SPAWNING AND REPRODUCTIVE PERFORMANCE OF

DOMESTIC WHITE STURGEON (*ACIPENSER TRANSMONTANUS*)

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Introduction

A collaborative University-Industry White Sturgeon Broodstock Development Program was established in California eight years ago to obtain information on sexual maturation of captive sturgeon and to help the industry in developing broodstock reproductive management. Currently used broodfish originated from wild parents caught in the Sacramento and Columbia Rivers. They are raised in tanks under natural photoperiod, temperatures 16-20° C and are fed salmonid diets.

The sturgeon industry initially relied on seedstock obtained by spawning wild-caught fish. Aquaculturists began rearing broodstock in the early 1980's and today there are increasing numbers of ripe fish available for spawning each year. Intensive culture has accelerated growth and sexual maturation of white sturgeon compared to the wild fish maturing for the first time at an age 12-20 years. Practically all cultured males reach sexual maturity at age 4 years and spawn annually. Females exhibit variable rates of maturation, but the majority of fish reach first sexual maturity at age 7- 10 years.

Sturgeon females do not ovulate spontaneously in captivity although males spermiate occasionally. Two pituitary gonadotropins are involved in regulation of gonadal development (stGTH I) and spawning (stGTH II) of white sturgeon. Before spawning, concentration of GTH II in the pituitary increases significantly, but its release in circulation occurs only after hormonal injection to induce ovulation (Moberg et al., 1995).

To induce ovulation or spermiation, fish are treated with either gonadotropin releasing hormone analog GnRHa ([D-Ala^6, Pro^9 NEt] GnRH) or/and common carp pituitary extracts (CPE). However, treatment is effective only if the female has reached the responsive stage of final ovarian maturation, which is manifested by the advanced stage of germinal vesicle migration and the oocyte response with germinal vesicle breakdown to a maturation inducing hormone.

The life cycle of white sturgeon is currently closed in captivity, and domestic offspring are now being used for commercial grow out. However, broodstock management remains a difficult task, due to the longevity of the sturgeon reproductive cycle and complexity of spawning procedures.

Estimation of Spawning Time

Although wild sturgeon of San Francisco Bay are spring breeders (Kohlhorst, 1976; Doroshov and Lutes, 1984), captive females reach a preovulatory state during an extended time

(February-September) depending on rearing temperature and endogenous cycle of individual fish. To estimate the spawning season on different farms, eggs from ripe females are sampled in early winter (December-January). Each fish is weighed, placed on a hooded stretcher ventral side up, with a flow of freshwater maintained across the gills. Ovarian follicles are sampled by abdominal catheterization (Conte et al., 1988) and kept in sturgeon Ringer solution (Dettlaff et al., 1993). Thirty follicles are boiled and fixed in 10% buffered formalin for measurement by image analysis of the polarization index (PI, a ratio of the distance of the germinal vesicle from the animal pole to the oocyte animal-vegetal axis diameter) which typically ranges from 0.20 - 0.35 at this time. Under optimal temperature conditions (generally, between 10° and 14°C) the rate of GV migration is relatively constant, allowing for estimation of the expected spawning month, when the oocyte PI reaches a value $\leq$ 0.10.

A second ovarian biopsy is collected at the expected spawning month of individual females to verify the physiologically responsive stage of oocyte development. The *in vitro* oocyte maturation assays are conducted in Ringer solution at 16°C for 16 hours. Fifteen follicles with two replicates are incubated in control and with $5\mu g$/ml progesterone (4-pregnene-3,20-dione, Sigma Co.). After incubation, the eggs are boiled, chilled on wet ice, stored in 10% buffered formalin, bisected and examined for GVBD. Females that exhibit a 70-100% GVBD response are spawned in 2-4 weeks after biopsy.

Spawning Procedures

Females are injected (IM) with a priming dose of ovulation-inducing hormone (10% of the total dose), and a resolving dose 12 hours later (the hormonal treatments are described below). Ovulation is expected 20-24 hours after the second injection at the holding temperature 15-16°C. Females are anesthetized in 100 ppm MS-222 for 15 minutes, and their eggs are removed by aseptic caesarian surgery (10-12 cm incision). Anesthesia is monitored during surgery lasting 40-60 minutes by alternating water supply to the gills between fresh water and 50 ppm MS-222 solution. The incision is closed by internal and external stitches (PDS suture, Ethicon). Fish are injected with antibiotics (oxytetracycline, 5mg/kg). Primary healing of the incision occurs within 1-2 months and complete healing in 4-5 months.

Eggs are inseminated *in vitro*, de-adhered and incubated in jars (Conte et al., 1988). Post-spawned females are held in a separate recovery tank, supplied with clean, flow-through well water. Spent females start vitellogenesis during the fall of the same year and can be spawned again in 2 years.

Data collected from spawned females included: estimation of the percent of eggs released into the body cavity at caesarian section (% ovulation); latency time (hours after the second injection to the time when eggs were first observed in the spawning tank); total number of eggs collected determined volumetrically, egg diameter measured by image analysis; fertilization success measured as a proportion of cleaving eggs at 5-7 hours after fertilization from a random sample of 200 eggs; and hatching success determined as a percent of hatched larvae to total number of eggs collected.

Males are selected by external appearance (full abdomen), held at temperatures 12-14°C, and are induced to spermiate with 1.5 mg/kg CPE. Water temperature is raised to 15-16°C at the time of injection and milt is collected by catheterization 24 hours later. The males are returned to the holding temperature if they are intended for repeated spermiation. They can be induced to spermiate 3-4 times at biweekly intervals.

Spawning Results

Increasing numbers of domestic females have been available for spawning induction during the past 4 years (Table 1). The body size and age of females spawned has been similar and individual females ranged from 17-80 kg in body weight and were 6-15 years old. Latency time, mean egg diameter and number of eggs removed were also similar, but fertility and hatching success improved significantly over time. Ovulatory response to hormonal injections increased from 43% in 1992 to 76% in 1995.

The number of domestic females induced to spawn increased from 9 females in 1992 to 29 in 1995, while the hatchery spawning of wild caught females decreased from 21 to 1. Improved management of broodstocks and induced spawning have led to an increase in the number of F_2 offspring from 131,000 during 1992 to 1,667,000 in 1995.

Almost all spawned females exhibited caesarian incision healing, although a small number of fish were lost after first spawning. Two factors appear to be important in reducing post-surgery mortalities. The first is careful control of anesthesia during the surgical procedures. Secondly, it appears critical to have a dedicated recovery tank for post-spawned females.

Table 1. Spawning performance of domestic females ovulated in 1992-1995. Data are means and standard errors.

Year (N=)	Body Weight (kg)	Age (yrs)	Latency (hr)	Egg Diameter (mm)	Eggs Removed (#)	Success Fert. (%)	Success Hatch (%)
1992	33.6	10	22.9	3.39	104,712	33.3	13.9
(n=9)	2.9	0.4	2.5	0.18	10,866	10.2	6.2
1993	36.9	10	21.9	3.52	126,250	50.0	30.4
(n=18)	2.9	0.2	1.4	0.16	14,756	8.0	7.3
1994	40.2	10	22.6	3.44	128,738	74.9	31.7
(n=16)	3.4	0.5	0.8	0.18	12,070	4.7	6.4
1995	36.3	10	21.4	3.50	115,515	73.5	49.1
(n=29)	2.7	0.8	1.3	0.18	8,959	5.3	6.3

Hormonal Injection Regimes

Previously, wild-caught white sturgeon females were induced to ovulate on farms by administration of CPE which provided high rates of ovulation but variable fertilization and hatching success (Doroshov and Lutes, 1984). A greater number of fish available in 1995 allowed us to conduct preliminary evaluation of different hormonal treatments for the induction of ovulation. Females were given one of four different injection regimes (total dose): GnRHa (21-40 μg/kg), GnRHa (11μg/kg) + pimozide (10mg/kg), GnRHa (10μg/kg) + CPE (4.5mg/kg), or CPE (4.5mg/kg) only. Application of the mammalian GnRHa, alone or in combination with antidopamine pimozide, yielded higher hatchability compared to treatments with CPE, or CPE in combination with a priming dose of GnRHa (Figure 1). While ovulatory response and fertilization success were similar in different treatments, the hatchability was significantly lower in the CPE treatment ($P<0.05$). Data suggest that CPE, being a strong inducer of ovulation in sturgeon at the dose applied, may negatively affect egg quality.

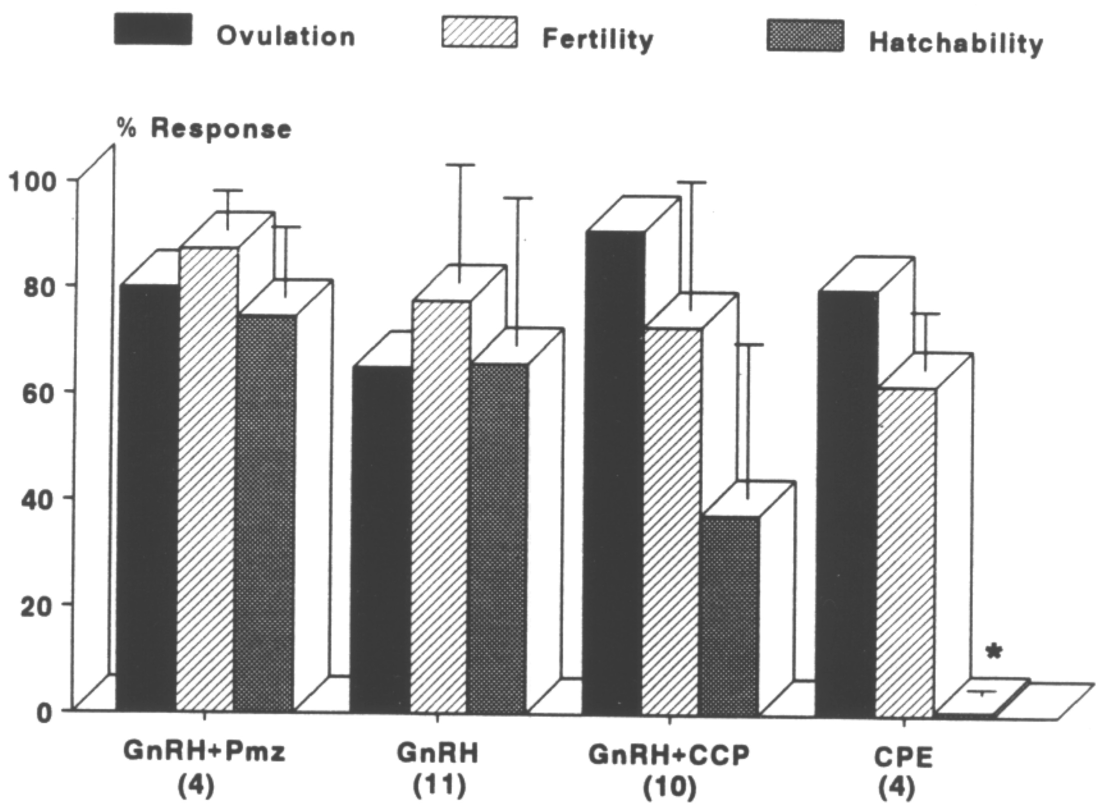

Figure 1. Ovulation response, egg fertilization and hatching success in four different hormonal treatments (pooled females spawned at UCD and collaborating farms). Females that did not release any eggs, or those with less than 50% ovulation (estimated during collection of eggs) were considered non-responsive. An asterisk denotes significantly different means ($P < 0.05$). Data (mean $\pm$ SD) were processed by the analysis of variance with arcsine-transformed proportions.

Performance of Iteroparous Domestic Female Sturgeon

Six females were repeatedly spawned a second time and four females a third time during the period 1993-1996 (Table 2). All iteroparous females exhibited regular biennial intervals between consecutive spawnings on odd or even years. The exception was a single female that spawned a second time after a 3 year interval. There were significant increases in body weight, egg diameter and number of ovulated eggs obtained by cesarian surgery between each consecutive spawning (Table 2). Number of eggs collected may not accurately reflect the trends in individual fecundity, but suggests a 65 percent increase in production of eggs at third spawning. A small but highly significant increase in egg diameter extrapolates into a 17% gain in egg volume between first and second spawning and 27% between the first and third spawning.

Table 2. Reproductive performance of iteroparous females. Data are mean $\pm$ s.e.m. Superscripts denote significant difference between means ($P<0.05$, paired T-test).

	Body Weight (kg)	Egg Diameter (mm)	Eggs Collected (th.)
First to Second Spawning			
	(n=10)	(n=10)	(n=6)*
1st	33 ± 4^A	3.36 ± 0.06^A	102 ± 9^A
2nd	40 ± 4^B	3.54 ± 0.03^B	142 ± 17^B
% increase	21	5	39
First to Third Spawning			
	(n=4)	(n=4)	(n=2)*
1st	37 ± 4^A	3.36 ± 0.08^A	113 ± 1^A
2nd	44 ± 5^B	3.53 ± 0.06^B	160 ± 5^B
3rd	51 ± 7^C	3.64 ± 0.03^C	186 ± 16^B
% increase 1-3:	38	8	65

* some data were deleted due to incomplete ovulation.

Discussion

The California sturgeon industry now relies entirely on spawning domestic broodfish. All techniques and methodologies to identify ripe females are now being transferred to the industry. During the next 2-3 years sturgeon breeders may become self sufficient in selecting and spawning white sturgeon broodstock.

The most critical aspects of broodstock care is the ability to maintain ripe fish at water temperatures $\leq 15°$ C during the spawning season. Spawning performance on the commercial farms was considerably improved in 1995 by holding gravid females at constant low temperatures (11-13°C), confirming observations of Kazanskii and Molodtsov (1973) with Russian sturgeon and Williot et al. (1991) with Siberian sturgeon.

During previous years the industry had some problems with caesarian incision healing and post-spawning mortalities, but with improved methods of using internal and external suture, the incision healing and survival of fish improved significantly. However, there are still some post-spawning mortalities. It appears that the best way to minimize these mortalities is to have a dedicated recovery tank that is used for maintaining post-surgery females for at least two months. This tank should be observed daily, have good water flow and water quality, feeding should start in about 3 days after surgery at approximately 0.3% body weight per day, and the feeding activity should be monitored.

Historically, ovulation in wild-caught white sturgeon was induced by administration of CPE (Doroshov and Lutes, 1984; Conte et al., 1988). However, these preliminary studies suggest that CPE, being a potent ovulatory stimulant for sturgeon, may negatively affect oocyte maturation (nuclear and cytoplasmic), resulting in abnormal gastrulation and variable hatching success. In contrast, the administration of GnRHa, particularly in combination with the dopamine antagonist pimozide, significantly improved survival from fertilization to hatching. Considering the small number of conducted spawning trials and variations in environmental conditions on different farms, this conclusion is preliminary, and further research for optimization of hormonal spawning induction should be conducted.

Observations on repeatedly spawned, individual females, provide the first evidence of a biennial breeding interval in domestic broodstock of white sturgeon. Iteroparous females may have significant value for breeding, because they produce a greater number of larger eggs and have a more predictable endogenous ovarian cycle, requiring minimum efforts in monitoring ovarian development. In addition, they can be used in a selective breeding program based on progeny evaluation. Their disadvantages are large body size, and difficulty in handling and spawning.

The eight years of the collaborative Broodstock Development Program has yielded new information on the reproductive development of white sturgeon and resulted in establishing a viable commercial sturgeon industry.

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