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

2007 Annual Summary Report on the Use of sGnRHa - Ovaplant® in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

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 2007 (CY07), seven trials were conducted under this INAD to evaluate the efficacy of Ovaplant® to induce gamete maturation in a variety of fish species. Trials involved 772 treated fish and 834 control fish and were conducted at five different hatcheries, including two U.S. Fish and Wildlife Service fish hatcheries, two state 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 from trials conducted in CY07 showed that treatments appeared efficacious in all trials.

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® 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 calendar year 2007 (CY07). 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 five different fish culture facilities during CY07, including two U.S. Fish and Wildlife Service fish hatcheries, two state hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 38.0 to 68.0°F.

2. Chemical material

Syndel International Inc. of Vancouver, British Columbia Canada 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 sGnRHa per pellet and administration as a single treatment event only. Drug dosages used by Investigators in CY07 ranged from 15 to 136 ug sGnRHa. Fish treated by pellet implant were euthanized at the hatchery and properly disposed after they were spawned.

Fish Species and Sex Treated

1. Fish Species Treated

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

Salmonids

chinook salmon *Oncorhychus tshawytscha*

steelhead trout *O. mykiss*

Non-salmonids

American Shad *Alosa sapidissima*

2. Gender of treated fish

Ovaplant® was used on 276 female and 496 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 a summary 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 CY07 under INAD #11-375.)

A. Efficacy of Ovaplant® on male fish treated between 15 and 136 ug/kg body weight (1 implant)

Male fish were treated in three trials and implanted one time with Ovaplant® at a dosage between 15 and 136 ug/kg body weight (Table 1). Fish will not be available for human consumption. Below are the treatment regimens used to induce gamete maturation in two fish species treated with Ovaplant® at the dosages described above:

1. Dose: 15 - 40 ug/kg

   Steelhead trout were used in two trials, and in each, fish were implanted with one Ovaplant®. Control fish were used in both of these trials. Results showed that there was 90 - 100% spermiation in treated fish; as compared to 78 - 100% spermiation in control fish. Overall, treatment appeared efficacious in both trials.

2. Dose: 136 ug/kg

American shad were used in one trial and were implanted with one Ovaplant®. Control fish were used in this trial. Results showed that there was 100% spermiation in treated fish; as compared to 100% spermiation in control fish. Treatment appeared efficacious in this trial.

Overall, treatment resulted in a 90 - 100% spermiation in the male treated fish; as compared to 78 - 100% in the control fish. Treatments appeared efficacious in all trials.

B. Efficacy of Ovaplant® on female fish treated at a dosage between 15 and 100 ug/kg body weight (1 - 2 implants)

Female fish were implanted one time with Ovaplant® pellets at a dosage between 15 and 100 ug/kg body weight (Table 2) in four different trials. Fish will not be available for human consumption. Below are the treatment regimens used to induce gamete maturation in three fish species treated with Ovaplant® at the dosages described above:

1. Dose: 15 - 40 ug/kg

Chinook salmon and steelhead trout were used in three trials and fish were implanted with 1 - 2 Ovaplants®. Control fish were used in all trials. Results showed that there was 94 - 100% ovulation in treated fish; as compared to 93 - 100% ovulation in control fish. Overall, treatment appeared efficacious in all trials.

2. Dose: 100 ug/kg

American shad were used in one trial and fish were implanted with one Ovaplant®. Control fish were used this trials. Results showed that there was a 50 - 90% ovulation in treated fish; as compared to 20 - 90% ovulation in control fish. Treatment appeared efficacious in this trial.

Overall, treatment resulted in a 50 - 100% ovulation in the female treated fish; as compared to 20 - 100% ovulation in the control fish. Treatments appeared efficacious in all trials.

2. Observed Toxicity

No toxicity or adverse effects relating to Ovaplant® treatments were reported in any trials conducted in CY07.

Number of Treated Fish under Slaughter Authorization

Total number of fish treated during CY07 was 772. The total number of treated fish to count against the slaughter authorization dated December 15, 2005 is 1,263. No changes have occurred to the current Ovaplant® INAD #11-375 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 CY07. Facilities not listed in

Appendix III-a of the current Ovaplant® INAD #11-375 study protocol have been highlighted.

The following facility received Ovaplant® during CY07 but never used the drug:

1. Edenton NFH

Summary of Study Results

Ovaplant® was used in seven efficacy trials to induce gamete maturation in three different fish species (n = 772 treated fish; 834 untreated control fish) at dosages ranging from 15 - 136 ug/kg bw. Ovaplant® was administered as a pellet implant. All treated fish administered Ovaplant® as a pellet implant were euthanized after the spawning season. Water temperature during treatments ranged from 38.0 - 68.0°F. All of the trials appeared efficacious. Data from the CY07 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.

References

Alvarino, J.M.R., S. Zanuy, F. Prat, M. Carrillo, and E. Mananos. 1992. Stimulation of ovulation and steroid secretion by $LHRH_a$ injection in the sea bass (Dicentrarchus labrax): effect of time of day. Aquaculture. 102: 177-186.

Almendras, J.M., C. Duenas, J. Nicario, N.M. Sherwood, and L.W. Crim. 1988. Sustained hormone release III: Use of gonadotropin-releasing hormone analogues to induce multiple spawnings in the sea bass, *Lates calcarifer*. Aquaculture. 74: 97-111.

Carolsfeld, J., N.M. Sherwood, H. Krieberg, and S.A. Sower. 1988. Induced sexual maturation of herring using GnRH 'quick release' cholesterol pellets. Aquaculture. 70: 169-181.

Crim, L.W., A.M. Sutterlin, D.M. Evans, and C. Weil. 1983a. Accelerated ovulation by pelleted LHRH analogs treatemtn by spring-spawning rainbow trout (*Salmo gairdneri*) held at low temperature. Aquaculture. 35: 299-307.

Crim, L.W., D.M. Evans, and B.H. Vickery. 1983b. Manipulation of the seasonal reproductive cycle of the landlocked salmon (Salmo salar) by LHRH analogues administered at various stages of gonadal development. Can. J. Fish. Aquat. Sci. 40: 61-67.

Crim, L.W. and B.D. Glebe. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture. 43: 47-56.

Crim, L.W., N.M. Sherwood, and C.E. Wilson. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (GnRHa) administration by either single time injection or cholesterol pellet implanted on plasma gonadotropin levels in bioassay model fish, the juvenile rainbow trout. Aquaculture. 74: 87-95.

Donaldson, E.M., G.A. Hunter, and H.M. Dye. 1981. Induced ovulation in coho salmon (Oncorhynchus kisutch). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. Aquaculture. 26: 129-141.

Donaldson, E.M., and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. Pages 351-403 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. Fish physiology, volume 9. Part B. Academic Press, New York.

Erdahl, D.A., and J McClain. 1987. Effect of LH-RH analogue treatment on egg maturation (ovulation) in lake trout broodstock. Progressive Fish-Culturist. 49: 276-279.

Fitzpatrick, M.S., B.K. Suzumoto, C.B. Schreck, and D. Oberbillig. 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (Oncorhynchus kisutch). Aquaculture. 43: 67-73.

Goetz, F.W. 1983. Hormonal control of oocyte maturation and ovulation in fishes. In: Fish Physiology Vol IX, Part B. Eds. W.S. Hoar, D.J. Randall and E.M. Donaldson. Academic Press, New York. pp. 117-169.

Goren, A., Y. Zohar, M. Fridkin, E. Elhanati, and Y. Koch. 1990. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata.* I. Cleavage of native salmon GnRH and LHRH in the pituitary. Gen. Comp. Endocrinol. 79: 291-305.

Hoar, W.S. 1969. Reproduction. In: Fish Physiology Volume III. Eds. W.S. Hoar and D.J. Randall. Academic Press, New York and London. pp.1-72.

Lee, C.S., C.S. Tamaru, J.E. Banno, C.D. Kelley, A. Bocek, and J.A. Wyban. 1986. Induced maturation and spawning of milkfish, Chanos chanos Forsskal, by hormone implantation. Aquaculture. 52: 199-205.

Marte, L.M., N. Sherwood, L. Crim, and J. Tan. 1988. Induced spawning of the maturing milkfish (*Chanos chanos*) using human chorionic gonadotropin and mammalian and salmon gonadotropin releasing hormone analogues. Aquaculture. 73: 333-340.

Millar, R.P., J.S. Davidson, C. Flanagan, N. Illing, I. Becker, G. Jacobs, and I. Wakefield. 1993. Gonadotropin-releasing hormone receptor structure and function. Proceedings of the “Perspectives in Comparative Endocrinology”. XII International Congress on Comparative Endocrinology. Toronto, Ontario, Canada. 16-21 May. pp. 264-268.

Ngamvongchon, S., J.E. Rivier, and N.M. Sherwood. 1992b. Structure-function studies of five natural, including catfish and dogfish, gonadotropin-releasing hormones and eight analogs on reproduction in Thai catfish (*Clarias macrocephalus*). Regul. Pept. 42: 63-73.

Peter, R.E., C.S. Nahorniak, M. Sokolowska, J.P. Chang, J.E. Rivier, W.W. Vale, J.A. King, and R.P. Millar. 1985. Structure-activity relationships of mammalian, chicken, and salmon gonadotropin releasing hormones *in vivo* in goldfish. Gen. Comp. Endocrinol. 58: 231-242.

Peter, R.E., C.S. Nahorniak, M. Sokolowska, J.P. Chang, J.E. Rivier, W.W. Vale, J.A. King, and R.P. Miller. 1987. Activity and position-8-substituted analogs of mammalian gonadotropin-releasing hormone (mGnRH) and chicken and lamprey gonadotropin-releasing hormones in goldfish. J. Comp. Endocrinol. 65: 385:393.

Powell, J.F.F., P. Swanson, and N.M. Sherwood. 1995. Induced ovulation in Pacific salmonids: gonadotropin levels in chinook salmon spawned out of seawater and freshwater. Proc. Amer. Fish. Soc. Apr 26-30, 1995. Victoria, B.C.

Powell, J.F.F., J. Brackett, and J. Battaglia. 1998. Induced and synchronized spawning of captive broodstock using Ovaplant and Ovaprim. Proc. Aquaculture Assoc. of Canada. 31 Jan - 4 Feb 1998, St. John's Nflnd. Canada.

Schultz, R.W., Bosma, P.T., Zanderbergen, M.A. van der Sanden, M.C.A., van Dijk, W., Peute, J., Bogerd, J, and Goos, H.J.Th. 1993. Two gonadotropin-releasing hormones in the African catfish, Clarias gariepinus: Localizationl, pituitary receptor binding, and gonadotropin release activity. Endocrinology. 133: 1569-1577.

Schultz, R.W., M.C.A. van der Sanden, P.T. Bosma, and H.J.Th. Goos. 1994. Effects of gonadotropin-releasing hormone during the pubertal development of the male African catfish (*Clarias gariepinus*): gonadotrophin and androgen levels in plasma. J. Endocrinol. 140: 265-273.

Sherwood, N.M., L.W. Crim, J.L. Carolsfeld, and S.M. Walters. 1988. Sustained release I: Characteristics of in vitro release of gonadotropin-releasing hormone analogue (GnRH-a) from pellets. Aquaculture. 74: 75-86.

Tao, Y.X. and H.R. Lin. 1993. Effects of exogenous hormones on serum steroid in female ricefield eel (*Monopterus albus*). Acta Zool. Sinica. 39: 315-321.

Taranger, G.L., S.O. Stefansson, and T. Hansen. 1992. Advancement and synchronization of ovulation in Atlantic salmon (Salmo salar L.) following injections of LHRH analogue. Aquaculture. 102: 169-175.

Van der Kraak, G., H.R. Lin, E.M. Donaldson, H.M. Dye, and G.A. Hunter. 1983. Effects of LHRH and desGly^{10}(D-Ala$_6$)LHRH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (Oncorhynchus kisutch). General Comparative Endocrinology. 49: 470-476.

Weil, C., B. Breton, S. Sambroni, N. Zmora, and Y. Zohar. 1992. *In vitro* activities of various forms of GnRH in relation to their susceptibility to degradation at the pituitary level in the rainbow trout *Oncorhynchus mykiss*. Ben. Comp. Endocrinol. 87: 33-43.

Zohar, Y., A. Goren, M. Fridkin, E. Elhanati, and Y. Koch. 1990. Degradation of gonadotropin releasing hormone in the gildhead seabream, *Sparus aurata*. II. Cleavage of native salmon GnRH and LHRH, and their analogs in the pituitary, kidney, and liver. Gen. Comp. Endocrinol. 79: 306-319.

Table 1. Summary of Year 2007 Ovaplant® Male Efficacy Results - Implant

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
1	effective	AMS	Central New England FRO	2 days	387	136	100	421	100
1	effective	STT	Dworshak NFH	14 & 21 days	70	15	90 - 100	12	78 - 80
1	effective	STT	Wells SFH	every 7 days	39	40	97	39	100

Table 2. Summary of Year 2007 Ovaplant® Female Efficacy Results - Implant

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	AMS	Central New England FRO	2 days	210	100	50 - 90	307	20 - 90
1	effective	SUS	Eastbank SFH	6 days	15	40	100	20	100
1	effective	CKS	Nez Perce Tribal Hatchery	7 - 14 days	36	15	94	15	93
1	effective	STT	Wells SFH	7 - 8 days	15	40	100	20	100

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2007 Ovaplant® Efficacy Studies

Total Number of Treatment Trials	7
Number of Trials that Appeared Efficacious:	7 (100%)
Total Number of Treated Fish:	772

Treatment Regimes Used:

15 - 40 ug/Kg body weight	5 trials
100 - 136 ug/Kg body weight	2 trials

Water Temperature (°F) Range: 38.0 - 68.0

Fish Species Treated:

Salmonids

chinook salmon *Oncorhychus tshawytscha*
steelhead trout *O. mykiss*

Non-salmonids
American shad *Alosa sapidissima*

Size Class of Treated Fish: Adults