

**Ovaplant® (Salmon Gonadotropin-releasing Hormone Analogue) Clinical
Field Trials - INAD 11-375**

**2010 Annual Summary Report on the Use of sGnRHa - Ovaplant®
in Clinical Field Efficacy Trials**

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Summary

Spawning aids such as Ovaplant® (Salmon Gonadotropin-releasing Hormone Analogue, sGnRHa), luteinizing hormone-releasing hormone analogue (LHRH_a), human chorionic gonadotropin, and common carp pituitary are routinely used in aquaculture to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of Ovaplant® under the Compassionate Investigational New Animal Drug (INAD) Exemption #11-375 for the purpose of gathering efficacy data to support a new animal drug approval for Ovaplant®. In calendar year 2010 (CY10), 16 trials were conducted under this INAD to evaluate the efficacy of Ovaplant® to induce gamete maturation in a variety of fish species. Trials involved 3,470 treated fish and 217 control fish and were conducted at eight different hatcheries, including three U.S. Fish and Wildlife Service fish hatcheries, three state hatcheries, one private hatchery, and one tribal hatchery during this period. Efficacy was determined by whether or not treated fish produced or yielded more eggs or milt

than untreated fish. Overall results of trials conducted during this period indicated that 100% of the trials appeared efficacious.

Introduction

The use of hormones to induce spawning in fish is critical to the success of many federal, state, private, and tribal fisheries programs. A wide variety of programs, including many that involve the restoration of threatened/endangered species, are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning.

The time of spawning is by its own nature a stressful period for all fish species. Both sexes are undergoing significant changes in physiology, morphology, and behavior (Hoar, 1969). The additional handling of fish required during the spawning process complicates an already delicate situation. This is particularly true for wildstock species that must endure the added stresses of capture, handling, and confinement in an unnatural environment. In fact, with respect to some wildstock species, the stress of capture alone is often sufficient to cause complete reproductive failure unless spawning is induced by hormone treatment. Hormone treatment in a variety of fish species is essential to ensure optimal spawning success.

Studies have shown that final gamete maturation (ovulation and spermiation) in fish can be induced by the administration of a variety of hormones (Donaldson and

Hunter 1983; Goetz 1983). Investigations have found that synthetic analogues of gonadotropin releasing hormones (GnRH_a) to be one of the most effective means of inducing final gamete maturation. These compounds, which may be similar to native gonadotropins found in either fish or mammals, are attractive choices as they typically exhibit both high biological activity and low species specificity. Although a number of these analogues are available, the most commonly used analogue for fish culture to date has been luteinizing hormone releasing hormone (LHRH_a; Alvarino et al. 1992; Donaldson et al. 1981; Erdahl and McClain 1987; Fitzpatrick et al. 1984; Taranger et al. 1992; and Van der Kraak et al. 1983). Effective treatment has been reported using both injection and pellet implant therapy.

The use of implants that contain GnRH analogues has been evaluated over the last 15 years (Crim et al., 1983a). In early attempts to use implants, peptide was imbedded in cholesterol pellets that contained cellulose to affect release rate (Sherwood et al., 1988). In this system, a 5% carboxymethyl cellulose / 95% cholesterol pellet containing mammalian GnRH_a (mGnRH_a) released an initial burst of mGnRH_a followed by a sustained release of peptide over the next 28 days. Several researchers have demonstrated that these types of implants were capable of inducing maturation in a variety of species including: Atlantic salmon (Crim et al., 1983a; Crim and Glebe, 1984), herring (Carolsfeld et al., 1988), sea bass (Almendras et al., 1988), rainbow trout (Crim et al., 1983b; Crim et al., 1988) and milkfish (Lee et al., 1986; Marte et al., 1988). In all of these studies, mGnRH_a was the imbedded peptide that induced maturation either in advance of, or synchronously within, a population.

The inclusion of salmon GnRHa (sGnRHa) instead of mGnRHa in Ovaplant[®] implants designed for inducing maturation in cultured fish is a logical one. In both in vitro (pituitary fragments or cell cultures) and in vivo studies sGnRHa has been found to be more potent in effect than mGnRHa for many species including: goldfish (Peter et al., 1985, 1987), Atlantic salmon (Crim et al., 1988), rainbow trout (Crim et al., 1988; Weil et al., 1992), winter flounder (Crim et al., 1988) and catfish (Namvongchong et al., 1992b; Schulz et al., 1994). This potency may be attributed to high pituitary binding affinity and gonadotropin hormone (GtH) releasing capacity, even though sGnRH itself may not be an indigenous form for some of the species tested (Schulz et al., 1993). Moreover, sGnRHa produces a sustained level of GtH from pituitary cells with a low therapeutic dose (Peter et al., 1987). Additionally, sGnRHa either as peptide alone or as Ovaprim[®] (sGnRH + a domperidone, Syndel International, Inc.) has proven to be effective in inducing final gamete maturation in a variety of cultured fish including, but not limited to, chinook salmon (Powell, 1995), coho salmon (Powell et al., 1998), catfish (Namvongchong et al., 1992b; Schulz et al., 1993), and ricefield eel (Tao and Lin, 1993). Furthermore, sGnRHa is an attractive therapy for aquaculture use as it has been shown to be ineffective in mammals (Millar et al., 1993), and has a short half life in fish (Goren et al., 1990; Zohar et al., 1990; Weil et al., 1992). Conversely, mGnRHa is superactive in humans and has a prolonged half-life in fish and water (Sherwood and Harvey, 1986) which potentially could constitute a human safety risk. Collectively, the above-described considerations indicate that sGnRHa (Ovaplant[®]) is an attractive choice for further evaluation and development as a candidate compound for a new

animal drug approval for use to induce final gamete maturation in a variety of fish species.

Purpose of Report

The purpose of this report is to summarize the results of Ovaplant[®] field efficacy studies conducted under INAD exemption #11-375 in CY10. Furthermore, it is expected that these data will be used to establish an Ovaplant[®] database for the purpose of developing an appropriate label claim for the legal use of this new animal drug in aquaculture.

Facilities, Materials, and Treatment Procedures

1. Facilities

Field efficacy trials were conducted at eight different fish culture facilities during CY10, including three U.S. Fish and Wildlife Service fish hatcheries, three state hatcheries, one private hatchery, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 38.0 to 80.0°F. Overall mean treatment temperature from all trials was 57.2 °F.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all Ovaplant[®] used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 250 ug sGnRH α per pellet and administration as a single treatment event only. Drug dosages used by Investigators in CY10 ranged from 10 to 176.72 ug sGnRH α . Male hickory shad received a higher than allowed dosage due to smaller than expected fish size and the pellet size that was used. Fish treated by pellet implant either 1) have been or will be euthanized at the hatchery and properly disposed of; or 2) will remain on station and will not be released.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on six different fish species under INAD #11-375 during the reporting period, including the following two salmonids, three non-salmonids, and one marine non-salmonid species:

Salmonids

fall chinook salmon *Oncorhynchus tshawytscha*

steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*

hickory shad *A. mediocris*

striped bass *Morone saxatilis*

Marine non-salmonid

cobia *Rachycentron canadum*

2. Gender of treated fish

Ovaplant[®] was used on 1,673 female and 1,797 male fish during the reporting period. Typically, females were treated with spawning hormone to shorten the gamete maturation period (i.e. advance maturation), while males were treated to ensure that sufficient milt would be available for egg fertilization.

Data Collected

1. Primary response variable (Maturation)

The primary response variable for evaluating the effect of Ovaplant[®] on fish was the percentage of ripe fish following treatment. These percentages reflected the number of female fish that ovulated and the number of male fish that reached active spermiation.

2. Egg development and milt evaluation

Secondary response variables for females included the relative number of eggs that reached the eyed stage and the number hatched. Secondary response

variables for males included the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa).

Discussion of Study Results

1. General observations on the efficacy of Ovaplant[®] to induce gamete maturation in salmonid and non-salmonid fish (Note: Tables 1 & 2 provides summaries of all efficacy trials; Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 4 describes all trials conducted during CY10 under INAD #11-375.)

A. Efficacy of Ovaplant[®] on male fish treated between 10 and 176.72 ug/kg body weight (1 - 2 implants)

Male fish were treated in eight trials and implanted 1 or 2 times with Ovaplant[®] at a dosage between 10 and 176.72 ug/kg body weight (Table 1). During three trials, the Investigators did not evaluate whether treatment induced gamete maturation. In these cases, it's implied that the relative level of gamete maturation was undetermined. The investigators noted fish were tank spawned so individual ripeness could not be determined; however, viable fry were produced. Fish will not be available for human consumption. Below are the treatment regimens used to induce gamete maturation in five fish species treated with Ovaplant[®] at the dosages described above:

1. Salmonids:

Ovaplant[®] was used at 10 - 40 ug/kg in two trials involving steelhead trout and were implanted with one pellet implant. Control fish were used in both trials. Results showed that there was a 92 - 93% spermiation in the treated fish; as compared to 64 - 100% spermiation in control fish. Overall, treatments appeared efficacious in both trials.

2. Non-salmonids

Ovaplant[®] was used at 43 - 176.72 ug/kg in five trials involving American shad, hickory shad, and striped bass and were implanted with 1 - 2 pellet implants. Control fish were used in one trial involving American shad. Results showed that there was an unknown level of spermiation in two trials of treated fish and 100% spermiation in the other three trials involving treated fish; as compared to 90% spermiation in the control trial. In the trials where the spermiation was unknown, individual fish were not checked to see if they were ripe after treatment; however, the investigator noted there were fry produced. Overall, treatment appeared efficacious in five trials.

3. Marine non-salmonid

Ovaplant[®] was used at 12.5 - 25 ug/kg in one trial involving cobia and were implanted with 1 - 2 pellet implants. Control fish were not used. Results showed that there was an unknown level of spermiation in this

trial. The investigator noted individual fish were not checked to see if they were ripe after treatment; however, fry were produced. Treatment appeared efficacious in this trial.

Overall, treatment resulted in an unknown spermiation or 92 - 100% spermiation in the male treated fish; as compared to 64 - 100% in the control fish. Treatments appeared efficacious in all trials.

B. Efficacy of Ovaplant[®] on female fish treated at a dosage between 12.5 and 150 ug/kg body weight (1 - 2 implants)

Female fish were implanted one to two times with Ovaplant[®] pellets at a dosage between 12.5 and 150 ug/kg body weight (Table 2) in eight different trials.

During two trials, the Investigators did not evaluate whether treatment induced gamete maturation. In these cases, it's implied that the relative level of gamete maturation was undetermined. Fish will not be available for human consumption.

Below are the treatment regimens used to induce gamete maturation in six fish species treated with Ovaplant[®] at the dosages described above:

1. Salmonids:

Ovaplant[®] was used at 12.5 - 40 ug/kg in two trials involving fall chinook salmon and steelhead trout and were implanted with one pellet implant.

No control fish were used. Results showed that there was a 98 - 100% ovulation in treated fish. Treatment appeared efficacious in both trials.

2. Non-salmonids

Ovaplant[®] was used at 41 - 150 ug/kg in five trials involving American shad, hickory shad, and striped bass and were implanted with 1 - 2 pellet implants. Control fish were used in one trial involving American shad. Results showed that there was an unknown level of ovulation in two trials of treated fish and 75 - 100% ovulation in the other three trials involving treated fish; as compared to 100% ovulation in the control trial. In the trials where the ovulation was unknown, individual fish were not checked to see if they were ripe after treatment; however, the investigator noted there were fry produced. Overall, treatment appeared efficacious in five trials.

3. Marine non-salmonid

Ovaplant[®] was used at 25 ug/kg in one trial involving cobia and were implanted with 1 pellet implant. Control fish were not used. Results showed that there was 100% ovulation in the treated fish. Treatment appeared efficacious in this trial.

Overall, treatment resulted in either an unknown percent ovulation (due to fish not evaluated for ovulation by the Investigator) or a 75 - 100% ovulation in the female treated fish; as compared to 100% ovulation in the control fish.

Treatment appeared efficacious in all trials.

2. Observed Toxicity

No toxicity or adverse effects relating to Ovaplant® treatments were reported in any of the trials conducted in CY10.

3. Observed Withdrawal Period

The investigators noted that treated fish will not be stocked, released, or harvested for human consumption. All treated fish will ultimately be destroyed.

Current Study Protocol for Ovaplant® INAD #11-375

Please see the attached current study protocol for Ovaplant® INAD #11-375. Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 5. Facilities and Names of Investigators” for facilities that signed-up to participate in the Ovaplant® INAD #11-375 during CY10. Facilities not listed in Appendix III-a of the current Ovaplant® INAD #11-375 study protocol have been highlighted.

Correspondence sent to Ovaplant® Participants

Please see the attached correspondence that was sent to all Ovaplant® participants after the AADAP Office received their sign-up form for CY10.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY10 was 3,470. The total number of treated fish to count against the treatment use authorization dated December 15, 2005 is 11,085.

Summary of Study Results

Ovaplant[®] was used in 16 efficacy trials to induce gamete maturation in six different fish species (n = 3,470 treated fish; 217 untreated control fish) at dosages ranging from 10 - 176.72 ug/kg bw. Ovaplant[®] was administered as a pellet implant. Fish treated by pellet implant will be euthanized at the hatchery and properly disposed of or will not be released from the facility. Water temperature during treatments ranged from 38.0 - 80.0°F. Overall, results showed that Ovaplant[®] treatment appeared efficacious in 100% of the trials. Data from the CY10 trials indicate that Ovaplant[®] treatment was efficacious in inducing gamete maturation in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #11-375 will also be ancillary data, efforts will be made to improve the quality of data whenever possible.

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Table 1. Summary of Year 2010 Ovaplant[®] Male Efficacy Results - Implant

Number of Trials	Efficacy	Species	Facility	Spawning Interval	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Spermiote	Number of Controls	% Spermaite
1	effective	AMS	Bears Bluff NFH	1 - 8 days	42	100 - 150	?	0	-
1	effective	AMS	Dennis Wildlife Center	15 - 72 hrs	454	75	?	0	-
1	effective	AMS	Muddy River Ecological Lab	48 hrs	528	79.3	100	117	90
1	effective	COB	Bears Bluff NFH	24 - 36 hrs	4	12.5 - 25	?	0	-
1	effective	HKS	Manning SFH	48 hrs	667	176.72	100	0	-
1	effective	STT	Dworshak NFH	13 & 34 days	72	10	93	11	64
1	effective	STT	Wells SFH	7 - 21 days	12	40	92	19	100
1	effective	STB	Warm Springs FHC & TC	7 - 15 days	18	43 - 67	100	0	-

Table 2. Summary of Year 2010 Ovaplant[®] Female Efficacy Results - Implant

Number of Trials	Efficacy	Species	Facility	Spawning Interval	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	AMS	Bears Bluff NFH	1 - 45 days	22	100 - 150	?	0	-
1	effective	AMS	Dennis Wildlife Center	15 - 72 hrs	471	75 or 150	?	0	-
1	effective	AMS	Muddy River Ecological Lab	48 hrs	360	56.6	100	70	100
1	effective	COB	Bears Bluff NFH	24 - 36 hrs	1	25	100	0	-
1	effective	FCS	Nez Perce Tribal Hatchery	1 - 4 wks	285	12.5	98	0	-
1	effective	HKS	Manning SFH	48 hrs	506	135.81	100	0	-
1	effective	STT	Wells SFH	7 - 14 days	16	40	100	0	-
1	effective	STB	Warm Springs FHC &TC	7 - 15 days	12	41 - 64	75	0	-

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY10 Ovaplant® Efficacy Studies

Total Number of Treatment Trials	16
Number of Trials that Appeared Efficacious:	16 (100%)
Total Number of Treated Fish:	3,470
Number of fish treated in efficacious trials	3,470

Treatment Regimes Used:

10 - 150 ug/Kg body weight	15 trials
176.72 ug/Kg body weight	1 trial

Water Temperature (°F) Range: 38.0 - 80.0

Fish Species Treated:

Salmonids

fall chinook salmon *Oncorhynchus tshawytscha*
steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*
hickory shad *A. mediocris*
striped bass *Morone saxatilis*

Marine non-salmonid

cobia *Rachycentron canadum*

Size Class of Treated Fish: Adults