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USE OF HUMAN CHORIONIC GONADOTROPIN (HCG) TO PROMOTE GAMETIC PRODUCTION IN MALE AND FEMALE LARGEMOUTH BASS[1]

by

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ABSTRACT

Fifty male and 106 female largemouth bass were injected with human chorionic gonadotropin (HCG) during the 1972 and 1973 spawning seasons. Milt production was increased or maintained in 80% of the males tested, and 63% of the females ovulated. Females with spent or immature gonads did not noticeably respond to HCG injections. Females tested during the latter half of both spawning seasons demonstrated lower percentages of successful ovulations and reduced numbers of eggs per ovulation. Ninety percent of the ovulated females required only one injection, whereas nearly half of the females that resorbed their eggs required two injections before resorption could be determined. Results indicate that some females can be ovulated twice or three times with multiple injections, but that the success rate is too low to enable practical application. Most females ovulated within 48 hours of injection. Ovulated eggs, if not stripped and fertilized, became inviable within 12 to 16 hours of ovulation.

INTRODUCTION

A sharp increase among anglers pursuing trophy largemouth (*Micropterus salmoides*) in the southeast has stimulated interest in hybridization and selective breeding as a means of enhancing both maximum potential growth and rate of growth. A prerequisite to selective breeding is that single females can be bred to many test males or conversely that one male can be bred to many test females. In that this would be practically impossible to carry out in pond culture, laboratory production of progenies is necessary. Ovulation of female largemouth must be induced and synchronized with spermatogenesis of her male partners. This is complicated by apparent blockage of reproductive pathways in largemouth bass removed from their natural habitats into laboratory containment.

Refining such a technique by over-riding this blockage was the first hurdle overcome in a selective breeding study presently being investigated by the Florida Game and Fresh Water Fish Commission. Although photoperiod and temperature controls have been successfully used to control gonadal recrudescence in a variety of fishes (Wiebe, 1968; Carlson, 1973; Henderson, 1963; McInerney and Evans, 1970), hormonal injection was considered to have superior potential in that there was no need for expensive temperature control equipment and fish holding time would be less.

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Although Haydock (1971), Stevens (1966), Clements and Snead (1962) and Atz and Pickford (1959) experimented with hormonal inducement of ovulation in fishes, particular application was found in the work of Stevens (1969). He reported that leuteurizing hormone (LH) was the primary hormone influencing ovulation in largemouth bass. While follicle-stimulating hormone (FSH) and thyroid stimulating hormone would produce ovulation, he ascribed this to a LH contamination of these preparations.

MATERIALS AND METHODS

Human chorionic gonadotropin (HCG), probably due to its LH activity, was reported by Stevens to produce ovulation of largemouth bass. Because it was cheapest and the most easily obtained it was selected for use in our work. Stevens reported that an injection of 4000 IU of HCG/Kg body weight was the threshold level for successful stimulation of ovulation with a single injection.

Male and female largemouth bass from several nearby lakes were collected with an electrical 220-volt shocking apparatus. These fish were held indoors in galvanized (inside coated with fiberglass resin) 6x2x2-ft water troughs set up for a constant flow of water. No more than three fish were held in a tank at any given time. Overhead fluorescent lights were on 24 hours a day, and water temperatures checked each day during the course of the study varied between 69 and 72° F.

Fish were held several days to a week before receiving an injection. A 2.5 cc tuberculin syringe with a 25-gauge needle was used. Stevens used a standard 0.5 cc injection volume, but for dilution simplicity we used a standard 1.0 cc volume containing 4000 IU of HCG/Kg body weight. The needle was inserted in the musculature below the first dorsal spine and 15 seconds were given to performing the injection. Another 15 seconds were allowed before removal of the needle. During the entire process, pressure was applied with an index finger over the point of needle insertion to prevent escape of the injected material.

Oocytes taken with a catheter tube were checked for maturity level at, and following, injection. This technique, reported Stevens (1969), "was based upon the fact that during the final maturation of fish oocytes just prior to ovulation, multiple oil globules appear and gradually coalesce to form one large globule. This globule then changes shape from oval to round and seems to rise from the interior of the egg to the surface." These changes could be readily seen through a dissecting microscope.

Stevens divided this maturation process into six, somewhat arbitrarily defined stages. Most fish were in Stage 1 at the time of injection, while Stage 6 was the last stage before ovulation. If the fish had not advanced at least one stage during the 24 hours following injection, a second injection was usually administered. Oocytes were sampled about every 8 hours (more frequently as ovulation approached) beginning 24 hours after injection.

Males were given injections of HCG at dosages of 4000 and 8000 IU/Kg. They were checked at injection for milt production by standard stripping techniques. Production was rated as none, slight (a few drops maximum), fair (about 4 to 6 drops), or good. Similar checks were conducted every 24 hours after injection to determine if milt production had measurably increased, decreased, or remained unchanged.

Test crosses to determine the viability of male and female sex products usually were not conducted during the 1972 spawning season, but were conducted in most instances during the 1973 spawning season. This consisted of stripping ovulated eggs into watch glasses and fertilizing the eggs with milt, collected by an eye dropper from the male's vent.

RESULTS

During the 1972 spawning season, 47 female largemouth bass were tested with HCG injections and 59 were tested during the 1973 season. Fish not ovulating typically

underwent what Stevens referred to as "oolysis" or "resorption." This condition was characterized first by a weakening of the oolema, which later burst to liberate egg contents. At this time, a fluid-like material containing some resorbing eggs, broken egg shells, and free oil globules could be stripped. Excluding females which had spent or immature gonads or that died following injection, 49% of the females tested in 1972 were ovulated and 75% of the females in 1973. This increase was primarily due to the testing of a greater number of fish early in the 1973 spawning season. Likewise, Stevens' 82% successful ovulations among the females he tested resulted from working with bass early in the spawning season.

Spawning activity had a significant effect on induced ovulation success. Although the extent of all spawning activity in nearby lakes was not always known, the first major spawn was usually easily documented. Its beginning was characterized by the sudden appearance of spawning pairs in daily shocking samples, and its synchorization with the first warming trend in early spring (usually late February or early March). It generally lasted four or five days after which virtually all females collected displayed significantly reduced gonad weights to body weights and many gonads had scattered unspawned ovulated eggs. Spawning activity was extremely low (perhaps nonexistant) for the next few weeks after which time intermittent spawning continued for about a month. At this time virtually all males and females collected had spent gonads.

Data in Table 1 demonstrates that the first half of the spawning season produced much higher percentages of successful ovulations than did the second half. Bass collected shortly after spawning could not be induced to ovulate with injections of HCG. After a recuperatory period of a week or two, ovulations could again be induced, although the percent of successful ovulations seemed to be held down by continued intermittent spawning throughout the remainder of the season.

It is also evident from Table 1 data that the average number of eggs ovulated in the first half of the spawning season greatly exceeded the last half. There also was a high correlation between the average number of eggs ovulated and percent hatch, or egg viability was greater during ovulations induced early in the spawning season.

Table 1. Data pertaining to success of induced-ovulation attempts with largemouth bass during the 1972-1973 spawning seasons.

	1972 Spawning Season				1973 Spawning Season			
	No. F. Inject.	Percent Ovulated	Avg. No. Eggs/Kg	Avg. % Hatch	No. F. Inject.	Percent Ovulated	Avg. No. Eggs/Kg	Avg. % Hatch
Jan. 15-31					8	63	2183	64
Feb. 1-14					7	100	2633	36[2]
Feb. 15-28	First major spawn: Feb. 22-27				7	86	1065	55
Mar. 1-15	9	0	--	--	11	73	293	29
Mar. 16-31	25	76	475	--	First major spawn: Mar. 15-20			
					8	0	--	--
Apr. 1-15	End of spawning: Apr. 1-7							
	5	0	--	--	7	29	408	53
Apr. 15-30	3	0	--	--	End of spawning: April 20-25			
					6	33	999	57
May 1-15					3	0	--	--

[2]Problems with aquarium heaters used caused water temperatures among these hatch-groups to exceed 85° to 90° F in some cases, and probably influenced low hatching success.

These results indicate that ovulation and spawning blocks successful inducement of ovulation of the remaining oocytes for a week or two. However, Stevens reported that 10 females in which he had induced ovulation were ovulated a second or third time with reinjections administered from 0 to 168 hours following initial ovulation. We attempted similar tests and found that of 7 female bass reinjected from 0 to 244 hours following initial ovulation, only 1 ovulated a second time.

Procedural differences seem to explain the difference in results. We found that females very forcefully stripped of all eggs immediately following initial ovulation would continue to produce additional stripable eggs at 12 hour checks up to as much as 48 hours following the initial ovulation. This was much more common among induced ovulations early in the spawning season. Whether these ovulations would be called separate multiple ovulations or a single ovulatory period composed of "mini-ovulations" is probably only a question of symantics. Stevens did not observe this, however, as he stripped only a small portion of the ovulated eggs to test viability. Consequently, whether the second ovulations reported by Stevens were sometimes carryovers of the initial ovulation is an unresolved possibility.

Regardless, double ovulations without some sort of natural recuperatory period as a practical tool to increase fry production does not appear to have merit at this point. Hatching success of second or third ovulations was generally low in both studies with Stevens reporting 0% hatch in 6 of the 12 double ovulations obtained.

Seven females were injected during 1972 testing at 6000 IU HCG/Kg body weight rather than at 4000 IU. All seven females ovulated within 48 hours, but other females of the same group had a correspondingly high ovulation percentage at 4000 IU. It was evident from this and from Stevens' work that 4000 IU was adequate, and further work with other dosages was not attempted.

The latent period between injection and ovulation was examined in Table 2. Most (47%) bass were found to ovulate between 36 and 48 hours following injection, while 35% ovulated between 24 and 36 hours. Of the 18% ovulating after 48 hours, most had required a second injection to stimulate ovulation. However, most (90%) of the 18 females requiring two injections toward their initial ovulation did not ovulate but resorbed their eggs. Consequently, repeat or double injections are probably a waste of time and hormone if only 10% can be expected to ovulate.

Five females had sample portions of ova stripped at ovulation and at 4-hour intervals thereafter. Hatching percentages from the resulting test crosses are shown in Table 3. These data indicate that viability is greatly reduced at 12 hours following ovulation and is nil at 16 hours. This is partially substantiated by observations made by Glen Lau in filming largemouth bass spawning at Silver Springs, Florida. He indicated (personal communications)[3] that spawning acts of most pairs early in the season was carried out over a 6 to 9 hour period. However, multiple "mini-ovulations" within an ovulatory period could have unrealistically extended the determined period of egg viability, so that eggs actually were viable for less than 12 hours. However, extended ovulatory periods were more common early in the spawning season, whereas these experiments were conducted on females late in the 1973 spawning season.

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Table 2. Latent periods between injection and ovulation of female bass induced to ovulate in the 1972-1973 spawning seasons.

Hrs. Between Injection and Ovulation	No. Requiring 2 Injections	No. Females Ovulating
24 Hrs	0	0
24-36 Hrs	0	17
36-48 Hrs	0	23
48-60 Hrs	3	5
60-72 Hrs	1	3
72 Hrs	1	1
Total	5	49

Table 3. Hatching percentages for test crosses made at 4-hour intervals following ovulation.

Test Cross	at Ovulation	at-4 hrs	at-8 hrs	at-12 hrs	at-16 hrs
1	73	--	68	--	--
2	68	68	61	26	--
3	84	80	--	40	0
4	63	44	--	24	0
5	53	--	--	--	0

Although Stevens had not injected male largemouth bass, Turner (1961) indicated that HCG causes release of spermatazoa in amphibians. Consequently it was tested on male largemouth bass. During t e 1972 spawning season, 7 male largemouth bass were injected, and 43 males were injected during the 1973 spawning season. Results of this work are summarized in Table 4. Increased milt production within 72 hours following the first HCG injection was noted in 70% of 50 males tested. Decreased production occurred in 6%, and a second injection given to one of these failed to reverse this direction. Seven males (14%) were producing no milt flow at injection and produced no detectable flow following injection. Two of these seven were given a second injection, and milt production was initiated in one but not the other. Five males (10%) were flowing at their first injection, but did not increase their flow following injection. However, four of these males were already flowing at a maximum level ("good") and increased flow could not have been detected.

Table 4. Milt production of largemouth bass at the following injections of HCG.

1st INJECTION		MILT PRODUCTION AT			2nd INJECTION	MILT PRODUCTION AT		
Date	IU/Kg	Inject.	48 hrs	72 hrs	IU/Kg	Inject.	48 hrs	72 hrs
1972								
3/2	4000	None	Poor	Fair	4000	Poor	Fair	Good
3/3	4000	None	Poor	Poor				
3/3	4000	None	None	Poor	4000	Poor	Fair	Good
3/3	4000	None	Fair	Fair				
3/13	4000	None	Good	Good				
3/13	4000	None	Good	Good				
3/25	4000	None	Fair	Good				
1973								
1/2	4000	None	Slight					
1/2	4000	None	None					
1/23	4000	None	Good	Good	4000	Good	Good	Good
1/23	4000	None	Fair	Fair				
1/24	4000	Slight	Good					
1/24	4000	None	Fair					
2/1	4000	None	None	Slight	8000	Slight	Good	Good
2/1	4000	None	None	None	8000	Died		
2/11	4000	None	Good		4000	Good	Good	Good
2/11	4000	None	None	None	8000	None	Good	Good
2/11	4000	None	Slight		8000	Died		
2/11	4000	None	None					
2/11	4000	None	Slight		8000	Slight	Good	
2/11	4000	None		Slight				
2/15	8000	None	None	None				
2/15	8000	None	Good	Good				

2/15	8000	None	None	Died				
2/27	8000	Slight	None	Slight	8000	Slight	Slight	
2/27	8000	Fair	Good		4000	Good	Good	
2/27	8000	Slight	None	None	8000	None	None	None
2/27	8000	None	None	None	8000	None	None	
3/5	8000	Slight	Good		4000	Good	Good	Good
3/5	4000	Slight	Fair	Fair	8000	Fair	Fair	Fair
3/5	4000	Slight	None	None	8000	Died		
3/5	8000	Slight	None	None	8000	Died		
3/9	8000	None	Fair	Fair	8000	Fair	Fair	Fair
3/9	8000	None	Fair	Fair	8000	Fair	Fair	Fair
3/16	8000	Fair	Good	Good	8000	Good	Good	
3/24	8000	Good	Good	Good				
3/24	8000	None	Slight	Fair	8000	Died		
3/24	8000	Good	Good	Good				
3/31	8000	Fair	Good	Good				
3/31	8000	Fair	Good	Good	8000	Good	Good	Good
4/16	8000	Fair	Good	Good	8000	Good	Good	Good
4/16	8000	Fair	Good	Good	4000	Good	Good	Good
4/24	8000	Fair	Good	Good	4000	Good	Good	Good
4/24	8000	Poor	Fair	Fair				
4/24	8000	Good	Good	Good				
4/24	8000	Good	Good	Good				
5/11	4000	Fair	Good	Good				
5/11	4000	Fair	Good	Good				
5/11	4000	Fair	Good	Good				

Injections of 4000 and 8000 IU/Kg were tested, but neither dosage produced a clearly greater degree of success than the other. Second injections were sometimes effective at stimulating increased milt production and were always effective at maintaining at least the flow present upon injection.

Aside from the males and females tested during the 1972 and 1973 spawning season, 15 spent females were routinely injected in May immediately following the 1973 spawning season. No ovarian response towards ovulation was detected. In September and October additional male and females were tested using prolonged series of low concentration injections to determine if gonadal recrudescence could be induced prior to normal spawning. Three fish were tested in each of three injection regimes:

1. Eight injections over an 18 day period increasing gradually from 200 to 800 IU/Kg.
2. Twelve injections over a 28 day period increasing gradually from 200 to 600 IU/Kg.
3. Fifteen injections over a 32 day period increasing gradually from 200 to 700 IU/Kg.

At the end of this period fish were autopsied. Gonadal condition of 6 females and 3 males tested was spent and similar in appearance and weight to fresh specimens from the field.

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