Ovaplant[7] (Salmon Gonadotropin-releasing Hormone Analogue) Clinical Field Trials - INAD 11-375

2012 - 2014 Annual Summary Report on the Use of sGnRHa - Ovaplant[7] in Clinical Field Efficacy Trials

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Summary

Spawning aids such as Ovaplant[7] (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[7] 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[7]. In calendar year 2012 - 14 (CY12-14), 41 trials were conducted under this INAD to evaluate the efficacy of Ovaplant[7] to induce gamete maturation in a variety of fish species. Trials involved 8,968 treated fish and 2,143 control fish and were conducted at 21 different hatcheries, including seven U.S. Fish and Wildlife Service fish hatcheries, one National Marine Fisheries Service facility, eight state hatcheries, four private hatcheries, 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 93% of the trials appeared efficacious and 7% 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 (GnRHa) 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 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 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 Ovaplant[7] 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 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 (Ovaplant[7]) 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[7] field efficacy studies conducted under INAD exemption #11-375 in CY12-14. We anticipate that data generated in these trials will be used to enhance data in the existing Ovaplant[7] database established from previous years, and will be considered in the Abody of evidence@ for the purpose of developing an appropriate label claim for the use of Ovaplant[7] in aquaculture.

Facilities, Materials, and Treatment Procedures

1. Facilities

Efficacy trials were conducted at 21 different fish culture facilities during CY12-14, including seven U.S. Fish and Wildlife Service fish hatcheries, one National Marine Fisheries Service facility, eight state hatcheries, four private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 41.9 to 80.6EF. Overall mean treatment temperature from all trials was 53.6 EF.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all Ovaplant[7] used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 250 ug sGnRHa per pellet and administration as a single treatment event only. Drug dosages used by Investigators in CY12-14 ranged from 11.3 to 171 ug sGnRHa. In one trial both male and female hickory

shad received a higher then allowed dosage (156 - 171 ug sGnRHa) 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; 2) will remain on station and will not be released; or 3) was a threatened or endangered species that is not legally available for human consumption

Fish Species and Sex Treated

1. Fish Species Treated

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

Salmonids

arctic char (*Salvelinus alpinus*)

Atlantic salmon (*Salmo salar*)

Chinook salmon (*Oncorhychus tshawytscha*)

coho salmon (*O. kisutch*)

rainbow trout (*O. mykiss*)

steelhead trout (*O. mykiss*)

Non-salmonids

American shad (*Alosa sapidissima*)

hickory shad (*A. mediocris*)

striped bass (*Morone saxatilis*)

Marine non-salmonid

cobia (*Rachycentron canadum*)

sablefish (*Anoplopoma fimbria*)

2. Gender of treated fish

Ovaplant[7] was used on 4,551 female and 4,417 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[7] 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[7] to induce gamete maturation in salmonid and non-salmonid 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 CY12-14 under INAD #11-375.) Due to the online database reporting system, both male and female studies may be reported under a single study number and reported as one trial. In past years the males and female reports were broken out.

A. Efficacy of Ovaplant[7] on male fish treated between 11.3 and 171 ug/kg body weight (1 - 2 implants)

Male fish were treated in 26 trials and implanted 1 or 2 times with Ovaplant[7] at a dosage between 11.3 and 171 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 10 fish species treated with Ovaplant[7] at the dosages described above:

1. Salmonids:

   Ovaplant[7] was used at 11.3 - 150 ug/kg in 11 trials involving arctic char, Atlantic salmon, Chinook salmon, rainbow trout, and steelhead trout and were implanted with one pellet implant. Control fish were used in eight trials and involved 1,189 fish. Results in the spawning records showed that there was a 0 - 100% spermiation in the treated fish; as compared to 0 - 100% spermiation in the control fish. Treatment appeared efficacious in all trials.

2. Non-salmonids

   Ovaplant[7] was used at 40 - 171 ug/kg in 13 trials involving American shad, hickory shad, and striped bass and were implanted with 1 pellet implant. Control fish were used in two trials involving American shad. Results in the spawning records showed that there was an unknown level of spermiation in two trials of treated fish and 14.3 - 100% spermiation in the other trials involving treated fish; as compared to unknown spermiation in the control trials. 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. Treatment appeared efficacious in 10 trials and was characterized as inconclusive in three trials.

3. Marine non-salmonid

Ovaplant[7] was used at 12.5 - 60 ug/kg in two trials involving cobia and sablefish; and were implanted with 1 or 2 pellet implants. Control fish were not used. Results in the spawning records showed that there was an unknown level of spermiation in one trial of treated fish and 100% spermiation in the other trial involving treated fish. 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. Treatments appeared efficacious in both trials.

Overall, treatment resulted in an unknown spermiation or 14.3 - 100% spermiation in the male treated fish; as compared to an unknown spermiation or 0 – 100% in the control fish. Treatments appeared efficacious in 23 trials and were characterized as inconclusive in three trials.

B. Efficacy of Ovaplant[7] on female fish treated at a dosage between 11.3 and 156 ug/kg body weight (1 - 5 implants)

Female fish were implanted one to five times with Ovaplant[7] pellets at a dosage between 11.3 and 156 ug/kg body weight (Table 2) in 36 different trials. During five 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 nine fish species treated with Ovaplant[7] at the dosages described above:

1. Salmonids:

   Ovaplant[7] was used at 11.3 - 150 ug/kg in 22 trials involving arctic char, Atlantic salmon, Chinook salmon, coho salmon, and steelhead trout and were implanted with 1 - 5 pellet implants. Control fish were used in 10 trials. Results showed that there was a 22.2 - 100% ovulation in treated fish; as compared to unknown percent ovulation or 26.3 - 100% ovulation in the control fish. Treatment appeared efficacious in all trials.

2. Non-salmonids

   Ovaplant[7] was used at 52 - 156 ug/kg in 12 trials involving American shad and hickory shad and were implanted with 1 pellet implant. Control fish were used in two trials involving American shad. Results showed that there was an unknown level of ovulation in five trials of treated fish and 50 – 100% ovulation in the other trials involving treated fish; as compared to an unknown percent of ovulation in the control trials. 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. Treatment appeared efficacious in nine trials and was characterized as inconclusive in three trials.

3. Marine non-salmonid

Ovaplant[7] was used at 25 - 60 ug/kg in two trials involving cobia and sablefish and were implanted with 1 - 2 pellet implants. Control fish were not used. Results showed that there was 77.8 - 100% ovulation in the treated fish. Treatments appeared efficacious in both trials.

Overall, treatment resulted in either an unknown percent ovulation (due to fish not evaluated for ovulation by the Investigator) or a 22.2 - 100% ovulation in the female treated fish; as compared to 26.3 - 100% ovulation in the control fish or an unknown ovulation in the control fish. Treatment appeared efficacious in 33 trials and was characterized as inconclusive in three trials.

2. Observed Toxicity

No toxicity or adverse effects relating to Ovaplant[7] treatments were reported in 38 of the trials conducted in CY12-14. Three trials did report toxicity or abnormal behavior. In one trial involving coho salmon the investigator noted that two fish seemed to be paralyzed. They did not die but laid on the bottom of the raceway. This was more than likely due to incorrect insertion of the hormone. In two trials involving American shad (CY 12 and 13) the investigator reported seeing whelps and lethal swelling at injection site this year; never been seen or reported before. The following year they again reported high mortality after injection; injection site would swell and eventually become infected. They tried various batches of ovaplant and sterilized equipment but it was inconclusive of what caused it.

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 or was a threatened or endangered species that is not legally available for human consumption.

Current Study Protocol for Ovaplant[7] INAD #11-375

No changes have occurred to the current study protocol for Ovaplant[7] INAD #11-375.

Facility Sign-up List

Please see ATable 4. Facilities and Names of Investigators@ for facilities that signed-up to participate in the Ovaplant[7] INAD #11-375 during CY12-14.

Correspondence sent to Ovaplant[7] Participants

Please see the attached correspondence that was sent to all Ovaplant[7] participants after the AADAP Office received their sign-up form for CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 8,968. The total number of treated fish since December 15, 2005 is 23,834.

Summary of Study Results

Ovaplant[7] was used in 41 efficacy trials to induce gamete maturation in 11 different fish species (n = 8,968 treated fish; 2,143 untreated control fish) at dosages ranging from 11.3 - 171 ug/kg bw. Ovaplant[7] was administered as a pellet implant. Fish treated by pellet implant will be euthanized at the hatchery and properly disposed of; will not be released from the facility; or is a threatened or endangered fish and is not legally available for human consumption. Water temperature during treatments ranged from 41.9 - 80.6EF. Overall, results showed that Ovaplant[7] treatment appeared efficacious in 93% of the trials and was inconclusive in 7% of the trials. Data from the CY12-14 trials indicate that Ovaplant[7] 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 2012 -2014 Ovaplant[7] Male Efficacy Results - Implant						**Treated**		**Control**	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (ug/kg b.w.)**	**Spawning Interval (days)**	**Number Treated**	**% Spermiate**	**Number of Controls**	**% Spermaite**
Bears Bluff NFH	Inconclusive	2	American Shad	100	1	118	? – 50	0	-
Dennis Wildlife Center/Bayless Hatchery	Effective	2	American Shad	75	2 - 3	587	? – 100	168	?
Muddy Run Lab	Effective	3	American Shad	80 - 89	2	1,037	100	0	-
Orangeburg NFH	Effective	1	American Shad	100	1	25	100	0	-
Welaka NFH	Inconclusive	1	American Shad	75	4	32	100	0	-
Troutlodge Rochester	Effective	1	Arctic Char	25	3	65	67.7	0	-
Richard Cronin NFH	Effective	1	Atlantic Salmon	150	5 - 6	2	100	5	100
Division of Sport Fish - Area Office	Effective	1	Chinook Salmon	20.6	2	6	100	0	-
Mt. Shasta SFH	Effective	1	Chinook Salmon	32.5	2 - 7	10	100	9	100
Pillar Creek Hatchery	Effective	1	Chinook Salmon	11.3	10	1	100	0	-
Bears Bluff NFH	Effective	1	Cobia	12.5	1	4	?	0	-
Matapeake	Effective	3	Hickory Shad	150 - 171	2	2,183	100	0	-
Mt. Shasta SFH	Effective	1	Rainbow Trout	75	14	78	90 - 100	1,090	90 - 95
Manchester Research Station	Effective	1	Sablefish	60	11 - 21	6	100	0	-
Dworshak NFH	Effective	3	Steelhead	12 - 150	7 - 28	239	0 - 100	30	0 - 100
Eastbank SFH	Effective	1	Steelhead	40	7	11	100	50	100

Table 1. Summary of Year 2012 -2014 Ovaplant[7] Male Efficacy Results - Implant						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (ug/kg b.w.)**	**Spawning Interval (days)**	**Number Treated**	**% Spermiate**	**Number of Controls**	**% Spermaite**
Wells SFH	Effective	1	Steelhead	40	weekly	6	0 - 100	5	100
Warm Springs FTC	Effective	1	Striped Bass	40 - 60	3	7	14.3	0	-

Table 2. Summary of Year 2012 -2014 Ovaplant[7] Female Efficacy Results - Implant						Control			
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (ug/kg b.w.)**	**Spawning Interval (days)**	**Number Treated**	**% Ovulate**	**Number of Controls**	**% Ovulate**
Bears Bluff NFH	Inconclusive	2	American Shad	150	1	85	50 – 66.7	0	-
Dennis Wildlife Center/Bayless Hatchery	Effective	2	American Shad	150	2 - 3	526	? - 100	133	?
Muddy Run Lab	Effective	3	American Shad	52 - 54	2	688	? - 100	0	-
Orangeburg NFH	Effective	1	American Shad	150	1	25	100	0	-
Welaka NFH	Inconclusive	1	American Shad	75	4	12	50	0	-
Troutlodge Rochester	Effective	1	Arctic Char	25	3	418	74.2	0	-
Richard Cronin	Effective	2	Atlantic Salmon	150	6	38	96.4 - 100	42	? - 100
Division of Sport Fish - Area Office	Effective	1	Chinook Salmon	20.6	2	64	100	7	83.3 – 100

Table 2. Summary of Year 2012 -2014 Ovaplant[7] Female Efficacy Results - Implant						**Control**			
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (ug/kg b.w.)**	**Spawning Interval (days)**	**Number Treated**	**% Ovulate**	**Number of Controls**	**% Ovulate**
Little White Salmon NFH	Effective	1	Chinook Salmon	75	3	86	53 - 100	0	-
Mt. Shasta SFH	Effective	1	Chinook Salmon	32.5	2 - 7	3	100	0	-
Nez Perce Tribal Hatchery Complex	Effective	1	Chinook Salmon	12.5	7 - 14	132	99.2	0	-
Pillar Creek Hatchery	Effective	2	Chinook Salmon	11.3	9 - 10	103	85.7 - 100	0	-
William Jack Hernandez SFH	Effective	2	Chinook Salmon	75	7	218	22.2 - 100	200	47.6 - 100
Bears Bluff NFH	Effective	1	Cobia	25	1	1	100	0	-
Medvejie Hatchery	Effective	2	Coho Salmon	48	10 - 12	281	81.1 - 97.2	153	26.3 – 59.7
Nez Perce Tribal Hatchery Complex	Effective	2	Fall Chinook Salmon	12.5	2 - 7	222	99.2 - 100	0	-
Matapeake	Effective	3	Hickory Shad	100 - 156	2	1,332	? - 100	0	-
Manchester Research Station	Effective	1	Sablefish	60	11 - 21	20	77.8 – 90.9	0	-
Little White Salmon NFH	Effective	1	Spring Chinook Salmon	75	6	200	99.5	0	-
Eastbank SFH	Effective	2	Steelhead	40	7	15	100	111	100
Kalama Falls	Effective	3	Steelhead	42	1 - 16	64	50 - 100	0	-
Wells SFH	Effective	1	Steelhead	40	7	18	100	140	100

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY12-14 Ovaplant[7] Efficacy Studies

Total Number of Treatment Trials	**41**
Number of Trials that Appeared Efficacious:	38
Number of Trials that were Inconclusive:	3
Total Number of Treated Fish:	**8,968**
Number of fish treated in efficacious trials	8,721
Number of fish treated in inconclusive trials	247

Treatment Regimes Used:
11.3 - 156 ug/Kg body weight female fish
11.3 - 171 ug/Kg body weight male fish

Water Temperature (EF) Range: 41.9 – 80.6

Fish Species Treated:

Salmonids

arctic char (*Salvelinus alpinus*)
Atlantic salmon (*Salmo salar*)
Chinook salmon (*Oncorhychus tshawytscha*)
coho salmon (*O. kisutch*)
rainbow trout (*O. mykiss*)
steelhead trout (*O. mykiss*)

Non-salmonids
American shad (*Alosa sapidissima*)
hickory shad (*A. mediocris*)
striped bass (*Morone saxatilis*)

Marine non-salmonid
cobia (*Rachycentron canadum*)
sablefish (*Anoplopoma fimbria*)

Size Class of Treated Fish: Adults