

**OvaRH[®] (Salmon Gonadotropin-releasing Hormone Analogue) Clinical Field
Trials - INAD 12-186**

**2013 - 2014 Annual Summary Report on the Use of sGnRH_a - OvaRH⁷
in Clinical Field Efficacy Trials**

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Summary

Spawning aids such as OvaRH⁷ (Salmon Gonadotropin-releasing Hormone Analogue, sGnRH_a), 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 OvaRH⁷ under the Compassionate Investigational New Animal Drug (INAD) Exemption #12-186 for the purpose of gathering efficacy data to support a new animal drug approval for OvaRH⁷. In calendar years 2013 - 2014 (CY13-14), 9 trials were conducted under this INAD to evaluate the efficacy of OvaRH⁷ to induce gamete maturation in a variety of fish species. Trials involved 1,397 treated fish and 42 control fish and were conducted at 4 different facilities, including one U.S. Fish and Wildlife Service fish facility and three private hatcheries 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 67% of

the trials appeared efficacious, while 22% were ineffective, and 11% were characterized as inconclusive.

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 un-natural 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 (LHRHa; 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 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 GnRHa (mGnRHa) released an initial burst of mGnRHa 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, mGnRHa 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 either implant or injection treatment 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 Ovaprim7 (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 (OvaRH⁷) 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 OvaRH⁷ field efficacy studies conducted under INAD exemption #12-186 in CY13-14. We anticipate that data generated in these trials will

be used to enhance data in the existing OvaRH⁷ database, and will be considered in the Abody of evidence@ for the purpose of developing an appropriate label claim for the use of OvaRH⁷ in aquaculture.

Facilities, Materials, and Treatment Procedures

1. Facilities

Efficacy trials were conducted at 4 different fish culture facilities during CY13-14, including one U.S. Fish and Wildlife Service fish facility and three private hatcheries.

Water temperature during treatments at the various testing facilities ranged from 55.0 to 86.0EF. Overall mean treatment temperature from all trials was 69.5 EF.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all OvaRH⁷ used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 50 ug sGnRH_a /kg fish body weight (bw). During this reporting period, the drug doses used ranged from 10 to 20 ug sGnRH_a /kg fish body. OvaRH⁷ was administered as either a single injection or as a series of 2 injections.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on five different fish species under INAD #12-186 during the reporting period, including the following four non-salmonids and one marine non-salmonid species:

Non-salmonids

bluehead sucker (*Catostomus discobolus*)

channel catfish (*Ictalurus punctatus*)

largemouth bass (*Micropterus salmoides*)

sauger (*Stizostedion canadense*)

Marine non-salmonid

giant grouper (*Epinephelus lanceolatus*)

2. Gender of treated fish

OvaRH⁷ was used on 1,223 female and 174 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 OvaRH⁷ 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 OvaRH⁷ to induce gamete maturation in non-salmonid fish and non-salmonid marine fish (Note: Tables 1 - 2 provides summaries of all efficacy trials; and Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used during CY13-14 under INAD #12-186.)

A. Efficacy of OvaRH⁷ on male fish treated between 10 and 15 ug/kg body weight (1 - 2 injections)

Male fish were treated in three trials and injected 1 or 2 times with OvaRH⁷ at a dosage between 10 and 15 ug/kg body weight (Table 1). Fish species treated included the largemouth bass and giant grouper. No trials included non-treated control groups.

Following treatment, there was a 0 - 100% spermiation among all treated fish. Treatments appeared efficacious in one trial, while one trial was ineffective, and one trial was characterized as inconclusive.

B. Efficacy of OvaRH⁷ on female fish treated at a dosage between 10 and 20 ug/kg body weight (1 - 2 injections)

Female fish were treated in nine trials and injected 1 or 2 times with OvaRH⁷ at a dosage between 10 and 20 ug/kg body weight (Table 2). Fish species treated included the bluehead sucker, channel catfish, largemouth bass, sauger, and giant grouper. One trial included non-treated control groups. Following treatment, there was a 0 – 100% ovulation among all treated fish; as compared to 14.3% ovulation in the control fish. Treatments appeared efficacious in six trials, while two trials were ineffective, and one trial was characterized as inconclusive.

2. Observed Toxicity

No toxicity or adverse effects relating to OvaRH⁷ treatments were reported in any of the trials conducted in CY13-14.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for OvaRH⁷ INAD #12-186

No changes have occurred to the current study protocol for OvaRH⁷ INAD #12-186.

Facility Sign-up List

Please see ATable 4. Facilities and Names of Investigators@ for facilities that signed-up to participate in the OvaRH⁷ INAD #12-186 during CY13-14.

Correspondence sent to OvaRH⁷ Participants

Please see the attached correspondence that was sent to all OvaRH⁷ participants after the AADAP Office received their sign-up form for CY13-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY13-14 was 1,397. The total number of treated fish to count against the current food use authorization dated October 24, 2012 is 1,397.

Summary of Study Results

OvaRH⁷ was used in 9 efficacy trials to induce gamete maturation in five different fish species (n = 1,397 treated fish; 42 untreated control fish) at dosages ranging from 10 - 20 ug/kg bw. OvaRH⁷ was administered using either 1 injection or a series of 2 injections. Water temperature during treatments ranged from 55.0 – 86.0EF. Overall, results showed that OvaRH⁷ treatment appeared efficacious in 67% of the trials; while ineffective in 22% of the trials, and was inconclusive in 11% of the trials. Data from the CY13-14 trials indicate that OvaRH⁷ 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 #12-186 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 2013 -2014 OvaRH⁷ Male Efficacy Results - <u>Implant</u>						Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (ug/kg b.w.)	Spawning Interval (days)	Number Treated	% Spermiate	Number of Controls	% Spermaite
Kampachi Farms	Ineffective	1	Giant Grouper	15	3	5	0	0	-
Kampachi Farms	Inconclusive	1	Giant Grouper	10	2	5	20	0	-
Dunns Fish Farm Inc	Effective	1	Largemouth Bass	10	1	164	100	0	-

Table 2. Summary of Year 2013 -2014 OvaRH⁷ Female Efficacy Results - <u>Implant</u>						Control			
Facility	Efficacy	Number of Trials	Species	Dose (ug/kg b.w.)	Spawning Interval (days)	Number Treated	% Ovulate	Number of Controls	% Ovulate
AADAP	Ineffective	1	Bluehead Sucker	10	3	34	0	0	-
Baxter Land Company	Effective	2	Channel Catfish	20	2	724	64.2 – 83.3	0	-
Kampachi Farms	Ineffective	1	Giant Grouper	18	3	1	100	0	-
Kampachi Farms	Inconclusive	1	Giant Grouper	20	2	1	100	0	-
Dunns Fish Farm Inc	Effective	1	Largemouth Bass	20	1	171	13.8 – 92.6	0	-
AADAP	Effective	3	Sauger	10	2 – 3.8	292	35 – 82.1	42	14.3

Table 4. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY13-14 OvaRH⁷ Efficacy Studies

Total Number of Treatment Trials	9
Number of Trials that Appeared Efficacious:	6
Number of Trials that Appeared Ineffective:	2
Number of Trials that were Inconclusive:	1

Total Number of Treated Fish:	1,397
Number of fish treated in efficacious trials	1,351
Number of fish treated in ineffective trials	40
Number of fish treated in inconclusive trials	6

Treatment Regimes Used:
 10 - 15 ug/Kg body weight male fish
 10 - 20 ug/Kg body weight female fish

Water Temperature (EF) Range: 55.0 – 86.0

Fish Species Treated:

Non-salmonids

bluehead sucker (*Catostomus discobolus*)
 channel catfish (*Ictalurus punctatus*)
 largemouth bass (*Micropterus salmoides*)
 sauger (*Stizostedion canadense*)

Marine non-salmonid

giant grouper (*Epinephelus lanceolatus*)

Size Class of Treated Fish: Adults