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OVULATORY AND STEROIDAL RESPONSES IN COHO SALMON AND STEELHEAD TROUT FOLLOWING ADMINISTRATION OF SALMON GONADOTROPIN AND D-Ala^6, des Gly^{10} GONADOTROPIN-RELEASING HORMONE ETHYLAMIDE (GnRHa)

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ABSTRACT

Sower, S.A., Iwamoto, R.N., Dickhoff, W.W. and Gorbman, A., 1984. Ovulatory and steroidal responses in coho salmon and steelhead trout following administration of salmon gonadotropin and D-Ala^6, des Gly^{10} gonadotropin-releasing hormone ethylamide (GnRHa). *Aquaculture*, 43: 35—46.

Hormone injection experiments were conducted for 2 years during the fall and early winter shortly before the normal spawning seasons of coho salmon and steelhead trout. Successive injections of partly purified coho salmon gonadotropin and a synthetic analogue of hypothalamic gonadotropin-releasing hormone advanced ovulation by 3—4 weeks in both coho salmon and steelhead trout. In these tests, the salmon gonadotropin (100 μg/kg fish) was followed by one of four dosages of GnRHa: 50, 5, 0.5, or 0.05 μg/kg. In all coho salmon so treated, there was a significantly depressed plasma estradiol level whereas plasma androgen levels were significantly elevated compared to controls. Treatment of steelhead trout with salmon gonadotropin followed by only one dose of GnRHa (60 μg/kg) also caused a significant decrease of plasma estradiol level. The 0.05 μg/kg dose of GnRHa preceded by injection of salmon gonadotropin is the lowest that has been reported to accelerate ovulation in salmonids. The decrease in estradiol levels concomitant with an increase in androgen levels in ovulatory salmonids indicate that gonadotropic stimulation evokes complex changes in patterns of steroidogenesis.

INTRODUCTION

Pituitary preparations alone or in combination with gonadotropin-releasing hormone (GnRH) and its analogues have increasingly been used for advancing ovulation in the salmonids (Jalabert et al., 1978; Donaldson et al., 1981; Sower et al., 1982; Crim et al., 1983). The most successful evocation in advancement of ovulation of coho salmon has been reported to be by use of doses of 100 μg/kg partly purified salmon gonadotropin (SG-G100) followed by 60 μg/kg of D-Ala^6, des Gly^{10} GnRH ethylamide

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(GnRHa) (Sower et al., 1982). It has been unclear whether so high a dose as 60 μg/kg is essential. We have sought here to establish the minimal effective dose (i.e., the most cost efficient procedure) for hormonal induction of ovulation in salmon. In addition, our study aimed to evaluate another effect of the gonadotropin—GnRHa treatment on ovarian function (steroidogenesis) aside from its action on ovulation in two salmonid species.

Prior research has shown that gonadotropin-releasing hormone and its analogues elevate plasma gonadotropin levels in goldfish (Crim et al., 1976; Peter, 1980), rainbow trout (Weil et al., 1978), brown trout (Crim et al., 1981), and coho salmon (Van der Kraak, 1983). Plasma 17α, 20β-dihydroxyprogesterone levels have been found to increase following injections of salmon pituitary extracts or GnRHa in rainbow trout (Scott et al., 1982) and in Atlantic and coho salmon (Wright and Hunt, 1982). The effects of hormonal treatments on plasma estradiol and/or androgens during the final reproductive period have not yet been reported in female salmon; yet a clear but unevaluated involvement of estradiol during final gamete maturation and ovulation has been indicated in various studies in female salmonids (Billard et al., 1978; Crim and Idler, 1978; Fostier et al., 1978; Whitehead et al., 1978, 1983; Sower and Schreck, 1982). The inverse changes noted in plasma estradiol and gonadotropin levels during final maturation and ovulation (Fostier et al., 1978; Whitehead et al., 1978) have suggested that these hormones are functionally interrelated (Whitehead et al., 1983). In certain mammals and some teleosts, priming with GnRH or pituitary gonadotropic preparation followed by GnRH has been the most effective in inducing pituitary responsiveness (see review by Chappel et al., 1983; Peter, 1980). In mammals, it is now recognized that prior estrogen exposure is important for expression of the priming effect of GnRH on the pituitary (Beck et al., 1978; Waring and Turgeon, 1980). Presumably, the priming action of a hormone is related eventually to its action on hormone receptor activity for the same or other hormones. Thus, one of our further objectives was to characterize, at least in part, the endocrine patterns during the period of hormonal induction by measuring plasma sex steroids following the hormonal treatments used in these tests.

MATERIALS AND METHODS

Hormonal preparations

Coho salmon pituitaries collected in 1978 at the Fall Creek Hatchery, Oregon, were used to prepare the partly purified salmon gonadotropin (SG-G100) by ethanol extractions and gel filtration (Sower et al., 1982). Gonadotropin-releasing hormone analogue, D-Ala^6, des Gly^{10}-LH-RH ethylamide, GnRHa, was obtained from Sigma Chemical Company.

During the morning of the day when the fish were injected, all peptides were dissolved in 0.6% NaCl in distilled water. The hormones or saline solutions were injected intraperitoneally into each fish. Blood samples

(200—500 μl) were collected in heparinized syringes from the caudal vein. Plasma samples were kept frozen at - 20°C until assayed for estradiol-17β (estradiol) and total androgens.

1980 and 1981 studies — coho salmon

Forty-three coho salmon used in 1980 were held in the spawning pond at the School of Fisheries, University of Washington. These salmon had returned from the Pacific Ocean to the spawning pond, the terminus of their anadromous spawning migration, about 1.5—2 months before their normal spawning time. Thirty-eight of the salmon were females. The temperature ranged between 8 and 12°C both years. The salmon used averaged 1.8 kg (1980) and 2.5 kg (1981) in body weight and were captured at each sampling and/or testing by seining the pond, followed by netting them into a tank containing an anesthetic bath.

On 24 October 1980, the salmon were anesthetized by immersion in 0.1 ml/l of 2-phenoxyethanol, tagged through the dorsal musculature with Peterson disk tags, weighed to the nearest 0.1 kg, and were treated as shown in Table I.

On 23 October 1981, the salmon were anesthetized by immersion in 0.1 ml/l of 2-phenoxyethanol, tagged both dorsally and on the opercle with Floy tags, weighed to the nearest 0.1 kg, and were treated as shown in Table II.

TABLE I

No. of salmon	Sex	First injection day 0 (μg/kg salmon)	Second injection day 3 (μg/kg salmon)
8	Female	Saline (control)	Saline
10	Female	SG-G100, 100	GnRHa, 0.5
10	Female	SG-G100, 100	GnRHa, 5
9	Female	SG-G100, 100	GnRHa, 50
5	Male	SG-G100, 100	No injection
1	Female	SG-G100, 100	No injection

TABLE II

No. of salmon	Sex	First injection day 0 (μg/kg salmon)	Second injection day 3 (μg/kg salmon)
10	Female	Saline (control)	Saline
10	Female	SG-G100, 100	Saline
10	Female	SG-G100, 100	GnRHa, 5
10	Female	SG-G100, 100	GnRHa, 0.5
10	Female	SG-G100, 100	GnRHa, 0.05
5	Male	SG-G100, 100	No injection

Plasma samples were taken on day 4 (1981) or day 5 (1980), 24 or 48 h after the GnRHa injection. The salmon were checked approximately every other day to determine if they had ovulated, as judged by external physical characteristics such as softness of the abdominal region and free-flowing eggs from the cloaca on application of gentle pressure on the anterior abdominal wall.

The salmon were killed on the day they were determined to have ovulated and plasma samples were taken. An ovulatory response was considered to have occurred if an individual female had more than 75% of her eggs loose (unattached to ovarian tissue) in the body cavity. Eggs were fertilized with sperm pooled from two males with 150 ml of added saline diluent (0.24 g $NaHCO_3$, 0.2 g Na_2CO_3, and 0.69 g NaCl in 150 ml H_2O), and were incubated at the University of Washington School of Fisheries hatchery to determine percentage survival to the eyed-egg and ponding stages.

1981 studies — steelhead trout

Twenty-two steelhead trout were held at the Seward Park, School of Fisheries facility. The animals used were a cross between female steelhead trout (*Salmo gairdneri*) and male rainbow trout (*Salmo gairdneri*), but will be referred to as steelhead trout. These trout had been maintained in freshwater in a 5 m circular concrete pond throughout their life, and were approximately 1 month from their first normal spawning time. The trout used averaged 0.6 kg in body weight. The water temperature averaged 7°C during the experiment.

On 23 January 1981, the trout were anesthetized by immersion in 0.1 ml/l of 2-phenoxyethanol, freeze branded with liquid nitrogen and tagged in the dorsal muscle with floy tags for identification. The female trout were treated as shown in Table III.

Plasma samples were taken on day 5, 48 h after the GnRHa injection. Ovulation determinations and fertilization of eggs in the trout followed the same procedures as for the salmon.

TABLE III

No. of female trout	First injection day 0 (μg/kg trout)	Second injection day 3 (μg/kg trout)
11	Saline (control)	Saline
11	SG-G100, 100	GnRHa, 60

Radioimmunoassay and statistics

Plasma estradiol-17β and total androgens were measured by radioimmunoassay as described and validated by Sower and Schreck (1982).

The percent ovulation data were analyzed by use of a 2 × 2 contingency table followed by the Benferonni approach (Neter and Wasserman, 1974). Data for hormone concentrations were analyzed by a Student—Newman—Keuls test after preliminary analysis of variance. Data for percent survival of progeny to eyed-egg stage and to ponding were evaluated by analysis of variance. In all tests, the level of significance for differing groups was $P < 0.05$.

RESULTS

1980 — coho salmon

The three dosage schedules of SG-G100 and GnRHa were equally effective in inducing over 75% ovulation by day 12, compared to 12.5% ovulation in control fish (Fig. 1). Maximal ovulation of control salmon occurred 2 weeks later than that of treated fish.

Plasma estradiol was significantly depressed 48 h (day 5) after the salmon were treated with all three doses of GnRHa of 50 μg/kg salmon (2.04 ± 0.21 SE ng/ml), 5 (2.75 ± 0.59), or 0.5 (2.36 ± 0.22) compared to controls (33.98 ± 7.88) (Fig. 2). Five days after the single injection of SG-G100 into one female salmon, plasma estradiol was not significantly different from the control level (Fig. 2). On the day of ovulation, plasma estradiol was significantly lower in the controls but did not differ from groups which had ovulated earlier.

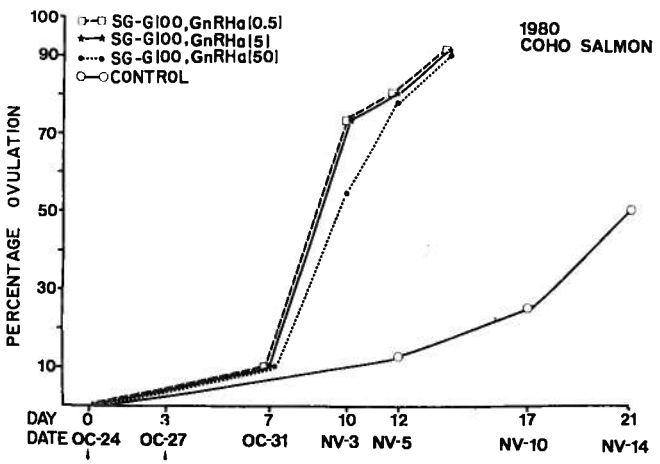


Fig. 1. Accumulative percent ovulation of female coho salmon injected in 1980 with saline (control) or SG-G100 (100 μg/kg) followed at 3 days by GnRHa (50 μg/kg), GnRHa (5), or GnRHa (0.5).

Plasma androgens were significantly elevated above the control level 48 h (day 5) after the salmon were treated with any of the three doses (50, 5, or 0.5 μg/kg salmon) of GnRHa (240.1 ± 21.6 ng/ml, 221 ± 21.5 ng/ml, or 266.8 ± 42.1 ng/ml). The androgen levels were higher also than in the one salmon injected with SG-G100 (Fig. 2). On the day of ovulation, plasma androgens were unchanged in the controls and did not differ significantly from levels in the hormone-injected groups.

Percent survival to eyed-egg stage of progeny of treated fish and controls was not significantly different between groups (Table IV).

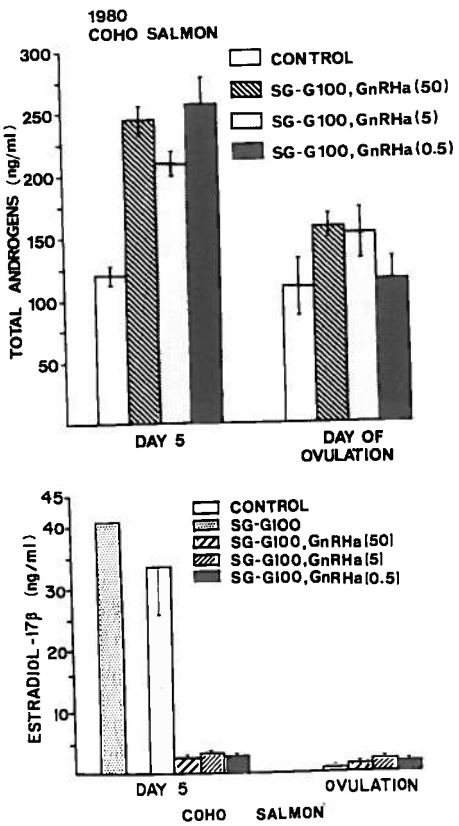


Fig. 2. Plasma estradiol-17β (ng/ml) and androgens (ng/ml) at day 5 and on the day of ovulation of female coho salmon following hormone treatments in 1980.

1981 — coho salmon

The three dosage schedules of SG-G100 and GnRHa were equally effective in inducing over 50% ovulation by day 12, compared to 30% ovulation in control fish or salmon treated with only SG-G100 (Fig. 3). Max-

TABLE IV

Summary data of progeny for 1980 and 1981 induced ovulation experiment

Coho salmon, 1980

Treatment	(μg/kg)	No. of salmon	% Survival to eyed-egg stage	
			X	SE
Control		2	67.2	10.3
SG-G100, GnRHa	(50)	5	49.5	15.8
SG-G100, GnRHa	(5)	6	29.5	11.2
SG-G100, GnRHa	(0.5)	4	59.7	20.8

Coho salmon, 1981

Treatment	(μg/kg)	No. of egg lots	% Survival to ponding	
			X	SE
Control		8	10.8	5.8
SG-G100		6	21.5	9.4
SG-G100, GnRHa	(5)	8	54.0	5.9
SG-G100, GnRHa	(0.5)	6	41.4	11.5
SG-G100, GnRHa	(0.05)	5	43.1	8.6

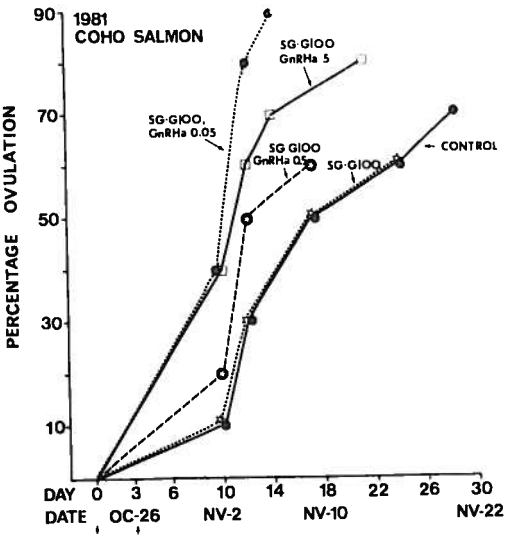
Steelhead trout, 1981

Treatment	(μg/kg)	No. of salmon	% Survival to eyed-egg stage
			X
Control		6	97
SG-G100, GnRHa	(60)	8	97

imal ovulation of control salmon occurred 1.5 weeks later than that of treated fish.

Plasma estradiol was significantly depressed 24 h after the salmon were treated with any of the three doses of GnRHa of 5 μg/kg salmon (2.82 ± 0.27 ng/ml), 0.5 μg/kg (3.00 ± 0.56), or 0.05 μg/kg (2.97 ± 0.77) compared to saline injected controls (6.08 ± 0.88) (Fig. 4). Plasma estradiol level was also significantly depressed on day 5 in the salmon treated with SG-G100 alone (3.49 ± 0.65) compared to saline-injected controls (6.08 ± 0.88) (Fig. 4).

Percent survival to ponding of progeny of treated fish except only for SG-G100 treated females was significantly higher compared to controls (Table IV).



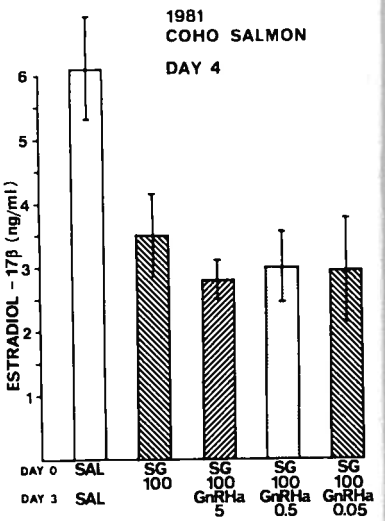


Fig. 3. Accumulative percent ovulation of female coho salmon injected in 1981 with saline (control), a single injection of SG-G100 or SG-G100 (100 μg/kg) followed at 3 days by GnRHa (5), GnRHa (0.5), or GnRHa (0.05).

Fig. 4. Plasma estradiol-17β (ng/ml) at day 4 following hormone treatments in coho salmon in 1981.

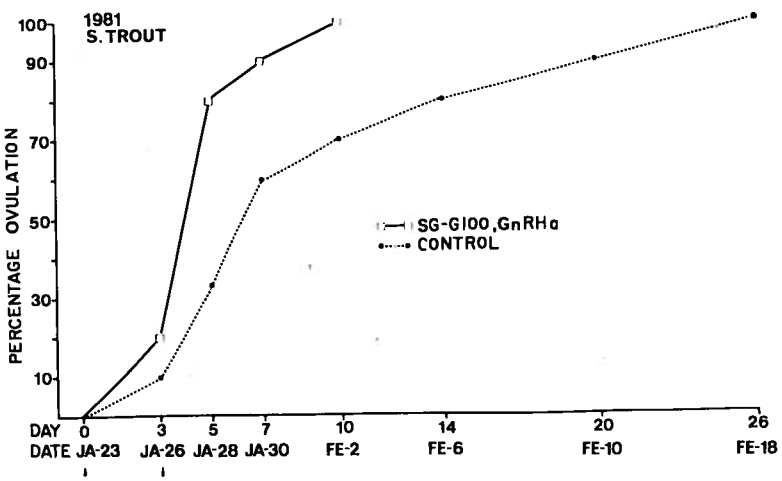


Fig. 5. Accumulative percent ovulation of female steelhead trout injected in 1981 with saline (control) or SG-G100 (100 μg/kg) followed at 3 days by GnRHa (60 μg/kg).

1981 — steelhead trout

In hormone-treated trout 100% ovulation was achieved by day 10 compared to day 26 for the same level of ovulation by control fish (Fig. 5).

Plasma estradiol was depressed, but not significantly, in hormone-treated fish, 6.77 ± 3.29 ng/ml compared to the controls, 23.26 ± 10.65 ng/ml on day 5 (Fig. 6). Plasma androgens were not significantly different between hormone-treated fish (91.6 ± 22.4 ng/ml) compared to controls (86.7 ± 17.3 ng/ml) and did not change significantly on the day of ovulation in both groups.

Percent survival to eyed-egg stage was 97% for progeny of both hormone-treated and control fish (Table IV).

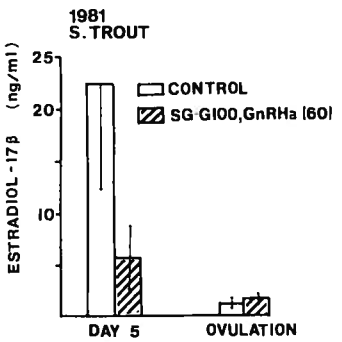


Fig. 6. Plasma estradiol-17β (ng/ml) at day 5 and on the day of ovulation of female coho salmon following hormone treatments in 1981.

DISCUSSION

In salmonids, the minimal effective dose for hormonally accelerated ovulation has yet to be established. In the present study, the three doses of GnRHa following administration of a partly purified coho salmon gonadotropin were equally effective for induction of ovulation in advance of natural spawning though they are lower dosages than any previously reported for this use. The biological potency of GnRHa, as shown in this study, is thus greater by at least 100-fold than has been shown previously in salmonids (Donaldson et al., 1981; Sower et al., 1982; Van der Kraak et al., 1983). The ovulatory success from treatment with coho SG-G100 (at a dose of 0.1 mg/kg) followed by several days with GnRHa at 0.05 μg/kg can be considered economically practical, costing a few cents per adult fish. Advancement of ovulation potentially increases the production of fish by hatcheries by yielding gametes from the fish before they die due to disease or other unfavorable conditions inherent in hatchery breeding.

It is clear from our experiments that receptors in salmon adenohypophysis and/or ovary are responsive to both the salmon pituitary preparation and GnRHa. However, the partly purified coho salmon gonadotropin preparation (SG-G100) used alone was not as effective in accelerating ovulation as the combination of coho salmon pituitary preparation and GnRHa. The first injection of this partly purified gonadotropin could be considered to have a priming action, as has been suggested in other studies of gonad stimulation of Pacific salmon (Jalabert et al., 1978; Donaldson et al., 1981; Sower et al., 1982). In studies on mammals, priming with GnRH or pituitary preparations followed by GnRH has proven the most effective in experimental induction of pituitary responsiveness (reviewed by Chappel et al., 1983). Similar priming appears to be demonstrated in the data from the present studies. Studies by Fitzpatrick et al. (1984) and S.A. Sower et al. (unpublished data, 1982) have shown that two successive injections of GnRHa at 0.5 μg/kg or 0.05 μg/kg, respectively, accelerate ovulation in at least 80% of the coho salmon. Hormone administration in single injections generally are less effective than a double injection, even when the total amount of hormone is the same (Peter, 1980; Donaldson et al., 1981; Chappel et al., 1983). The increased responsiveness of the pituitary gonad to two injections occurs whether the first injection is the pituitary preparations or GnRHa. However, greater effectiveness has been claimed when the first (priming) injection is the pituitary hormone.

In salmonids, the precise series of endocrine events that actually motivate hormonal induction of ovulation cannot yet be defined. Until it is known how many salmonid gonadotropins and hypophysiotropic factors are present and their physiological functions are specified, many questions will persist. However, it has been shown in mammalian studies that estrogen is required for the priming effect of GnRH on pituitary responsiveness (Beck et al., 1978; Waring and Turgeon, 1980). The elevated estradiol levels we have noted in normal female salmon prior to ovulation (Fostier et al., 1978; Sower et al., 1982) may play such a role in the salmonid response to the administered hormones. An inverse relationship between plasma estradiol and gonadotropin has been observed during final maturation and ovulation in coho salmon (Jalabert et al., 1978) and rainbow trout (Fostier et al., 1978; Whitehead et al., 1983). In these studies, an increase in circulating gonadotropin levels at or near the time of ovulation is consistently preceded by a drop in estradiol levels, indicating that these hormones may be functionally interrelated. Plasma gonadotropin levels are stimulated and remain elevated for at least 96 h following injection of GnRHa in salmon in which induced ovulation eventually occurs (Van der Kraak et al., 1983). We can add to this evidence the data reported here, in which administered GnRHa was followed by depressed estradiol levels 24—48 h after injection. Thus, the case for a functional interrelationship between estradiol and gonadotropin during final maturation and ovulation

is further enhanced. Androgens may also have a direct role in ovarian maturation. The fact that plasma androgens increased while estradiol decreased following GnRHa injection suggests that aromatase activity may be influenced by the hypothalamo-pituitary gonadotropic axis. Further detailed studies would be required to determine the role of estradiol in the endocrine events during the pre-ovulatory period.

Steelhead trout responded to the gonadotropin—GnRHa treatment in a pattern similar to that of coho salmon in that estradiol levels were depressed and ovulation was accelerated. As evident in other studies, the use of pituitary preparations from a variety of Pacific salmon species appears to be effective in other species of salmonids (Hunter et al., 1981; Wright and Hunt, 1982).

The development of the eggs from hormonally accelerated ovaries to the eyed stage or later to the juvenile stage (at ponding) appeared to be equal to or better than that of controls. The juveniles from the 1981 coho salmon studies were released into the Pacific Ocean via Lake Washington, and the returns from hormone-treated and control fish were not significantly different. The fish from the appropriate treatments and controls were spawned in the fall 1983, and evaluation of progeny development from these fish will be monitored and will provide further necessary information for a complete evaluation of hormone treatments for induced ovulation in subsequent generations.

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