

# Field Trials to Determine the Efficacy of Human Chorionic Gonadotropin for Spawning Walleye

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*Abstract:* Human chorionic gonadotropin (HCG) is often used to induce spawning of fish. However, the U.S. Food and Drug Administration (FDA) has not approved HCG for use in fish because they claim additional efficacy data are required. Efficacy of HCG for spawning walleye (*Stizostedion vitreum*), was evaluated at 3 hatcheries for 3 years. During the 1993–1995 spawning seasons, 17.5% (54 of 308) of female walleyes spawned after a single injection of HCG. Of 253 females receiving a second injection, 92.1% spawned for an overall spawning success of 93.2%. For females, a HCG dose as low as 330 IU/kg in each injection was as satisfactory as higher doses. Fish from ponds had a significantly lower spawning rate (8.7%) than wild fish (30.6%) after a single injection of HCG, but there was no significant difference in spawning success for these groups after 2 injections. Eggs spawned/kg body weight averaged  $51,065 \pm 1,379$  during 1994 and 1995, and hatching rates, based on total eggs spawned, averaged  $49.0\% \pm 2.1\%$  during these years. All of the control and HCG-injected male walleyes produced milt. This study demonstrated HCG was effective for inducing spawning; 2 injections were often beneficial for spawning female walleyes.

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Walleye is an important species for both sportfish programs and commercial aquaculture (Malison et al. 1990). Artificial propagation of walleye is used to overcome shortages in the supply of fish for stocking. Nelson et al. (1965) used carp pituitary (CP) to induce walleyes to spawn, and all 6 female walleyes used in their trial ovulated after 1 or 2 injections. Lessman (1978) tested HCG, CP, and luteinizing hormone for inducing ovulation of walleyes. He found walleyes could be induced to ovulate either by HCG alone with 2 injections or by a combination of HCG and CP. During a 2-year efficacy trial, Hearn (1980) injected pond-reared walleyes with different doses of HCG and reported HCG enhanced ovulation. Pankhurst et al. (1986) found HCG accelerated walleye oocyte development.

Human chorionic gonadotropin has been used to induce spawning of many fish species (Lam 1982, Legendre and Otémé 1995, Grizzle et al. 1995, Watanabe et al. 1995). However, HCG is not approved by the FDA for use in fish, and the FDA indicated additional efficacy data were required for approval of HCG (S.D. Vaughn, pers. commun.).

The objective of this study was to document the efficacy of HCG for spawning walleye. Field trials were conducted at 3 hatcheries for 3 years. Both spawning success and percentage of eggs that hatched were considered in this study.

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## Methods

Efficacy trials for HCG were conducted at 3 hatcheries during 1993–1995 (Table 1). Experimental protocols were approved by the FDA, and these field trials were conducted under investigational new animal drug (INAD) exemptions INAD 8860 and INAD 9115. A total of 330 female and 102 male walleyes were used for the trials. Fish were from ponds and wild stocks (Table 1). Walleyes from ponds had originally been captured from rivers or lakes and were held for at least 1 year in hatchery ponds. After wild fish were transported to hatcheries or hatchery fish were removed from ponds, they were weighed and injected with HCG or an equivalent volume of saline. During the 1994 and 1995 trials, about 10% of all the fish were chosen to serve as controls by designating every tenth fish as a control. Dose of HCG in each injection ranged from 264 to 3,667 IU/kg for females and from 160 to 1,833 IU/kg for males. Males were injected with HCG at 1 or 2 of the hatcheries each year, but most males were not injected. After 3–4 days, a second injection of HCG, at the same dosage as

**Table 1.** Hatcheries and broodfish sources for field trials of HCG for spawning walleyes, 1993–1995.

Investigator and hatchery	Broodfish source
James M. Smith Eagle Bend Fish Hatchery Clinton, Tennessee	ponds, Norris Lake, and Dale Hollow Reservoir
Michael C. Hearn Minor Clark Fish Hatchery Morehead, Kentucky	ponds, Carr Fork Lake, Paintsville Lake, Nolin Lake, Licking River, and Laurel Lake
Mallory G. Martin and William E. Wilson Table Rock Fish Hatchery Morgantown, North Carolina	ponds and Catawba River

the first injection, was given to females that did not spawn or have oocytes near maturation. Some females were given third and fourth injections, but only results after 2 injections were considered in this study because the number of fish injected more than twice was insufficient to evaluate the effectiveness of additional injections.

When oocytes ovulated, eggs and milt were stripped. Egg samples were taken to determine the number of eggs spawned, and eggs were transferred to hatching jars. After hatching, larvae in each jar were counted, and hatching rates were calculated by dividing the number of larvae by the number of eggs spawned. Numbers of eggs and hatching rates were determined during 1994 and 1995.

The HCG used in 1993 was from Crescent Research Chemicals (Phoenix, Ariz.). That used in 1994 and 1995 was Chorulon® (Intervet, Millsboro, Del.), and saline injected into controls was the diluent for Chorulon®.

Spawning success of different groups of fish was compared by Chi-square analysis (SAS Inst. 1989). Effects of HCG dose on spawning, number of eggs spawned, and hatching rates were determined by analysis of variance. Probabilities  $\leq 0.05$  were considered statistically significant.

## Results and Discussion

For all 3 years, spawning rates for female walleye averaged 17.5% and ranged from 13.0% to 22.3% after a single injection of HCG (Table 2). A second injection of HCG was given to 253 females that did not spawn after 1 injection; 92.1% spawned, with yearly averages from 88.6% to 96.0%. Including fish spawning after either 1 or 2 injections of HCG, a total of 93.2% of female walleyes spawned; yearly averages ranged from 91.1 to 95.7%. For control females, spawning success totaled 18.2% after 2 injections. After 1 injection, there was no significant difference in spawning rate of HCG-injected and control females, but after 2 injections, significantly more HCG-treated females had spawned. Barry et al. (1995) reported a single injection of

**Table 2.** Percentage of female walleyes spawning at 3 hatcheries (*N* in parentheses) following injection with HCG. Fish were given a second injection of HCG if they did not spawn after the first injection.

Injection	1993 <sup>a</sup>	1994	1995
One injection			
Control		18.2 (11)	9.1 (11)
HCG	17.3 (81)	13.0 (115)	22.3 (112)
Two injections			
Control		0.0 (9)	11.1 (9)
HCG	91.0 (67)	96.0 (99)	88.6 (87)
Total spawning			
Control		18.2 (11)	18.2 (11)
HCG	92.6 (81)	95.7 (115)	91.1 (112)

<sup>a</sup>No control fish used in 1993.

HCG (500 IU/kg) could induce oocyte maturation and ovulation in walleyes captured from the wild 2 to 3 weeks prior to the normal spawning season. However, the spawning success was low in their small-scale experiment; spawning rates were 40% and 25% for trials in 2 years.

The percentage of females spawning after 1 injection of HCG and the percentage of control females spawning varied among hatcheries (Table 3). Overall, the percentage of females spawning after 1 injection was low, similar to the percentage of control fish spawning; however, at Eagle Bend Fish Hatchery in 1995, all females spawned after 1 injection of HCG. This indicates that 1 injection of HCG can sometimes be sufficient to induce ovulation. Lack of standardization among hatcheries, especially regarding temperature and stage of egg development when fish were brought into the hatchery, probably accounted for the variation in spawning after 1 injection.

For females receiving 1 or 2 injections of HCG, there was no significant effect of dose on spawning success. A dose as low as 330 IU/kg was as satisfactory as higher doses. Hearn (1980) found 220 IU/kg resulted in ovulation rates similar to those after higher doses ranging from 440 to 1,540 IU/kg.

Walleye brood fish are generally obtained from 2 sources, ponds and wild stocks. The relative importance of fish from ponds depends on availability of wild fish suitable for spawning. With a single injection of HCG, spawning success for wild females (30.6%) was significantly higher than for fish kept in ponds (8.7%). This difference was consistent during all 3 years of this study. However, there was no significant difference in spawning success after 2 injections.

All of the control and HCG-injected male walleyes produced sperm. Only the production of milt was considered during this study, and the usefulness of HCG in male walleye was not established based on this criterion. However, other factors such as milt volume and viscosity, sperm motility, and fertility rate should be considered before concluding that HCG has no value as a spawning aid for males.

The average number of eggs/kg body weight was  $50,986 \pm 1,169$  and  $51,197 \pm$

**Table 3.** Percentage of female walleyes spawning at 3 hatcheries during 1993–1995 (*N* in parentheses) following injection with HCG. Fish were given a second injection of HCG if they did not spawn after the first injection.

Injection	Hatchery		
	Minor Clark	Table Rock	Eagle Bend
One injection			
Control	0 (13)	25.0 (4)	40.0 (5)
HCG	4.9 (183)	11.4 (44)	49.4 (81)
Two injections			
Control	0 (13)	33.3 (3)	0 (3)
HCG	91.3 (173)	87.2 (39)	97.6 (41)
Total spawning			
Control	0 (13)	50.0 (4)	40.0 (5)
HCG	91.8 (183)	88.6 (44)	98.8 (81)

2,378 in 1994 and 1995, respectively, and hatching rates during these years averaged  $49.3\% \pm 2.3\%$  and  $48.6\% \pm 4.1\%$ , respectively. The number of eggs/kg produced was typical for walleye (Stickney 1986). These data were obtained to determine if injection of HCG had adverse effects on egg numbers or hatching rates; however, the low number of spawns from control fish prevented a statistical comparison between HCG-injected fish and controls. There was no significant effect of HCG dose on number of eggs spawned or hatching rates.

This study demonstrated HCG is effective for inducing spawning of female walleye. Although some species of fish, such as striped bass (*Morone saxatilis*), can be successfully spawned with 1 injection of HCG (Grizzle et al. 1995), 2 injections are often beneficial for spawning female walleye. In addition to demonstration of efficacy, which has been accomplished by several studies, data required for approval of HCG by the FDA are target animal safety, human food safety, environmental assessment, and manufacturing information.

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