with oocytes in which the GV had migrated further towards the oocyte surface prior to incubation (figures 9 and 10).

From these results it appears that the incidence of oocytes ovulating prior to GVBD is dependent on the degree of GV migration prior to incubation. If the GV has migrated sufficiently prior to incubation then GVBD will be complete before ovulation occurs. In contrast, if the GV has not migrated sufficiently, ovulation can occur prior to GVBD and in many cases the oocytes will die.

It was especially interesting that there were several lower 17α-20β-PROG concentrations inducing GVBD but not ovulation within the 48 hr incubation period (figures 9-11). This phenomenon was observed in incubates with all donors regardless of the initial stage of the GV prior to incubation. To determine whether or not ovulation would eventually occur in incubates in which GVBD but not ovulation occurred within the 48 hr incubation period, we observed the longterm effects of low 17α-20β-PROG concentrations on GVBD and ovulation. When oocytes in which the GV was initially one-third the distance between the oocyte center and surface were incubated with 0.48-0.04 ng 17α-20β-PROG/ml no ovulation was observed after 48 hr though a large percentage of oocytes underwent GVBD (table 1). By 96 hr more than 90% of the oocytes had

Table 1 *Longterm effects of 17α-20β-PROG on GVBD and ovulation of yellow perch oocytes. Prior to incubation the GV was one-third the distance from the oocyte center to the periphery*
(**Note** : *Each figure is the mean of three individual donor trials*)

17α-OH-20β-D-PROG Concentration	48 hr		96 hr	
(ng/ml)	GVBD (%)	Ovulation (%)	GVBD (%)	Ovulation (%)
0.48	96.7	0	95.8	77.6
0.36	90.6	0	100.0	63.1
0.27	16.8	0	98.9	21.7
0.20	0	0	98.9	2.2
0.15	0	0	95.4	0
0.11	0	0	32.5	1.0
0.08	0	0	8.5	0
0.06	0	0	13.9	1.1
0.04	0	0	1.1	0
0	0	0	0	0

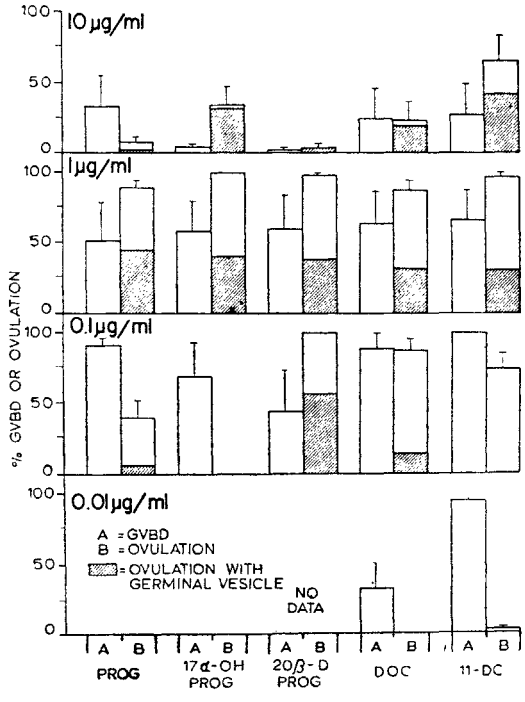


Figure 8 Effects of 17α-dihydroprogesterone (20α-D-PROG), progesterone (PROG), 20β-dihydroprogestrone (20β-D-PROG), deoxycorticosterone (DOC), and 11-deoxycortisol (11-DC) on GVBD and ovulation of yellow perch oocytes
A, GVBD; *B*, ovulaton and hatching indicates oocytes ovulated with intact GVs. Data for GVBD and ovulation are the mean ± 1 standard error of the mean or 4 donor trials
(*From Goetz and Bergman 1978*)

undergone GVBD in incubates with 0.48-0.15 ng 17α-20β-PROG/ml and many ovulated oocytes were observed in the incubates containing 0.48-0.27 ng 17α-20β-PROG/ml. From these results it was apparent that ovulation would eventually follow GVBD at low 17α-20β-PROG concentrations.

In our more recent investigations, 17α-20β-PROG was the most effective steroid in inducing GVBD or ovulation of yellow perch oocytes regardless of the stage of the oocyte (denoted by the GV position) prior to incubation (figures 9 and 10 and Goetz & Theofan 1979). We have previously shown that 11-deoxycortisol and 20β-dihydroprogesterone are more effective in the induction of GVBD and ovulation of yellow perch oocytes than various 11-oxygenated corticosteroids or androgens (Goetz & Bergman 1978). Although the differences in potency are more subtle within the progestogens and 11-deoxycorticosteroids, there appears to be a dramatic difference between these steroids and the 11-oxygenated steroids (Goetz & Bergman 1978). Apparently, this is also true for other fish species (Jalabert et al. 1973, Jalabert 1976).

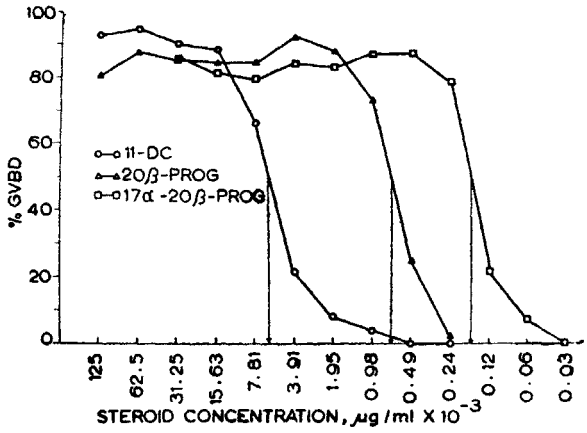


Figure 9 Effects of 11-deoxycortisol (11-DC), 20β-dihydroprogesterone (20β-PROG) and 17α-hydroxy-20β-dihydroprogesterone (17α-20β-PROG) on GVBD in yellow perch oocytes. All figures are the means for 5 donor trials. Prior to incubation the GVs of all donor oocytes were located approximately one-third the distance from the center of the oocyte to the surface. Arrows indicate the mean steroid concentrations producing a 50% response.

(Modified from Goetz and Theofan 1979)

17α-20β-PROG is the most effective steroid in inducing GVBD in rainbow trout, goldfish and northern pike oocytes (Fostier et al. 1973, Jalabert 1976). We have also found it to be the most potent steroid in brook trout (unpublished results). The possibility that 17α-20β-PROG is the most effective maturational steroid in oocytes of all fish species is doubtful, since in *in vitro* incubations with zebrafish oocytes, 17α-20β-PROG was less effective than deoxycorticosterone (Van Ree et al. 1977).

In view of the potency of 17α-20β-PROG in inducing GVBD and ovulation of yellow perch oocytes it is tempting to suggest that it is the maturational steroid in yellow perch. Whether this steroid is produced in yellow perch is currently under investigation.

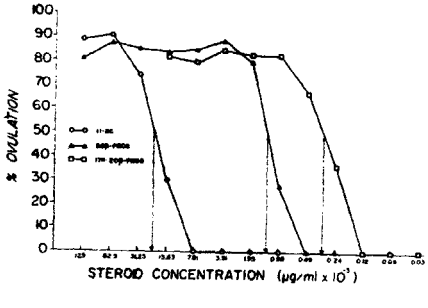


Figure 10 Effects of 11-deoxycortisol (11-DC), 20β-dihydroprogesterone (20β-D-PROG) and 17α-hydroxy-20β-dihydroprogesterone (17α-20β-PROG) on ovulation of yellow perch oocytes. Conditions as in figure 9

(Modified from Goetz and Theofan 1979)

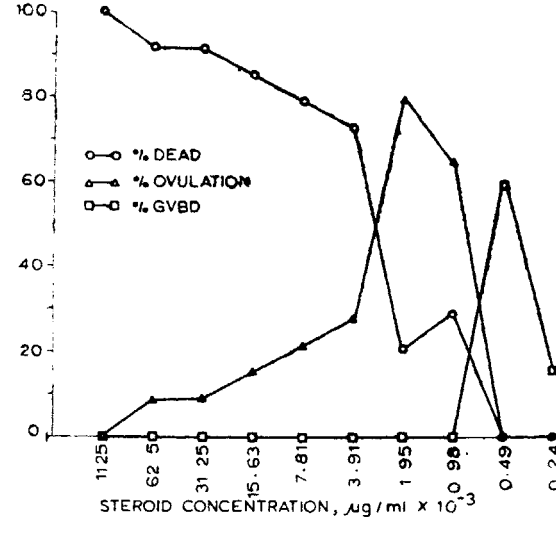


Figure 11 Effects of 17α-20β-PROG on yellow perch oocytes (from one donor) in which the GV was located in the center of the oocyte prior to incubation

(Modified from Goetz and Theofan 1979)

Involvement of prostaglandins in steroid-induced ovulation of yellow perch oocytes

Results of several investigations have indicated that prostaglandins (PGs) may be involved in ovulation of fish oocytes (Jalabert & Szollosi 1975, Stacey & Pandey 1975). We were interested in determining whether or not PGs were involved in steroid-induced ovulation of yellow perch oocytes. Oocytes from seven donors were treated from the initiation of incubation with 0.031μg 17α-20β-PROG/ml or 0.031μg 17α-20β-PROG and 10μg indomethacin/ml. At 33 hr of incubation oocytes treated with steroid and indomethacin were further treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) E_1 (PGE_1) or E_2 (PGE_2). These oocytes were assayed two hours later for ovulation. At the time of assay (35 hr incubation) 67.5$\pm$49.8% of the oocytes treated only with 17α-20β-PROG ovulated, whereas in incubates with steroid and indomethacin no ovulation was observed. Therefore, indomethacin was 100% effective in blocking ovulation by 35 hr PGE_1, PGE_2 and $PGF_{2\alpha}$ induced ovulation in the indomethacin-blocked follicles in a dose-dependent manner (figure 12). PGE_2 was the most potent, while $PGF_{2\alpha}$ and E_1 were approximately equipotent.

Stacey and Pandey (1975) reported that indomethacin could block ovulation in goldfish, induced *in vivo* by human chorionic gonadotropin and elevated holding temperatures. PGE_1, PGE_2 and $PGF_{2\alpha}$ restored ovulation in indomethacin-inhibited fish, though no apparent differences in potency were observed. Jalabert and Szollosi (1975) reported that $PGF_{2\alpha}$ but not PGE_2, induced *in vitro* ovulation of rainbow trout oocytes that had undergone GVBD *in vivo*. Steroid-induced ovulation could be blocked in our investigation by a prostaglandin synthesis inhibitor and restored by prostaglandin replacement. This suggests that prostaglandins are involved in yellow perch ovulation under *in vitro* conditions and may be acting directly on the ovary to induce ovulation.

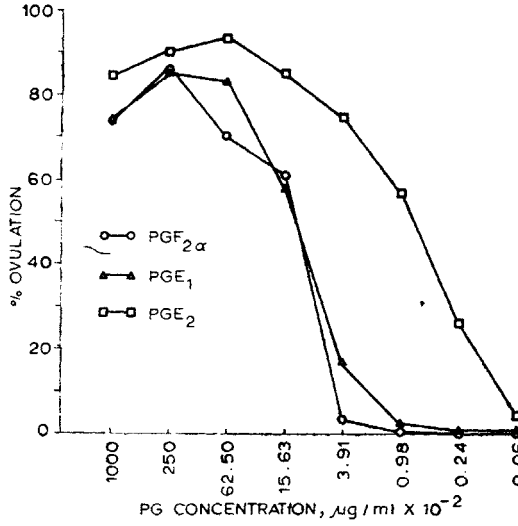


Figure 12 Effects of prostaglandins E_1 (PGE_1), E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) on yellow perch ovulation from indomethacin-blocked follicles. Each figure is the mean for seven individual donor trials
(see text for details)

(*Modified from Goetz and Theofan 1979*)

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