

PRELIMINARY DATA ON THE INDUCTION OF OVULATION IN WHITE STURGEON (*ACIPENSER TRANSMONTANUS* RICHARDSON)

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ABSTRACT

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Forty-six white sturgeon females captured prior to their spawning migration in the San Francisco Bay and during their spawning run in the Sacramento River were induced to ovulate with three different hormonal substances: white sturgeon and common carp crude pituitary extracts, and [D-Ala⁶] GnRH analogue. All three inducing agents were potent at the respective doses of 2.5 mg, 4.0 mg and 0.1 mg dry matter per kilogram of female body weight. The best ovulatory responses and egg fertilities were observed in fish administered hormonal treatment during the months of March and April. Fish captured prior to and during their spawning migration exhibited similar spawning success.

INTRODUCTION

Sturgeon broodstock captured in the wild and held captive in the hatchery do not spawn without hormonal stimulation. Early attempts to develop hatchery procedures for white sturgeon, a highly valued food and game fish of the North American West Coast, were based on the techniques developed for artificial recruitment of sturgeon stocks in the Caspian and Azov Seas. These techniques were of limited use because of the minimal data available on the white sturgeon reproductive cycle, and the lack of readily available sturgeon pituitary glands necessary for the induction of ovulation.

This paper reviews spawning induction trials conducted at the University of California during 1980—1982. The objectives of these trials were to determine the appropriate inducing agent, optimal time of hatchery spawning, and the specific sites for the broodstock procurement. As induction of spermiation has always been successful in these and earlier trials (Doroshov et al., 1983), attention was focused on responses of the females.

MATERIAL AND METHODS

Brooders were collected from two different locations: near the mouth of the San Francisco Bay prior to their spawning migration (November and December), and in the mid-portion of the Sacramento River during the spawning run (January through April). A total of 46 females, ranging from 14 to 52 kg in body weight, were used in this study.

Fish from the Bay, where water salinity fluctuated from 20 to 32‰, were acclimated to fresh water and kept in the hatchery for a period of from 1 to 4 months prior to spawning induction. They refused to feed and were held starved. Fish from the Sacramento River were spawned immediately after their capture.

Selection and administration of hormonal material were based on previous studies (Doroshov et al., 1983), and information available for Russian sturgeon (Milstein, 1972) and Pacific salmon (Donaldson et al., 1981). The following substances were injected in saline at the given dosages of dry matter per kilogram of female body weight: (1) white sturgeon pituitary extract (collected in the laboratory), 2.5 mg; (2) common carp pituitary extract, 4.0 mg; (3) [D-Ala⁶] GnRH analogue, 0.1 mg. Each fish was administered two intramuscular injections, the first 10% and the second 90% of the total dose, after an interval of 12 h.

After injection, females were held in individual tanks at a water temperature of 14–15°C. Ovulation was recognized by the discharge of a few adhesive ova and was observed within 19–26 h after the second injection. About one-third of the ova were removed surgically from each fish through an abdominal incision. The incision was then sutured and the fish tagged and released. The insemination, de-adhesion and incubation of eggs were carried out as previously described (Doroshov et al., 1983). Fertilization success was examined in samples from individual batches at the 4–6th stages of cleavage.

The irregular broodstock supply did not permit a rigorous trial design, or an adequate factorial data analysis. All data obtained were pooled in groups corresponding to hormonal treatments, spawning time and capture sites (Table I). Heterogeneity between the groups was tested with log-likelihood, Mann-Whitney and Kruskal-Wallis statistics. Means and confidence intervals for egg fertility were computed using an arcsine transformation.

RESULTS

The results of the trials are shown in Table II. Seventy-five percent of the fish injected with homoplastic pituitary extracts (6 out of 8 fish) and the GnRH agonist (3 out of 4 females) responded with ovulation. The ovulatory success was 65% (22 out of 34 females) in common carp pituitary treatment. The mean egg fertility was 53%, 52% and 83% in sturgeon

TABLE I

The number of white sturgeon females used in spawning induction trials. The fish captured in the Sacramento River (numerator) and San Francisco Bay (denominator) are in parentheses

Hormonal material	Spawning time			
	Nov.—Dec.	Jan.—Feb.	Mar.—Apr.	Total
Sturgeon hypophysis	—	3 (0/3)	5 (4/1)	8 (4/4)
Carp hypophysis	3 (0/3)	22 (4/18)	9 (7/2)	34 (11/23)
[D-Ala ⁶] GnRH	—	2 (1/1)	2 (0/2)	4 (1/3)
Total	3 (0/3)	27 (5/22)	16 (11/5)	46 (16/30)

TABLE II

Spawning response of the white sturgeon to hormonal induction. Data are means and confidence intervals (in parentheses)

Groups	Ovulatory success (%)	Fertilization success (%)
Hormonal material		
Sturgeon hypophysis	75	53 (10—94)
Carp hypophysis	65	52 (35—68)
[D-Ala ⁶] GnRH	75	83 (10—90)
Spawning time		
Nov.—Dec.	33	0
Jan.—Feb.	59	49 (32—72)
Mar.—Apr.	75	70 (48—88)
Capture sites		
River	75	62 (37—70)
Bay	53	55 (35—74)

and carp pituitary treatments, and GnRH agonist treatment, respectively. Fertilization success in all cases exhibited high individual variation. Neither the ovulatory response nor the egg fertility were significantly different between the three treatments ($P < 0.75$ and $P < 0.50$, respectively).

The spawning of only three fish was attempted during the months of November and December, prior to the onset of freshwater spawning migration. One of the three females ovulated, producing non-fertile eggs. The proportion of fish responding with ovulation increased in January and February (59%, 16 out of 27) and, particularly, during the spring, in March and April (75%, 12 out of 16). Mean egg fertility exhibited a similar increasing trend — from 49% in January and February to 70% in

March and April. Despite these trends, neither the ovulatory response nor the egg fertility differed significantly between the winter and spring spawning trials ($P < 0.50$ and $P < 0.20$, respectively).

Females procured during their spawning run in the Sacramento River exhibited a 75% (12 out of 16) ovulatory response, compared with 53% (16 out of 30) captured in the Bay and held for 1 to 4 months prior to spawning. The mean egg fertility in these two groups was 62% and 55%, respectively. No significant differences were revealed in either ovulatory response ($P < 0.25$) or fertilization success ($P < 0.50$).

The data obtained in this study are preliminary and should be interpreted with caution. Nevertheless, they show that: (1) all three inducing agents, at the doses applied, were equally potent for the induction of ovulation and production of fertile eggs; (2) a successful induced spawning of San Francisco Bay--Sacramento River white sturgeon stock should be anticipated during the winter and, particularly, during the spring months; (3) the females procured from the Bay, prior to their spawning migration, can be spawned after prolonged holding in the hatchery with a success similar to that of the river-caught broodstock.

DISCUSSION

Inducing agents

Sturgeon pituitary glands are used solely for the induction of ovulation in the sturgeon hatcheries of the U.S.S.R. (Milstein, 1972). Exclusive use of the homoplastic hormonal material assumes that two or more potentially spawnable animals are available for sacrifice to achieve a single spawning. Such an approach is impractical for white sturgeon due to its low abundance and the difficulties in procuring sexually mature fish for pituitary gland extraction. An alternative inducing agent is a necessity for the maintenance of a viable hatchery.

Unpublished observations have shown that crude common carp pituitary extract was effective *in vitro* for white sturgeon oocyte maturation at approximately twice the dose of white sturgeon pituitary extracts. The response obtained *in vivo* parallels these observations and eliminates the need for sturgeon hypophysis in practical hatchery applications. Common carp pituitary material is commercially available and adds relatively little cost to hatchery production. It does, however, suffer the disadvantages of variable GtH activity and quality control in preparation, stimulating a further search for better inducing agents.

Synthetic mammalian luteinizing hormone-releasing hormone (LH-RH) has been found to be relatively ineffective for ovulation induction in various fish species. Several synthetically produced gonadotropin-releasing hormone (GnRH) agonists are considered to be more potent, due to either a longer biological half-life within the organism (Buckingham, 1978; Don-

aldson et al., 1981) or an enhanced ability of the altered molecular structure to affect the GnRH receptors of the hypophysis (Coy et al., 1979). Administration of these agonists has resulted in successful induced spawning of coho salmon, *Oncorhynchus kisutch* (Donaldson et al., 1981), stellate sturgeon, *Acipenser stellatus* (Barannikova et al., 1982), and paddlefish, *Polyodon spathula* (K. Semmens, personal communication, 1983).

The success obtained with the application of GnRH analogue for inducing white sturgeon to spawn supports the hypothesis that the structure of GnRH has been highly conserved throughout vertebrate evolution (McCreery et al., 1982). The use of GnRH may potentially eliminate various problems associated with crude pituitary material, such as species-specificity, variable GtH content, and a possible immune response from the organism injected. However, GnRH agonists produced at the present time are expensive for large-scale practical application, and the optimal administration doses are not yet defined.

Spawning time

The reproductive cycle and spawning season of white sturgeon stocks have not been investigated adequately. Other anadromous acipenserids exhibit a complex pattern of spawning behaviour, including the presence of several spawning ecotypes within one river stock (Barannikova, 1972). This factor is of critical importance in hatchery operation for both domestic seed supply and artificial recruitment of wild stocks.

During three years of work the spawning migration of white sturgeon in the Sacramento River was observed to begin in January and extend through April. Females enter the river in a relatively uniform and advanced ovarian stage, with an oocyte diameter ranging from 3.7 to 4.0 mm. The egg size does not increase significantly over the period of the spawning run, but major changes occur internally, i.e. germinal vesicle migration and the polarization of yolk and ooplasm. In January, when the Sacramento River temperatures are low (5–7°), the germinal vesicle is positioned at approximately one-half the radial distance from center to animal pole of the oocyte. Females induced to spawn at this time exhibit a poor ovulatory response and low egg fertility. During the late winter and spring, when the water temperatures rise to 10–15°, the occurrence of fish in the pre-ovulatory condition (the germinal vesicle is in close proximity to the animal pole) increases dramatically. Spawning induction conducted at this time results in higher ovulatory and fertilization success.

These observations correspond well with the data on seasonal distribution of sturgeon larvae in the Sacramento River, which indicate that the major spawning of white sturgeon occurs in March and April at a river temperature 14° (Kohlhorst, 1976). It appears that Sacramento River white sturgeon stock has a single spawning season, extending throughout the spring. The existence of discrete spawning ecotypes, similar to those

in Russian sturgeon, *Acipenser guldenstadti* (Barannikova, 1972), appears to be only a remote possibility in the white sturgeon stock investigated.

Broodstock procurement sites

This study provides the first example of an acipenserid species captured in sea water and manipulated to spawn in captivity. The data on development occurring in the ovary of these fish during the hatchery holding period until their spawning will be presented later. Procurement and prolonged holding of Russian sturgeon broodstock is currently practiced in soviet sturgeon hatcheries (Molodtsov, 1979). The species migrating up the Volga River for spawning accumulates a vast amount of energy reserves prior to the spawning migration, which enables the fish to complete vitellogenesis with little or no contribution from exogenous food (Krivobok and Tarkovskaya, 1970). Apparently, white sturgeon exhibit similar metabolic patterns, i.e. prolonged starvation during hatchery holding does not influence normal vitellogenesis, egg maturation and ovulation.

Early procurement of sturgeon broodstock offers some advantages for a sturgeon hatchery. One may plan in advance the spawning induction and hatchery production schedule. Extension of the hatchery production season through environmental control of gonadal development may also be possible.

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