GREAT LAKES FISHERY COMMISSION

1987 Project Completion Report[1]

The Use of Lamprey Gonadotropin-Releasing Hormone and its Analogues to Control Reproduction in the Sea Lampreys

by:

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FINAL REPORT

June, 1987

Great Lakes Fishery Commission
Project Completion Report *

PROJECT TITLE:

The Use of Lamprey Gonadotropin-Releasing Hormone and its Analogues to Control Reproduction in the Sea Lampreys

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SUMMARY

1. **OBJECTIVE**: To determine the effects of administered lamprey GnRH and its analogues on steroidogenesis and gametogenesis in the adult lampreys held under various temperatures.

RESULTS: The biological activities of lamprey GnRH, a lamprey agonist ([D-Ala^6, Pro^{9}NEt] GnRH) and a lamprey GnRH antagonist (D-Phe^{2,6}, Pro^3] lamprey GnRH were determined in female lamprey held under different temperatures of 50^{0}C or 66^{0}C. Lamprey GnRH and lamprey GnRH agonist and to a much lesser extent, the lamprey GnRH antagonist, stimulated the pituitary-gonadal axis as determined by steroidogenesis and ovulation. The responses noted were dependent on the water temperature and dosage of the compound tested.

2. **OBJECTIVE**: To test lamprey GnRH in lampreys in the parasitic phase to determine biological activity. Additionally, we tested an antibody to lamprey GnRH in the parasitic phase lampreys to see if we could induce immunoneutralization.

RESULTS: Lamprey GnRH significantly reduced plasma estradiol compared to controls. This is in sharp contrast to the response noted in adult spawning lampreys. The lamprey GnRH antibody decreased plasma progesterone indicating that in parasitic phase lampreys, lamprey GnRH is biologically active in reproductive processes. Importantly, however, the hypothalamus appears to have different mechanisms of control of the pituitary-gonadal axis in the parasitic phase lampreys compared to the adult lampreys.

3. **OBJECTIVE**: To investigate the specificity of the lamprey GnRH by administering this compound to another fish species besides the lamprey.

RESULTS: Lamprey GnRH stimulated reproductive activity in coho salmon as measured by elevated gonadotropin compared to controls.

4. **OBJECTIVE**: To test the effects of the lamprey GnRH antagonist on spawning behavior of adult lampreys. This study is to test for a potential non-mutagenic sterilization compound.

RESULTS: The lamprey GnRH agonist and antagonist inhibited reproductive behavior in the female lampreys. However, in the males these compounds stimulated reproductive behavior but in a slightly less reponse compared to control males. In a smaller study, sperm from control and treated males were tested in a 24 hr fertilization test. LGnRH agonist and controls appeared to fertilize the eggs, however the lGnRH antagonist had less than 10% fertilization.

CONCLUSION:

My investigations over the past three years of the structure and function of GnRH in lampreys indicate that the use of one of the GnRH antagonists has a very strong potential for use as a non-mutagenic sterilant. Physiological and chemical data of GnRH and its analogues has expanded enormously in mammals. Over 1500 agonist and antagonist GnRH analogs have been synthesized and researched. The use of these compounds are not only being used now for conceptive and contraceptive measures but they are also being used in therapeutic clinical applications (prostatic cancer, breast cancer,etc.). The present advanced status of our knowledge of the mechanism of interaction of the hypothalamus and adenohypophysis in the mammal rests on the solid foundation laid down by many scientists over the past four decades. This basic information was crucial to the understanding and application of the GnRH analogues. We have advanced very rapidly in our studies on lamprey neuroendocrinology particularly compared to the mammalian studies. We now have solid evidence for hypothalmic-pituitary control in these lampreys and have identified that potentialy a GnRH antagonist may be effective for a sterilization program. However, these kinds of studies will take a few years before any analogue can be implemented for such a program. A comprehensive state-of-the-art analysis should lead to the application of GnRH analogues as a potential management tool.

RESULTS

OBJECTIVES 1 and 2

Experiments A and B: Steroidogenesis

1. The purpose of this study was to determine the effects of lamprey GnRH, a lamprey GnRH agonist ([D-Ala^6, Pro^{9}NEt]GnRH), and a lamprey GnRH antagonist ([D-Phe^{2,6},Pro^3]lGnRH) on steroidogenesis in female adult lampreys that were approximately 1 1/2 months from their normal spawning period. The lampreys were treated with two injections two days apart followed by blood sampling at 0, 5, 24 and 48 after the second injection. There were two groups held at two different temperatures: A)50^{0}F or B)66^{0}F. Except for temperature, they were treated in identical fashion. There were thirteen treatment groups in each of Experiment A and Experiment B. Each of the treatment groups contained 10 female lampreys.

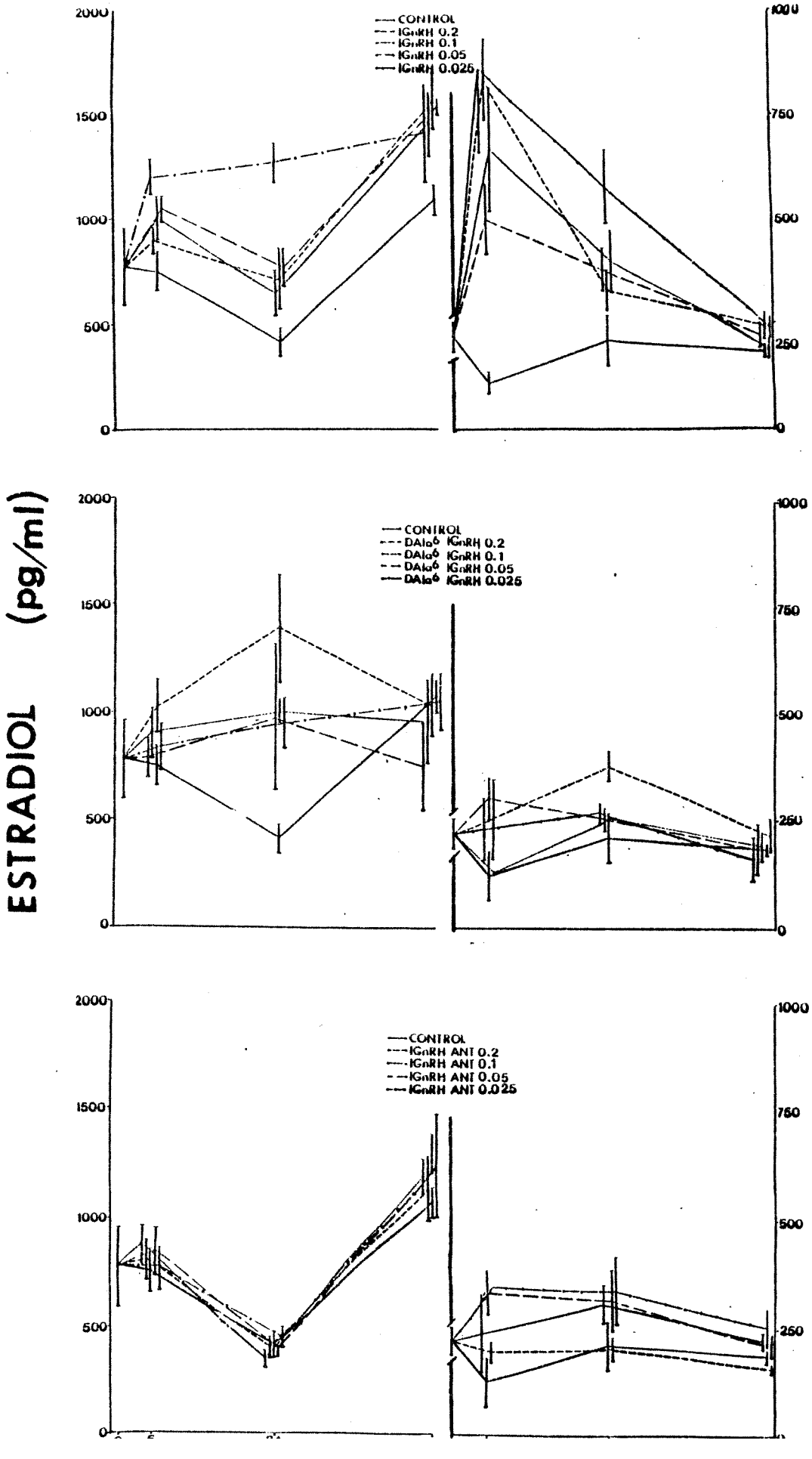
Within 30 min of injection, all peptides were dissolved in 0.6% NaCl in distilled water and were injected intraperitoneally in a dose relative to body weight. The animals were anesthetized with ethyl m-amino benzoate methanesulfonate (MS222), prior to injection or blood sampling. Blood samples (about 300 ul) were collected in heparinized syringes by cardiac puncture. Plasma was drawn off and stored frozen at -20^{0}C until assayed by radioimmunoassay for estradiol and progesterone. Plasma estradiol and progesterone were measured by radioimmunoassay as previously described (Sower et al., 1983).

Plasma estradiol and progesterone increased significantly in the lamprey GnRH and lamprey GnRH agonist treated lampreys compared to controls in both experiments A and B (Fig. 1 and 2). Estradiol was more dramatically increased in those treated lampreys held at the higher temperature of 66^{0}C and the response was also greater at 5 1/2 hr. The lamprey GnRH antagonist treated lampreys had a varied estradiol or progesterone response compared to controls. Only in Experiment B (water temperature 66^{0}C) with one exception the lamprey GnRH antagonist treated lampreys had significantly higher progesterone or estradiol levels compared to controls but this was only at the lower dosages.

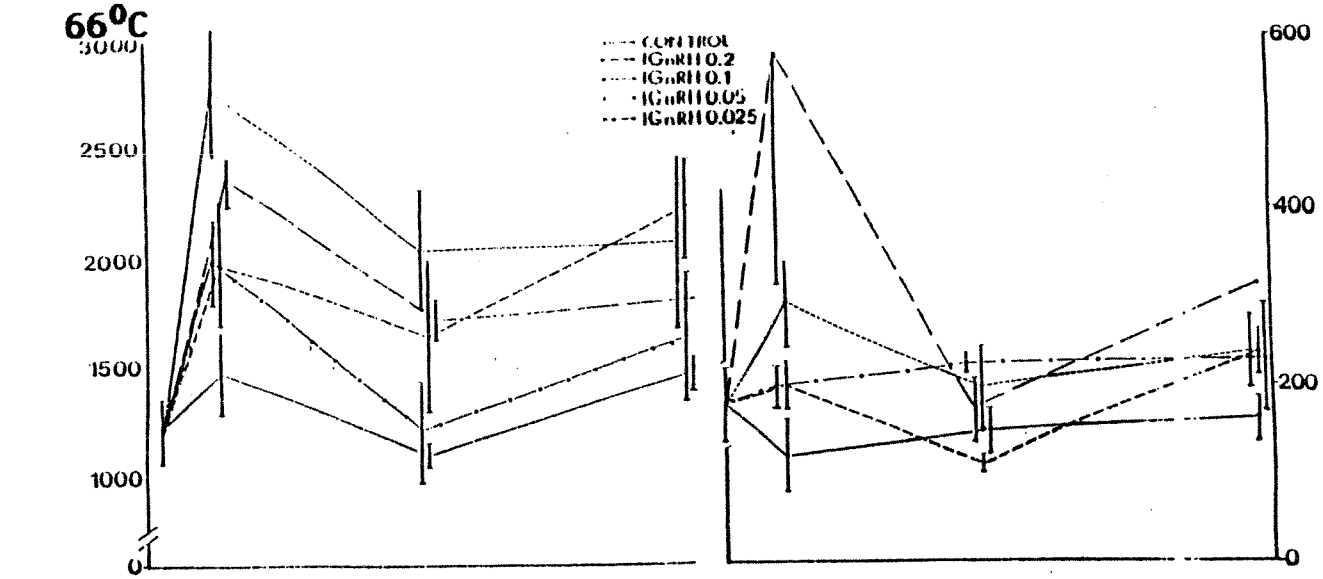
EXPERIMENT ..

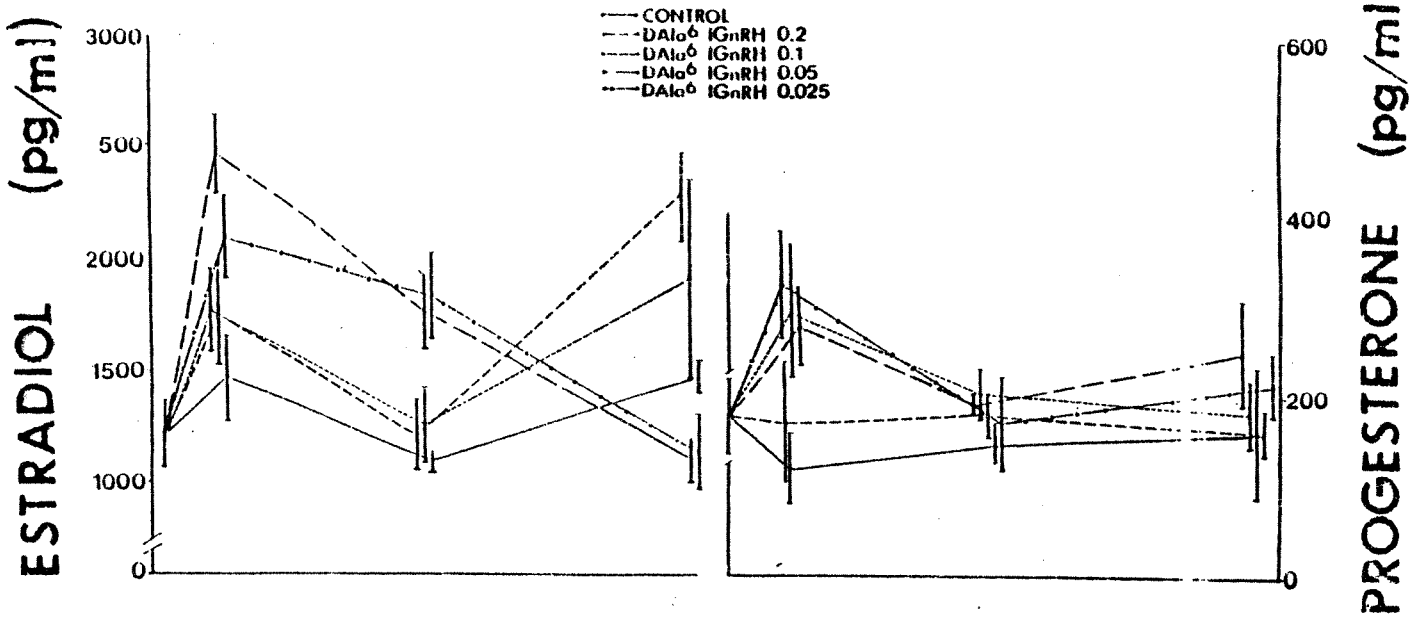
1986

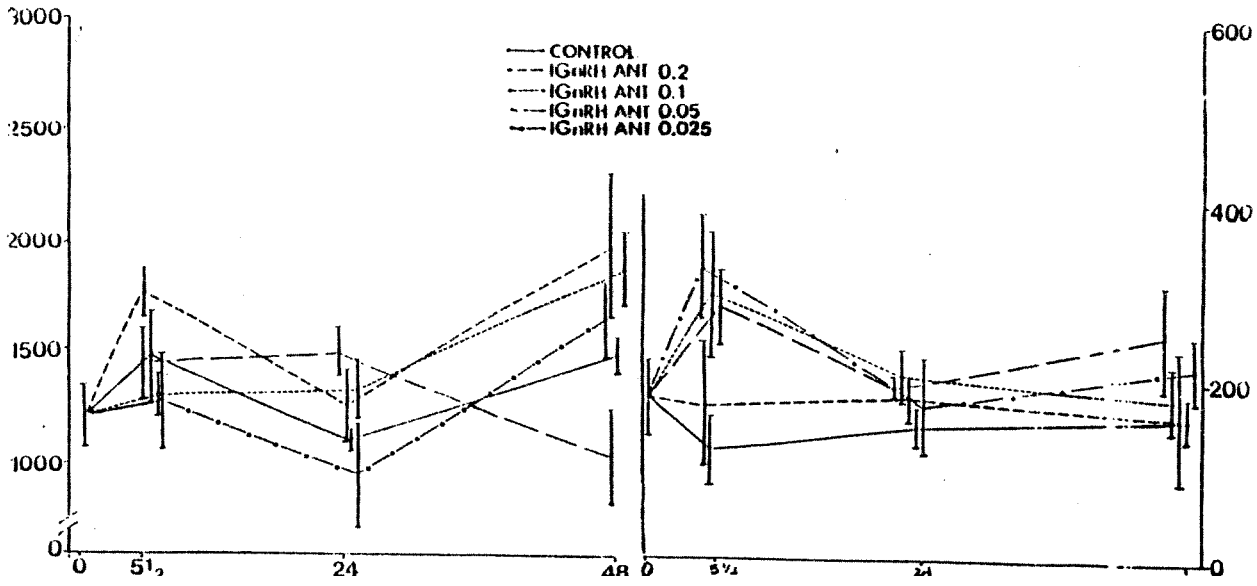
50°C



EXPERIMENT B
1986
66°C







These data provide further evidence that lamprey GnRH and its analogues are biologically active in stimulating the hypothalamo-adenohypophysial axis as determined by steroidogenesis. The varied response of the lamprey GnRH analogues appears similar to the mammalian GnRH data such that the replacement or deletion of one or more amino acids in GnRH results in effective binding at the pituitary, but induces varied or hinders the functional effect.

Experiment C: Induced Ovulation:

The purpose of this study was to determine the effects of lamprey GnRH and its analogues on ovulation in female lampreys that were approximately 1 1/2 months from their normal ovulatory period. The lampreys were treated with four injections two days apart held at a water temperature of 60^{0}C. This experiment was similar to that of the previous year however the timing,and number of injections were different. Additionally, these lampreys were sampled for plasma unlike the previous year where we did not sample the injected lampreys for plasma. The lampreys were sampled for blood 24 hr after their fourth injection.

The data are summarized in Table 1 which includes the list of the various treatment groups. Each treatment group had 12 lampreys.

The lampreys were checked every other day for a period of 41 days to determine if they had ovulated as judged by external physical characteristics as described in my earlier studies. The lampreys were killed on the day they had ovulated. At day 12 after the fourth injection ovulation had occured in those lampreys treated with lamprey GnRH at 0.1 or 0.05 ug/g lamprey; lamprey GnRH antagonist at 0.2 ug/g; or lamprey GnRH agonist at 0.1 ug/g. The control lampreys did not ovulate until day 35. The lower doses of the various compounds were similar to controls.

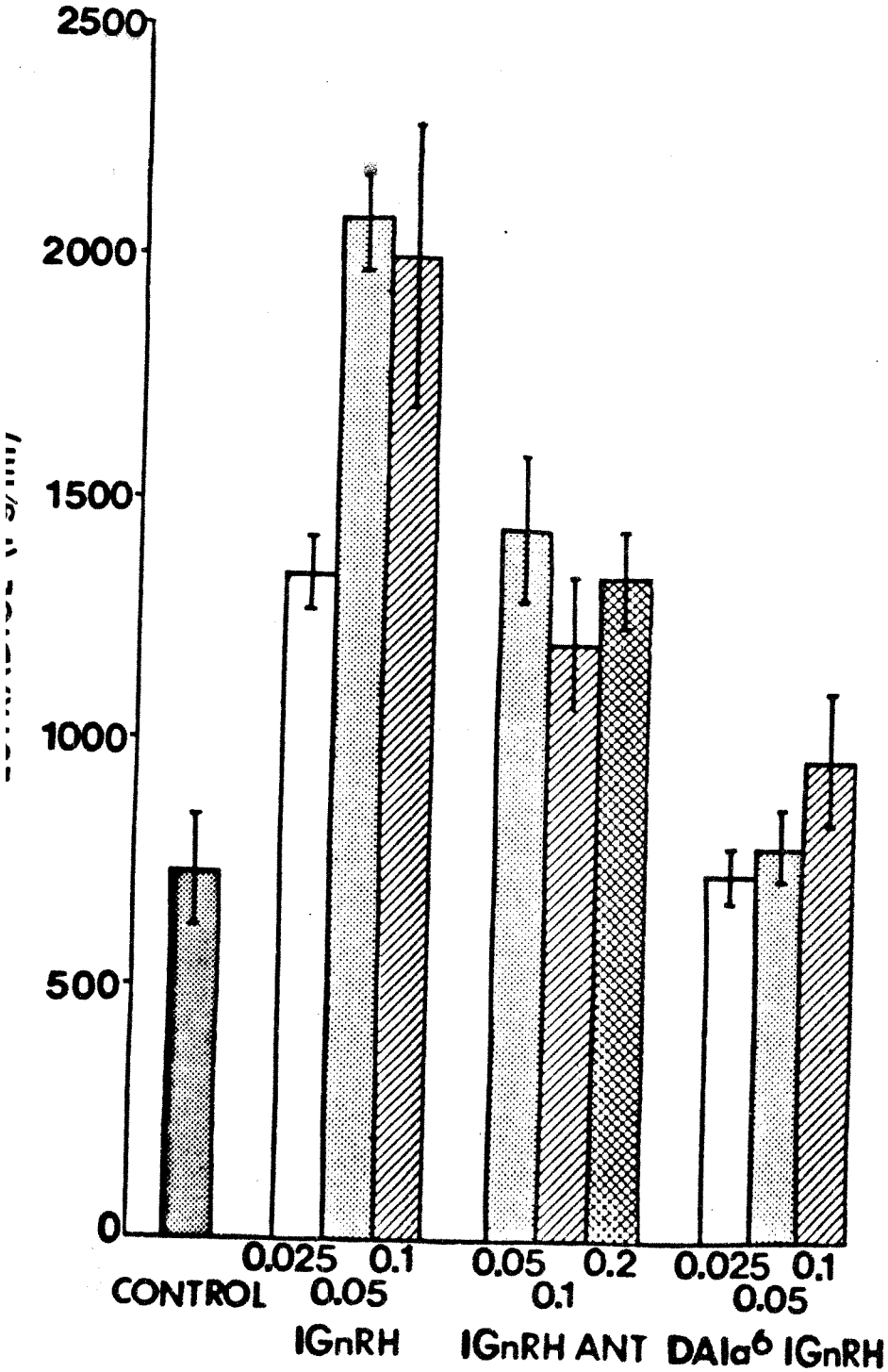
EXPERIMENT C
1986
INDUCED OVULATION

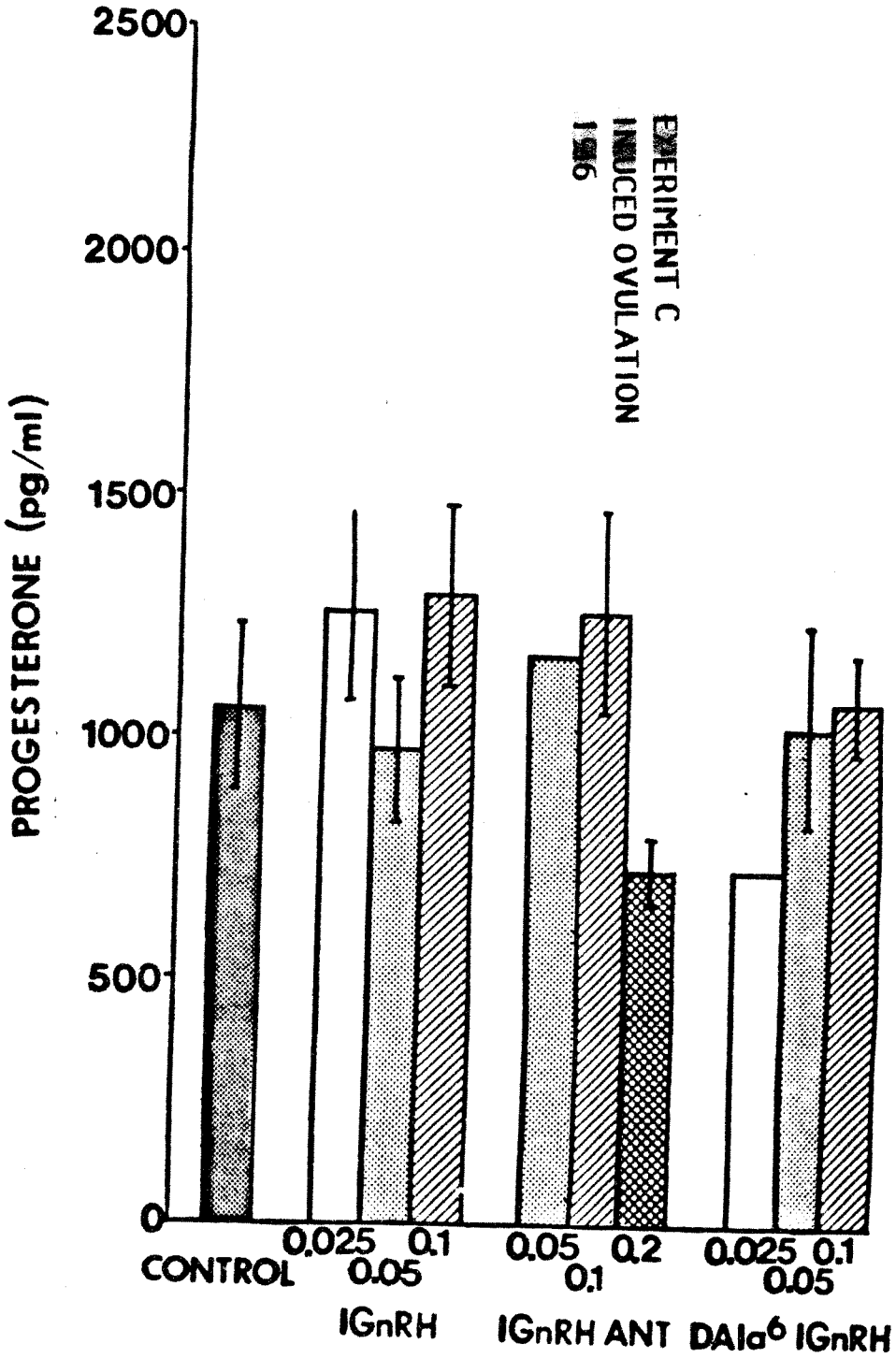
TABLE 1

TREATMENT ug/g	% OVULATION Day 12
Control	0
Lamprey GnRH-0.1	11.1
Lamprey GnRH-0.05	22.2
Lamprey GnRH-0.025	0
Lamprey GnRH ANTAG-0.2	10
Lamprey GnRH ANTAG-0.1	0
Lamprey GnRH ANTAG-0.05	0
Lamprey GnRH Agonist-0.1	10
Lamprey GnRH Agonist-0.05	0
Lamprey GnRH Agonist-0.025	0

Lamprey GnRH ANTAG: D-Phe^{2,6},Pro ^{3} lamprey GnRH

Lamprey GnRH Agonist: D-Ala^{6}, Pro ^{9} NEt lamprey GnRH


2500
2000
1500
1000
500
0
CONTROL
0.025
0.05
0.1
0.05
0.1
0.2
0.025
0.05
0.1
IGnRH
IGnRH ANT
DAla6 IGnRH


PROGESTERONE (pg/ml)
2500
2000
1500
1000
500
0
CONTROL
0.025
0.05
0.1
0.05
0.1
0.2
0.025
0.05
0.1
IGnRH
IGnRH ANT
DAla6 IGnRH

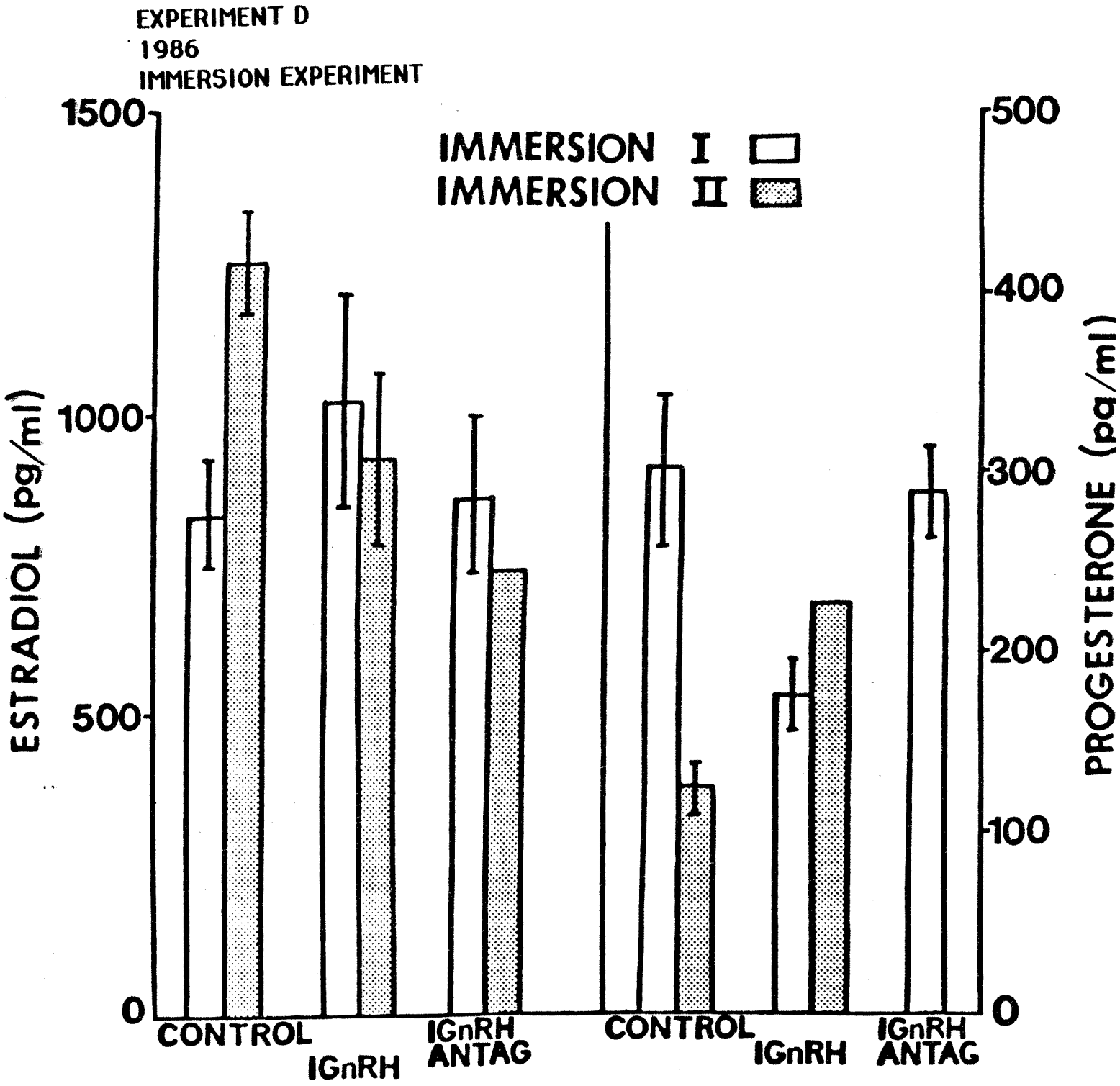
The results are similar to last year, however, the ovulatory response is less than that of the previous year. This may be due to the plasma sampling which stressed the lampreys which was evident by the mortalities that occured the day after the blood sampling which was approximately 10% in each of the groups.

Estradiol levels were significantly higher in the treated lampreys at the higher doses corresponding to the ovulatory response (Fig. 3). These data provide further evidence that pituitary receptors for GnRH are specific and can distinguish between molecular variants of these peptides based on the steroid responses.

Experiment D--Immersion Experiment

The purpose of this study was to determine if lamprey GnRH and the lamprey GnRH antagonist can effect steroid production by immersing the adult females in water containing the peptides. Three groups of 3 lampreyes each were immersed in aerated lake water daily for 3 days for 1 hr. Immediately before the immersion, the peptides were added to the water. The lampreys were sampled for plasma on the thrid day immediately following the last immersion treatment. The results similar to last year were not significant (Fig. 4).

The immersion experiments were being conducted with the concept of applying the lamprey GnRH antagonist to the streams to inhibit spawning. Our results to date indicate that this may not be a feasible means of application. In addition, we have preliminary evidence indicating that lamprey GnRH, despite its unique structure, does influence reproduction in salmonids, carp, and mammals.


EXPERIMENT D
1986
IMMERSION EXPERIMENT
IMMERSION I
IMMERSION II
ESTRADIOL (pg/ml)
PROGESTERONE (pg/ml)
1500
1000
500
0
500
400
300
200
100
0
CONTROL
IGnRH
IGnRH ANTAG
CONTROL
IGnRH
IGnRH ANTAG

Experiment E-- Parasitic Phase Lampreys

The purpose of this study is to determine the role of the hypothalamic-pituitary-gonadal axis in parasitic phase lampreys. To date, most of our experiments have been performed on adult spawning lampreys. It is very important to understand the role of neuroendocrine system in other phases of lampreys particularly the earlier stages to fully understand the development and role of the hypothalamus during the life cycle of the sea lamprey.

In the previous year, we had demonstrated that a mammalian GnRH analogue significantly decreased plasma estradiol levels compared to controls. This is in direct contrast to the response noted in the adult lampreys.

Two groups of parasitic lampreys were injected with Normal rabbit serum (control) or with an antibody to lamprey GnRH. The objective is to determine if the lamprey GnRH antibody can effectively immunoneutralize the reproductive system. Eighteen parasitic lampreys were shipped to the University of New Hampshire on 28 June 1987. The next day, the lampreys were injected at 9:00 am and were reinjected at 9:00 pm. Blood samples were taken the following morning at 9:00 pm by cardiac puncture. The blood was centrifuged and the plasma collected until analyzed for progesterone (Table 2). As noted the antibody significantly decreased plasma progesterone levels compared to controls. These data are very exciting and indicate that there is hypothalamic control in parasitic phase lampreys and that this response appears to be under a different mechanism than in adult lampreys. Further experiments similar to this will be done in metamorphosing phase lampreys.

EXPERIMENT E
1986
PARASITIC PHASE LAMPREYS

TABLE 2

TREATMENT	PROGESTERONE pg/ml X +SE
CONTROL (NRS)	264+96
Lamprey GnRH Antibody	44+6

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OBJECTIVE 3

Specificity of Lamprey GnRH in Coho Salmon

The purpose of this study was to investigate the specificity of Lamprey GnRH in a representative of another vertebrate class, Coho salmon. Adult female coho salmon were maintained at NHFG Milford Hatchery following their return from the Atlantic Ocean. The salmon were injected three days apart followed by a blood sample 24 hr after the second injection. The results are as follows:

TREATMENT ug/g salmon	GTH -pg/ml X±SE
Control	17.3±3.8
Salmon GnRH-0.1	34.8±10.5
Salmon GnRH-0.05	20.4±8.7
Lamprey GnRH-0.1	33.4±14.1
Lamprey GnRH-0.05	10.9±2.3

Lamprey GnRH at 0.1 ug/g was equally effective as salmon GnRH at 0.1 ug/g in significantly stimulating plasma gonadotropin (GTH) levels in adult female coho salmon. Lamprey GnRH at 0.05 ug/g had little effect on plasma GTH levels compared to controls. These data are the first data to demonstrate that lamprey GnRH has biological activity in a teleost fish.

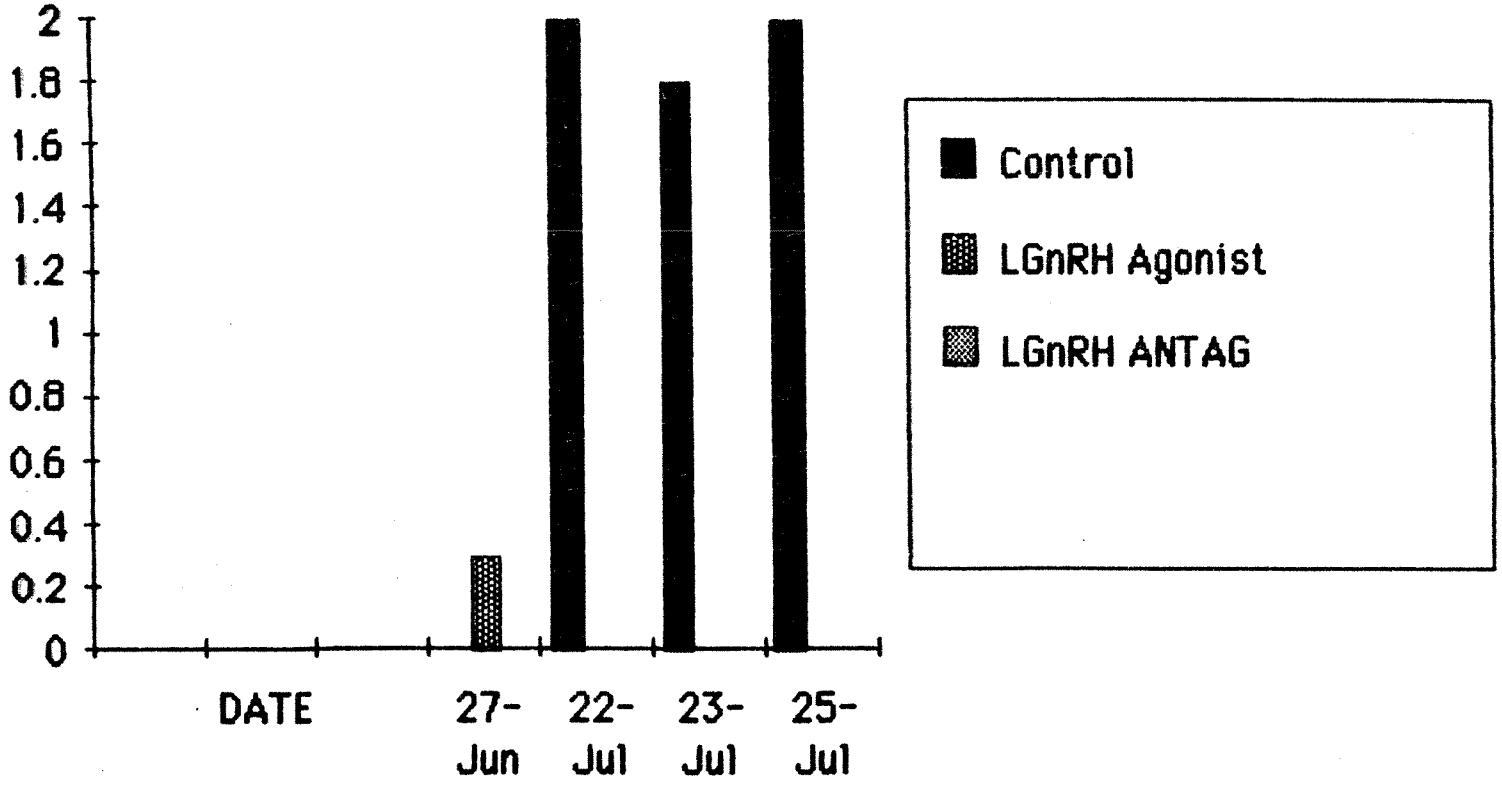
OBJECTIVE 4

Lamprey Spawning Behavior Experiment

The purpose of this study was to test the effects of GnRH analogues on spawning behavior of adult female and male lampreys. These experiments were conducted at the AFAIRS Building (UNH). Three groups of 12 sea run lampreys each were injected two times with saline; lamprey GnRH agonist or lamprey GnRH antagonist. After the second injection, the lampreys were introduced into the artificial stream channel and behaviors monitored. The lampreys were observed for 10 minute periods over 1/2 hr for 2 hr periods 3 to 4 times daily. The responses of the behavior from the treatment of the lamprey analogues compared to last year's data using mammalian GnRH analogues were not similar. In females, the lamprey GnRH agonist and the GnRH antagonist inhibited spawning behavior. However, in the males, lamprey GnRH agonist and antagonist stimulated the spawning act approximately 1 week earlier than controls. Two of the male lampreys each of a different treatment were used for a small fertilization study. The lamprey GnRH agonist and controls appeared to induce over 33% fertilization after 24 hr compared to 8% fertilization in the lamprey GnRH antagonist treated males.

These data indicate that lamprey GnRH influences behavior, either directly or indirectly, in the lampreys and that this response is under different hypothalamic control in the female versus male lamprey. Additionally, these data indicate that the GnRH antagonist stimulated reproductive behavior with a possibility that the sperm were not viable. In mammals, it has become very clear that the GnRH analogues are being used for sterilization, conception and other therapeutic and clinical applications. Further information is needed on the basic biological actions of lamprey GnRH as well as further studies on the actions of variant lamprey GnRH antagonist molecules as potential sterilants. Further studies on other lamprey GnRH antagonist molecules with various amino acid subsitutions shows promise for the eventual use of sterilizing the lampreys as a potential management tool. The potential of using GnRH analogues is exciting since these various antagonists like GnRH are proteins and easily degraded within the body and nontoxic to humans.

LAMPREY SPAWNING BEHAVIOR EXPERIMENT
1986
FEMALE



LAMPREY SPAWNING BEHAVIOR EXPERIMENT
1986
MALE