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Hormonal changes during meiotic maturation and ovulation in the brook trout (*Salvelinus fontinalis*)

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Abstract

The plasma levels of estradiol-17 β (E2), 17 α , 20 β -dihydroxy-4-pregnen-3-one (17,20-P) and gonadotropin (GTH) were measured in brook trout (*Salvelinus fontinalis*) during the period from the end of vitellogenesis to postovulation. Blood samples were taken according to specific stages of maturation, including germinal vesicle breakdown (GVBD) and ovulation. E2 levels were quite high (~ 45 ng/ml) at the end of vitellogenesis (and prior to GVBD) and dropped precipitously by GVBD (~ 2 ng/ml). They remained low through ovulation and postovulation. 17,20-P levels were low prior to GVBD (~ 0.7 ng/ml) and increased dramatically at GVBD (~ 148 ng/ml). The levels of 17,20-P remained high at ovulation (~ 142 ng/ml) and then dropped significantly within 24 h to approximately half of the ovulatory values. They decreased even further by 7 days postovulation. GTH levels rose gradually through GVBD and ovulation from a postvitellogenic level of approximately 3 ng/ml to a 7 day postovulatory value of approximately 10 ng/ml. The overall results; 1) decrease in estradiol prior to GVBD, 2) increase in 17,20-P at GVBD and 3) gradual GTH rise through GVBD and ovulation, are similar to those reported for other salmonids.

Introduction

Over the last five years there have been many papers describing the changes in circulating steroids and gonadotropins in fish during the period from vitellogenesis until ovulation. Most of these investigations have been conducted on salmonids and major trends in the results of these studies appear reasonably similar. Circulating estradiol (vitellogenic hormone) is relatively high prior to final oocyte maturation and drops precipitously prior to germinal vesicle breakdown (GVBD). On the other hand, the levels of 17 α , 20 β -dihydroxy-4-pregnen-3-one (17,20-P: maturational hormone) are low prior to final maturation and begin to increase just prior to GVBD. They generally increase dramatically

during GVBD and may increase even further following ovulation. Gonadotropin levels increase gradually prior to ovulation and generally continue to increase postovulation. Although this general pattern of hormonal changes is very similar from one salmonid species to another, there are some subtle differences. The most evident of these is in the absolute level of steroids and gonadotropins measured. For example, reported peak levels of 17,20-P have varied from approximately 60 ng/ml in amago salmon (Young *et al.* 1983) to between 300 and 400 ng/ml in rainbow trout (Fostier *et al.* 1986; Scott *et al.* 1983; Springate *et al.* 1984). Also, the rate and duration of steroid changes have varied between species.

For several years, we have been investigating the

hormonal control of oocyte final maturation and ovulation in the brook trout (*Salvelinus fontinalis*) using an *in vitro* incubation system. One of the objectives of the present study was to determine the changes in circulating steroid and gonadotropin levels prior to and during final maturation so that they could be compared to results from other salmonid species and to our past *in vitro* results. Compared to other salmonids, there have only been a few reports concerning hormonal changes in members of the genus *Salvelinus* (Kagawa *et al.* 1981; Tam *et al.* 1986). Since this species is now being used in the assay of acidic environmental conditions (Tam and Payson 1986), it is important to describe the basic biology of this species; particularly reproduction.

A second important objective of this study was to critically observe the hormone levels, especially 17,20-P, at the time of GVBD and ovulation. Although a large number of papers have reported on hormonal changes during the reproductive season, only a few of these have critically correlated the changes with specific maturational stages (e.g. germinal vesicle position, GVBD and ovulation). In many past studies, blood samples have been taken on a chronological basis which frequently will not allow for strict correlations between hormone levels and specific maturational stages. In the present study, samples were obtained according to the attainment of specific stages such as GVBD or ovulation. We were particularly interested to see if there was a consistent change in 17,20-P levels between GVBD and ovulation. Past investigations in this laboratory have studied the possible interactions of prostaglandins in brook trout ovulation (Goetz *et al.* 1982; Cetta and Goetz 1982; Goetz and Cetta 1983) and future investigations are directed at studying the possible hormonal regulation of prostaglandin synthesis by steroids and gonadotropins. Thus, it would be helpful to know more about the basic hormonal events that occur in this species during final maturation and ovulation.

Materials and methods

Animals

Female brook trout (300–400 g) were purchased during the reproductive season from private fish hatcheries in northern Wisconsin. They were held under natural photoperiods in tanks supplied with continuously flowing wellwater at 12.5°C. Oocyte samples were obtained from fish by gentle pressure applied to the abdomen as previously described (Cetta and Goetz 1982). These oocytes were treated in a fixative (Goetz and Bergman 1978) to determine germinal vesicle position within the oocyte. At the time of the first blood sample (October 4, 1984), fish contained ovaries in which the germinal vesicle was approximately 4/5 the distance from the center to the egg membrane. A total of nine fish were used in the investigation and they were serially blood sampled, relative to maturational stage, as described below.

Blood sampling

Initial blood samples were taken from fish prior to any extensive cytoplasmic clearing and before the germinal vesicle had reached the egg membrane. Following this, the ovaries of these fish were periodically monitored to determine the time of GVBD, and a second blood sample was taken at this time. Obtaining fish exactly at the time of GVBD is extremely difficult. As a result, of the 9 fish in the experiment only 6 were sampled at exactly this period. When it was determined that a fish had undergone GVBD, it was examined at least every 6 h to determine when ovulation occurred. This was accomplished by applying slight pressure to the abdomen to observe the extrusion of ovulated eggs. A blood sample was taken when eggs could be easily extruded from the ovipore and the abdomen had softened. Although this ovulatory criterion may be considered somewhat arbitrary, we feel that, as a result of past experiments involving similar sampling, in which fish were actually killed at each period (Cetta and Goetz 1982), this time represents a point at which fish are just fully

ovulated or nearly so. This would not be considered the beginning of ovulation which is extremely difficult to ascertain in salmonids without actually killing the fish. Serial blood samples were also taken 24 h and 7 days postovulation. In all post-ovulatory samples, fish still contained ovulated eggs in the body cavity. Very little spontaneous oviposition could be observed up to 7 days postovulation.

At each sampling time, a 1.0 ml sample of blood was removed from the caudal vasculature using a 1.0 ml syringe fitted with a 23 gauge needle. Syringes were pre-rinsed in a 1,000 IU/ml heparin solution. Blood was centrifuged at 1,500 g for 20 minutes at 4°C after which the plasma was removed and stored at -20°C until assayed.

Hormone assay

For steroid assays, 400 µl of plasma was extracted twice with 2.0 ml of a mixture of ethyl acetate:cyclohexane (1:1) and the extracts separated on LH-20 according to the methods described by Fostier and Jalabert (1986). Radioimmunoassays (RIA) of 17,20-P and estradiol-17β were performed as described by Fostier *et al.* (1981) and Jalabert and Fostier (1984). Cross-reactivities of various steroids with the antisera used in the RIAs have been described (Fostier and Jalabert 1986; Jalabert and Fostier 1984).

Gonadotropin was measured by RIA using an antibody directed against salmon gonadotropin. The assay and its characteristics have been described previously (Breton *et al.* 1978).

Analysis and statistics

RIA data was analyzed using a logit-log transformation and all potency estimates for steroids were corrected for the procedural loss of material during extraction and separation. Potencies given in the text and figures are expressed as the mean ± SEM. Potency estimates at various stages were analyzed using a one-way ANOVA followed by Tukey's multiple comparison test.

Results

GVBD and ovulation

The first blood sample was taken on Oct. 4 (1984) at which time all fish contained oocytes with sub-peripheral germinal vesicles. The first fish to undergo GVBD did so on Oct. 7 while the last underwent GVBD on Oct. 28. The time from GVBD to ovulation varied between 1 and 2 days. For 6 fish, the exact time of GVBD could be determined (i.e. we were able to follow the germinal vesicle as it dispersed). For 5 of these fish we were reasonably sure that an ovulatory sample was taken within 6 h of the time of ovulation. For an additional 3 fish we were not able to determine the exact time of GVBD but were able to determine that they had undergone GVBD but had not yet ovulated. These fish were followed at 6 h intervals and we were reasonably certain that an ovulatory blood sample was taken from them within 6 h of ovulation. Hence, the sample size of 8 at ovulation and at 24 h postovulation (Figs. 1–3). One of the 8 fish died between the 24 h postovulatory sample and the 7 day postovulatory sample, hence the sample size of 7 for the last sample.

Estradiol-17β

Circulating estradiol levels were high (45.1 ± 5.8 (SEM) ng/ml) in fish containing oocytes with intact, subperipheral germinal vesicles (Fig. 1). At the time of GVBD the levels had dropped to 2.3 ± 0.5 ng/ml and remained at approximately this level through 7 days postovulation. Pre-GVBD levels were significantly ($P < 0.05$) higher than the mean levels at all other stages, but no other significant differences were observed.

17α, 20β-Dihydroxy-4-pregnen-3-one

Females sampled prior to GVBD had very low circulating 17,20-P levels (0.7 ± 0.1 ng/ml-Fig. 2). At GVBD the levels had increased significantly ($P < 0.05$) to a mean of 147.8 ± 22.9 ng/ml and at ovu-

Fig. 1. Circulating estradiol-17 β levels in brook trout at different stages of maturation and ovulation. Stage 1 = prematuration; 2 = GVBD; 3 = ovulation; 4 = 24 h postovulation; 5 = 7 days postovulation. All values are means $\pm$ SEM for N fish. * indicates significant difference from all other means at $P < 0.05$.

Fig. 2. Circulating 17,20-P levels in brook trout at different stages of maturation and ovulation. Stages as in Fig. 1. All values are means $\pm$ SEM for N fish. Bars with similar * designation are not significantly different. All other comparisons significantly different at $P < 0.05$.

Fig. 3. Circulating gonadotropin levels in brook trout at different stages of maturation and ovulation. Stages as in Fig. 1. All values are means $\pm$ SEM for N fish. * indicate significant differences at $P < 0.05$.

Table 1. 17,20-P levels in brook trout females serially sampled at GVBD and within 6 h of ovulation

Fish	17,20-P (ng/ml)	
	GVBD	Ovulation
1	92.5	217.5
2	124.1	149.3
3	202.0	150.5
4	85.1	111.4
5	218.7	97.9

lation the levels remained at approximately this level (142.0 ± 15.7 ng/ml). Of five fish in which a blood sample had been obtained exactly at GVBD and within 6 h of ovulation, the 17,20-P levels rose in 3 between GVBD and ovulation, and dropped in 2 of the fish (Table 1).

The levels of 17,20-P measured 24 h postovulation (65.2 ± 11.7 ng/ml) were significantly ($P < 0.05$) lower than those measured at GVBD or ovulation but were still significantly ($P < 0.05$) higher than pre-maturation levels. By seven days postovulation, 17,20-P levels (16.5 ± 8.9 ng/ml)

Table 2. Peak values of circulating 17,20-P in salmonids during natural reproduction

Species	17,20-PG (ng/ml)	Stage	Reference
Rainbow trout	320 (X)	GVBD	Fostier <i>et al.</i> (1986)
	300–400	4 days prior to ovulation	Scott <i>et al.</i> (1983)
	400 (X)	ovulation	Springate <i>et al.</i> (1984)
	113 (X)	postovulation	Liley <i>et al.</i> (1986)
Brook Trout	148/142	GVBD/ovulation	present paper
Chum Salmon	332 (X)	spawning population*	Ueda <i>et al.</i> (1984)
Coho Salmon	270	4 days prior to ovulation	Van der Kraak <i>et al.</i> (1984)
	70–80	GVBD	Fitzpatrick <i>et al.</i> (1986)
	120–280	ovulation	Wright and Hunt (1982)
	68 (X)	GVBD but prior to ovulation	So <i>et al.</i> (1985)
Amago Salmon	50–70	GVBD/ovulation	Young <i>et al.</i> (1983)
Sockeye Salmon	74 (X)	GVBD-presumed	Truscott <i>et al.</i> (1986)
Masu Salmon	30–70	2–4 days prior to ovulation	Yamauchi <i>et al.</i> (1984)

* sample contained ovulated and nonovulated females; (X) = mean value.

were not significantly different from prematurational ones, however at this time the levels were extremely variable ranging from a high of 51.9 ng/ml to a low of 0.7 ng/ml.

Gonadotropin

Gonadotropin (GTH) levels rose gradually from a pre-GVBD level of 3.3 ± 0.4 ng/ml to a postovulatory (7 days) level of 9.9 ± 1.6 ng/ml (Fig. 3). GTH levels measured at 7 days postovulation were significantly ($P < 0.05$) higher than those measured prior to and during GVBD and ovulation. They were not significantly different from the levels measured 24 h postovulation (8.5 ± 0.9 ng/ml). In addition, although the mean GTH levels at GVBD (5.5 ± 0.5 ng/ml) and ovulation (5.4 ± 0.6 ng/ml) were higher than those prior to GVBD, this difference was not significant.

Discussion

Levels of circulating steroids and gonadotropins during meiotic maturation and ovulation have been

reported in a number of species in the Salmonidae. A summary of some of these results, pertinent to this discussion, are given in Tables 2 and 3. From the results given for 17,20-P (Table 2), it appears that the absolute values of 17,20-P measured at GVBD or ovulation are greatest in rainbow trout in which levels are generally between 300 and 400 ng/ml and much lower in several other species including amago salmon (~ 60 ng/ml), masu salmon (30–70 ng/ml) and sockeye salmon (74 ng/ml). Thus, at levels of approximately 145 ng/ml, brook trout appear to be intermediate between these species. The absolute level of steroid measured may be a result of the degree of domestication of the trout stock (Liley *et al.* 1986). Liley *et al.* (1986) observed that the maximum level of 17,20-P measured in wild rainbow trout females was approximately 113 ng/ml just following ovulation. This is considerably lower than that reported for domestic rainbow stocks (see Table 2). However, the wild trout were able to spawn which is not usually the case in studies on domestic rainbow trout. Social stimuli may also have an effect on the absolute level of hormones (Liley *et al.* 1986). In our study on brook trout, females were held together but isolated from males. In addition, the tanks used to hold females

Table 3. Circulating levels of estradiol-17 β at two stages of maturation in various species of salmonids

Species	Estradiol (ng/ml)		Reference
	Premat.*	OV or POV**	
Rainbow trout	~ 20	~ 2	Fostier <i>et al.</i> (1986)
	~ 18	~ 2	Scott <i>et al.</i> (1983)
	~ 60	30–2.8 (spawning)	van Bohemen and Lambert (1981)
	~ 50	~ 2	Scott and Sumpter (1983)
	~ 48	~ 2	Scott <i>et al.</i> (1982)
	~ 7	~ 2	Breton <i>et al.</i> (1983)
Brown Trout	~ 45	~ 2	present paper
Brook Trout	~ 40	~ 5	Tam <i>et al.</i> (1986)
Coho Salmon	~ 16	1–2	van der Kraak <i>et al.</i> (1984)
	3–6	2–3	Sower and Schreck (1982)
	13–18	~ 3	Fitzpatrick <i>et al.</i> (1986)
Chum Salmon	~ 13	~ 5***	Ueda <i>et al.</i> (1984)
Atlantic Salmon	34	0.9	Stuart-Kregor (1981)
Sockeye Salmon	~ 21	~ 1	Truscott <i>et al.</i> (1986)
Masu Salmon	4–25	~ 1	Yamauchi <i>et al.</i> (1984)
White-spotted Char	~ 13	~ 2	Kagawa <i>et al.</i> (1981)

* time period varied from several days to one month prior to final maturation

** OV = ovulation and POV = postovulation

*** sample contained ovulated and nonovulated females

did not contain any gravel substrate and the females did not spontaneously release eggs.

The observation that 17,20-P levels were significantly elevated in brook trout at GVBD further supports a role for this steroid in the induction of meiotic maturation in this species. Past studies on this species have demonstrated that 17,20-P is the most potent maturational steroid when tested *in vitro* (Duffey and Goetz 1980), and its synthesis by brook trout ovarian tissue has been tentatively demonstrated (Theofan and Goetz 1983). The results presented here also clearly demonstrate that in brook trout 17,20-P levels are elevated at GVBD and ovulation and that there is not a consistent increase or decrease in the circulating levels of this progestagen between these two periods. In coho salmon there was also no consistent change in the level of 17,20-P from GVBD to ovulation (Fitzpatrick *et al.* 1986), while in Atlantic salmon, Truscott *et al.* (1985) suggested that there was a slight decrease from the time just following GVBD until ovulation, though it is doubtful that this was sta-

tistically significant. Regardless, in all investigations that have been reported so far, it cannot be precisely determined how long, following GVBD, that an ovulatory sample was taken. In several cases (Fostier *et al.* 1981; Fitzpatrick *et al.* 1986) serial samples were taken from fish at two or three day intervals and, therefore, 48–72 h may have elapsed between GVBD and ovulation. In other investigations the sampling periods were even wider apart or sampling was not done serially, making it impossible to determine how long, before the ovulatory sample, that the fish had undergone GVBD. Although we cannot say that our ovulatory sample was taken during the time of ovulation, because of our sampling regime it is possible to state that it was taken within 6 h of ovulation and this appears to be much closer to the time of GVBD than in any other investigation yet reported. Of course, the circulating levels may not accurately reflect synthetic changes that are occurring in the follicle, and these may be important in the stimulation of ovulation. Another interesting observation was that in brook

trout, 17,20-P levels quickly dropped following ovulation in fish that still retained ovulated eggs in the body cavity. In rainbow trout, the levels of 17,20-P also drop following ovulation (Fostier *et al.* 1981) but since they are at least twice as high at ovulation as in brook trout it appears to take a longer time for them to decrease.

As Table 3 indicates, estradiol levels are relatively high prior to GVBD in all salmonids while at the time of ovulation they are generally low; 1–2 ng/ml. It has been suggested that the fall in estradiol is a consequence of a shift in the steroidogenic pathway prior to GVBD (Fostier and Jalabert 1986) and physiologically the decrease may be indirectly involved in the control of oocyte final maturation (Jalabert and Fostier 1984a,b). There appears to be quite a range of prematurational values for estradiol levels, though this may be related more to the exact time at which the sample was taken rather than a species difference in absolute concentrations. In brook trout the estradiol levels are very high, even shortly before GVBD. For example, in several fish that underwent GVBD within 9 days following the first blood sample, the mean was 37.7 ng/ml. In contrast, from the literature it appears that estradiol levels in rainbow trout at a similar period are lower; in the range of 10–20 ng/ml (Fostier *et al.* 1986).

The seasonal changes in estrogen levels in brook trout have recently been reported (Tam *et al.* 1986). Although these levels cannot be strictly related to the stages reported here since the sampling was not done on a stage basis, it does appear that near the end of vitellogenesis the levels reach a maximum of approximately 40 ng/ml (Tam *et al.* 1986). This value is nearly the same as the one we obtained for fish just prior to GVBD; presumably at a similar stage. In addition, our post-GVBD value of approximately 2.0 ng/ml, is also similar to those reported for postovulatory brook trout (Tam *et al.* 1986). From the data presented here it would be assumed that in brook trout the estradiol levels remain high until just prior to GVBD; inferring that the drop is closely tied to final maturation. However, Tam and coauthors (1986) reported that, of brook trout obtained from two hatcheries, there was a decrease in estrogen in brook trout obtained

from one of the hatcheries even though none of them had ovulated. Although it was not explicitly stated, it could be inferred from their figures that the decrease in this case was from approximately 40 ng/ml to 25 ng/ml. It could be that these fish were undergoing GVBD at just about the time that blood sampling was conducted and this was the reason for the decrease.

In the present study, gonadotropin levels increased gradually during GVBD and ovulation and reached peak values at seven days postovulation. This has also been observed in several other salmonids (Crim *et al.* 1975; Fostier *et al.* 1981; Scott *et al.* 1983). It should be pointed out that even though levels were greatest at 7 days postovulation, they were also quite variable at this time, ranging from a high of 15.1 ng/ml to a low of 4.0 ng/ml. In rainbow trout, GTH levels continue to increase following ovulation and can reach values as high as 100 ng/ml in fish still holding eggs in the body cavity and 50 ng/ml in fish spent immediately following ovulation (Jalabert and Breton 1980). Truscott *et al.* (1986) also reported GTH values of 90 ng/ml in post-spawned sockeye salmon. It is possible that higher values would have been observed in some brook trout females if sampling was continued. In addition, in rainbow trout, GTH levels decline somewhat following ovulation and then increase even further following this decline (Fostier *et al.* 1981). Of the 7 females sampled at 7 days postovulation, GTH levels had begun to decline in 3 and this might indicate that sampling may have occurred in a transient postovulatory trough. In comparison to rainbow trout, the absolute values of GTH measured were lower in brook trout but this may have been the result of using a RIA based on salmon GTH. Using a RIA based on salmon gonadotropin, Crim *et al.* (1975) also reported an increase in plasma GTH in brook trout through the time of ovulation. Although it is difficult to exactly compare the two studies, it appears that the GTH levels measured were slightly higher in the earlier study by Crim *et al.* (1975) compared to the present one.

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