Transactions of the Illinois State Academy of Science
(2003), Volume 96, #3, pp. 229-233
received 12/30/02
accepted 5/13/03

Enhancement of Semen Production in Sauger with Human Chorionic Gonadotropin

Richard P. Bushman, Edwin J. Hansen and Randall P. Petges
Illinois Department of Natural Resources, LaSalle Fish Hatchery
2649 N. 21st. Rd., Marseilles, Illinois, 61341

ABSTRACT

In 1995, too few male sauger (*Stizostedion canadense*) were available to supply the volume of semen needed to meet the hatcheries production goals for sauger and walleye (*S. vitreum*) x sauger hybrids. Thus, in 1996 we initiated a study to determine whether injecting male sauger with human chorionic gonadotropin (hCG) at a rate of 550 IU/kg body weight (BW) would increase the production of semen. The total volume of semen produced from fish injected at a rate of 550 IU/kg was 3.3 times greater than the total volume produced from noninjected fish. There was a highly significant difference ($P<0.01$) between the mean volume of semen produced from fish injected at a rate of 550 IU/kg (3.0 ml/kg) compared with noninjected fish (1.1 ml/kg). In 2001, we compared the effect of two hCG injection rates (550 and 1,100 IU/kg BW) on semen production. The total volume of semen collected from fish injected at 550 IU/kg was 94.0 ml compared with 99.5 ml for fish injected at 1,100 IU/kg. There was no significant difference between the mean volume of semen produced from fish injected at 550 IU/kg (3.4 ml/kg) compared with fish injected at 1,100 IU/kg (4.2 ml/kg). A second injection (550 IU/kg) following the first stripping was used to maintain the volume of semen produced at a second stripping. An hCG injection rate of 550 IU/kg has been incorporated into fish production methods as a means to maximize fish production with a limited number of male sauger.

INTRODUCTION

The production of sauger (*Stizostedion canadense*) and walleye (*S. vitreum*) x sauger hybrids is a large component of annual fish production by the Illinois Department of Natural Resources. Combined total annual production of fry and fingerlings of sauger and hybrid walleye ranged from 11.1 to 22.7 million from 1996 through 2001. Male and female sauger broodfish are collected over a two day period at the end of March at the Masters Walleye Circuit Tournament held on the Illinois River at Spring Valley, Illinois. The total number of sauger weighed in at this tournament between 1996 and 2001 ranged from 814 to 1,825. However, an average of only 13.2% of the fish were male sauger. Male sauger are typically small (< 0.7 kg) and do not produce a large volume of semen. Insufficient volume of sauger semen has been a limiting factor for fish production as well

as for use in trades with other state agencies. The total volume of extended sauger semen shipped out of state increased from 180 ml in 1996 to 1,860 ml in 2001.

Hormones have been used by others to stimulate semen production and to induce ovulation in fish. Injections of an analog of luteinizing hormone releasing hormone (LHRHa) was successfully used to induce (Ciereszko et al. 1993) or increase (Dabrowski et al. 1994) semen production in yellow perch (*Perca flavescens*). Billard and Marcel (1980) used partially purified salmon gonadotropin (PPSG) and carp pituitary extract (CPE) to increase semen production in northern pike (*Esox lucius*). Human chorionic gonadotropin (hCG) can be used to induce ovulation in female walleye (Heidinger et al. 1996). Personnel of the Illinois Department of Natural Resources LaSalle Fish Hatchery have injected 97% of the female sauger spawned between 1996 and 2001 with hCG to stimulate ovulation. The goal of the present study was to evaluate the benefit of using hCG injections to enhance the volume of semen produced from male sauger broodfish. An increase in semen production from male sauger collected at fishing tournaments would make the fertilization of millions of eggs possible and provide extended semen for trade obligations with other state agencies.

METHODS

Fish collection

This research was carried out in 1996 and 2001. On March 23, 1996, a total of 26 male sauger were collected at a fishing tournament on the Illinois River, near Spring Valley, Illinois. On March 9 and 10, 2001, a total of 74 male sauger were collected at a Walleye Anglers Trail fishing tournament held at the same location. Experiments were carried out only when an adequate number of male sauger were collected prior to female broodfish collection.

In each year, the fish were transported to the LaSalle Fish Hatchery, Marseilles, Illinois, immediately following collection. At the hatchery, fish were placed in 1.1 m^3 tanks with a water temperature of 7.8 °C. The fish were held overnight prior to hormone injection.

Hormone injection

In both years, on the day after collection, fish were weighed individually and marked with a numbered jaw tag (Recreation Supply Company, Bismark, North Dakota) prior to injection. In 1996, 13 fish were given an intraperitoneal injection of 550 IU/kg body weight (BW) hCG (Chorulon; Intervet, Charlotte, North Carolina) and 13 fish were not injected. In 2001, 37 fish were given an intraperitoneal injection of hCG at 550 IU/kg and 37 fish were given the same type of injection at 1,100 IU/kg. Injections were administered with a 22 gauge hypodermic needle and 3 cc syringe. In 2001, a second hCG injection was given to each fish at 550 IU/kg five days after the first injection.

Semen collection

In each year, fish were held for five days after injection at 7.8 °C before they were hand stripped using a water powered aspirator (Moore 1996). The volume of semen from each male was measured to the nearest 0.1 ml in a graduated test tube. To ensure genetic diversity in semen collected for use in egg fertilization, semen from 3-4 fish was stripped into the same test tube. However, the volume of semen collected from each fish was

measured. Injected and noninjected fish and fish injected at different rates were separated by jaw tag number prior to stripping.

After the second injection in 2001, the fish were held seven days before they were hand stripped a second time. After collection, the semen was diluted with an extender solution provided by the Colorado Division of Wildlife at a rate of 1 part semen:3 parts extender. The extended semen was stored in 25 cm^2 tissue culture flasks at 6 ml/flask, and refrigerated at 2.2 °C for up to five days before being used to fertilize 24.2 million and 31.3 million sauger eggs in 1996 and 2001, respectively.

Data Analysis

Differences between injected and noninjected fish and between fish injected at different rates were determined using a non-paired t-test (Steel and Torrie 1980). The 0.05 alpha value was used to determine significance.

RESULTS

In 1996, the total volume of semen produced from noninjected fish was 6.5 ml compared with 21.6 ml for the injected fish. There was a highly significant difference ($P<0.01$) between the mean volume of semen produced from noninjected fish compared with fish injected at a rate of 550 IU/kg (Table 1). In each year, the mean weight was not significantly different ($P>0.05$) between injected and noninjected fish or between fish injected at different hCG rates.

In 2001, the total volume of semen produced from fish injected at 550 IU/kg was 94.0 ml and 99.5 ml from fish injected at 1,100 IU/kg . The mean volume of semen produced from fish injected at 550 IU/kg was not significantly different ($P>0.05$) from fish injected at 1,100 IU/kg. Because all fish were given a second injection of 550 IU/kg, differences in the volume of semen produced between the first and second stripping could not be statistically compared due to lack of controls. However, the average volume of semen produced at the second stripping (3.4 ml/kg) was not substantially different than the average volume produced at the first stripping (3.8 ml/kg).

DISCUSSION

In male yellow perch, injections of LHRHa was effective at inducing (Ciereszko et al. 1993) and increasing the amount of semen produced compared to controls (Dabrowski et al. 1994). Billiard and Marcel (1980) used injections of PPSG, CPE and pike pituitaries to significantly increase the amount of sperm produced from northern pike. However, Lin et al. (1996) found that injections of either CPE or hCG did not significantly increase sperm production in muskellunge (*Esox masquinongy*). In the present study, an hCG injection of 550 IU/kg significantly increased the volume of semen produced in sauger compared to noninjected fish. However, an increase in the injection rate from 550 IU/kg to 1,100 IU/kg did not result in a significant increase in the volume of semen produced. This could be due to the occupancy of the gonadotropin receptor by excess hCG. The volume of semen produced at first stripping was not substantially different than the volume produced at the second stripping.

The use of hCG and LHRHa have each been shown to have similar effects on sperm production in percid fishes. However, hCG is approved for use on fish by the Food and Drug Administration (FDA). The use of LHRHa requires an Investigational New Animal Drug (INAD) permit for legal use on fish. Also, the appropriate preparation and dosage of hCG is less expensive than LHRHa.

Billiard and Marcel (1980) reported that sperm obtained from northern pike following hormonal treatment had a fertilization capacity of 67 - 90%. The quality of semen in the present study was not quantitatively compared between injected and noninjected fish. However, prior to the use of extended semen, it was checked under a binocular microscope to ensure adequate motility. No obvious differences in motility were evident between extended semen from injected and noninjected fish. Semen collected from male sauger injected with hCG was used to fertilize a total of 55.5 million sauger eggs in 1996 and 2001 resulting in an average hatch rate of 63.2%.

ACKNOWLEDGMENTS

In 1996, the use of hCG was performed under INAD permit number 8106. hCG is currently labeled for use on fish by the FDA and marketed by Intervet as Chorulon. We would like to thank Larry Willis and Steve Shults from the Illinois Department of Natural Resources, Jake Wolf Memorial Fish Hatchery for ordering the hCG used in these experiments and filing the INAD reports. We thank the Colorado Division of Wildlife for providing the extender used in these studies. We would also like to thank Robert Summerfelt and Ron Brooks for their review of this manuscript.

LITERATURE CITED

Billiard, R. and J. Marcel. 1980. Stimulation of spermiation and induction of ovulation in pike (*Esox lucius*). Aquaculture 21:181-195.

Ciereszko, A., L. Ramseyer, and K. Dabrowski. 1993. Cryopreservation of yellow perch semen. Progressive Fish-Culturist 55:261-264.

Dabrowski, K., A. Ciereszko, L. Ramseyer, D. Culver, and P. Kestemont. 1994. Effects of hormonal treatment on induced spermiation and ovulation in the yellow perch (*Perca flavescens*) Aquaculture 120:171-180.

Heidinger, R.C., B.L. Tetzlaff, and R.C. Brooks. 1996. Hormone induced spawning of walleye. Pages 59-62 *in* R.C. Summerfelt, editor. Walleye culture manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames.

Lin, F., A. Ciereszko, and K. Dabrowski. 1996. Sperm production and cryopreservation in muskellunge after carp pituitary extract and human chorionic gonadotropin injection. Progressive Fish-Culturist 58:32-37.

Moore, A. 1996. Use of semen extenders for walleye. Pages 51-53 *in* R.C. Summerfelt, editor. Walleye culture manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames.

Steel, R.G. and J.H. Torrie. 1980. Principles and procedures of statistics, 2nd edition. McGraw-Hill, New York.

Table 1. Semen production comparison. Data from 1996 compares sauger injected with human chorionic gonadotropin (hCG) versus noninjected fish. Data from 2001 compares results from sauger injected with different doses of hCG. Asterisk * denotes semen volume was significantly larger for that treatment group ($P<0.01$).

Injection Rate (IU/kg BW)	Fish (n)	Fish Weight (kg)	Semen Volume (ml/kg)	Semen Volume (ml/fish)
		1996 Experiment		
0	13	0.48 ± 0.12	1.1 ± 0.7	0.5 ± 0.3
550	13	0.57 ± 0.13	3.0 ± 0.7*	1.7 ± 0.2*
		2001 Experiment		
550	37	0.75 ± 0.24	3.4 ± 1.5	2.5 ± 1.4
1,100	37	0.68 ± 0.19	4.2 ± 2.5	2.6 ± 1.5