Asian Fisheries Science 7(1994):225-231.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1994.7.4.004

Use of Human Chorionic Gonadotrophin (hCG) for Induced Spawning in Tilapia Under Laboratory Conditions

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Abstract

A study was conducted to determine the optimum dosage of Chorulon®, a commercial preparation of human chorionic gonadotrophin (hCG), for increasing spawning rates in the Nile tilapia, *Oreochromis niloticus*, under laboratory conditions. Dosages of 500-3,500 IU·kg^{-1} body weight hCG were injected intramuscularly into sexually-mature females reared in fiberglass aquaria. Spermiating males were provided at 1:1 sex ratio. The number of ovulated and mouthbrooding females were determined 24 h after injection. The eggs from ovulated females were stripped and fertilized artificially while those from mouthbrooding females were removed from the oral cavity of the fish. All eggs were transferred to individual zuger jars for determination of pigmentation rates as an indicator of fertilization rates. Results showed that dosages of 2,000-3,500 IU·kg^{-1} hCG were effective in significantly increasing the percentage of females either ovulating or mouthbrooding 24 h after injection (25-75%), with pigmentation rates ranging from 36 to 83%. The optimum dosage of the hormone was 3,500 IU·kg^{-1}.

Introduction

Induced spawning in tilapia is important for chromosome manipulation work, where predictable ovulation is a prerequisite to permit artificial fertilization of gametes. The success of various efforts to improve the yield of gynogenetic fish has depended partly on the availability of a large number of females that may be stripped to provide gametes at any one time.

Spawning induction in tilapia was attempted by various authors using carp pituitary extract (Dadzie 1970), human chorionic gonadotrophin (hCG) (Itzkovich 1981; Srisakultiew and Wee 1988; Garcia et al., in press) and gonadotrophin-releasing hormones administered alone or in combination with dopamine antagonists (Gissis et al. 1991; Garcia et al., in press). Previous

studies on the induction of spawning in *Oreochromis niloticus* using hCG were generally unsuccessful (Srisakultiew and Wee 1988; Garcia et al., in press).

Chorulon®, a commercial preparation of hCG, is being used in the standardized induced breeding procedure for the African catfish, *Clarias lazera* (Eding et al. 1982). It was of interest to evaluate its effectivity in some other freshwater fish of commercial importance, such as tilapia. The use of hCG as an induced spawning agent in tilapia may not be directed primarily towards commercial production of fry, but for the provision of a reliable supply of eggs for research on chromosome manipulation, for synchronization of spawning to mass produce fry of the same age, and for experimental purposes.

This study aims to determine the optimum dosage of hCG that could lead to increasing the incidence of ovulation and spawning among female *O. niloticus* held in aquaria.

Materials and Methods

Hormone Composition

The hormone used in this study (Chorulon®) is a commercial preparation of hCG (Intervet, the Netherlands). Chorulon® comes as a white, lyophilized crystalline plug containing 5,000 international units (IU) per vial. Each vial of freeze-dried hCG is supplied with 5 ml of solvent containing phosphate-buffered water.

Experimental Conditions

All experiments were conducted in sixteen 122 x 46 x 46 cm fiberglass aquaria provided with a 5-8 cm gravel bed and adequate aeration. Temperature ranged from 29 to 30°C.

The fish used belong to a Philippine strain of *O. niloticus* derived from a number of strains imported into the Philippines from Israel and other Asian countries, and were 8-10 months old. These were obtained from the stock maintenance program of the FAC/CLSU-UCS Project on Genetic Manipulations for Improved Tilapia at the Freshwater Aquaculture Center (FAC) of Central Luzon State University (CLSU). Prior to stocking in aquaria, each female was selected from a pool of broodstock based on the presence of fully-yolked oocytes, using the ovarian biopsy procedure (Garcia et al., in press). Each female was conditioned in their respective aquaria for 1 d before the experiment commenced. Spermiating males of similar size were provided at 1:1 sex ratio immediately after injection. Spawning success was determined 24 h after injection by summing the total number of mouthbrooding and ovulated females. Mouthbrooding females were those incubating eggs in their mouth, while ovulated females were those releasing fertilizable eggs upon gentle abdominal pressure. The position of the germinal vesicle was not used as a basis for ovulation considering the extreme difficulty of visualizing the germinal nucleus in tilapia eggs. Among ovulated females, the stripped eggs were fertilized using the

sperm of their respective male partners. All eggs collected were incubated artificially in upwelling zuger jars connected to a continuous water source. Pigmentation rates, the proportion of eggs with pigment cells scattered over the yolk sac (Galman 1980), were determined 2 d after spawning as an indicator of fertilization rates. Two separate experiments were performed according to the following designs:

EXPERIMENT 1

Dosages of 500-3,500 IU·kg^{-1} with 500 IU increments of hCG were administered to the test fish using single, intramuscular injections, with the control fish receiving 0.05 ml of solvent (Table 1). For each dosage tested, control fish were always paired with hormone-treated fish. The different treatments were evaluated over a 1-month period, with 84 females receiving hCG treatments (Table 1), and 28 females used as controls.

EXPERIMENT 2

This study was conducted to assess the potency of 3,500 IU·kg^{-1} hCG using a larger number of females than in Experiment 1. Five trials were conducted over 15 d, utilizing 80 females which were equally divided into treated and control groups, receiving 3,500 IU·kg^{-1} and 0.05 ml of solvent, respectively.

Data Analysis

The percent spawning success observed after injection of each hormone dosage was compared to the untreated control using the chi-square (χ^2) contingency test (Strickberger 1976). The differences in the number of ovulating and mouthbrooding females as well as their respective pigmentation rates were compared using paired samples t-test analysis. The degree of relationship between hormone dosage and spawning success were determined using simple correlation analysis.

Results and Discussion

Table 1 summarizes the results obtained from Experiments 1 and 2. In Experiment 1, the number of females which ovulated was not significantly different from the number that spawned for all the dosages used ($t=1.18$, $P=0.3$). Differences in pigmentation rates were also insignificant ($t=2.51$, $P=0.1$). Thus, the number of females which ovulated or spawned were pooled and represented as spawning success in Table 1.

Results of Experiment 1 showed that dosages of 2,000-3,500 IU·kg^{-1} can significantly increase the proportion of females either mouthbrooding or ovulating 24 h after injection. There is a positive relationship ($r=0.91$, $P=0.03$) between hormone dosage and spawning success (Fig. 1). Pigmentation rates for artificially and naturally fertilized eggs ranged from 36 to 83% (Fig. 2). In the control

Table 1. Number of ovulated and spawning females following injection with varying dosages of hCG for Experiments 1 and 2.

Dosage ($IU \cdot kg^{-1}$)	Injected females	Body weight ±SD (g)	Egg size ±SD (mm)[2]	Ovulated females	Pigmentation rate ±SD (%)	Mouthbrooding females	Pigmentation rate ±SD (%)	Spawning success (%)	Contingency χ^2
Experiment 1. Optimization of hormone dosage									
Control	28	63.4 ± 17.8	2.0 ± 0.2	0	-	0	-	0	0
500	8	67.8 ± 11.3	2.1 ± 0.2	0	-	0	-	0	0
1,000	8	67.1 ± 8.4	2.0 ± 0.3	0	-	0	-	0	0
1,500	20	68.8 ± 27.0	2.0 ± 0.2	1	29	1	39	10.0	0.95^{ns}
2,000	12	59.4 ± 21.7	2.0 ± 0.2	1	36	2	71 ± 9.9	25.0	4.39*
2,500	20	63.7 ± 24.0	2.0 ± 0.2	3	42 ± 14.0	2	65 ± 12.7	25.0	5.36*
3,000	8	66.2 ± 28.0	2.2 ± 0.1	2	75 ± 8.5	1	80	37.5	7.07*
3,500	8	56.8 ± 16.0	2.0 ± 0.1	4	80 ± 6.1	2	83 ± 5.7	75.0	20.1***
Experiment 2. Potency of 3,500 $IU \cdot kg^{-1}$									
Control	40	68.9 ± 24.0	2.0 ± 0.2	0	0	0	0	0	0
3,500	40	58.7 ± 14.6	2.1 ± 0.3	20	78.5 ± 8.0	6	74 ± 6.8	65	35.61***

ns=not significant; *=significant ($P<0.05$); ***=highly significant ($P<0.001$).

[1]Spawning success refers to the number of ovulated females plus number of mouthbrooding females as a percentage of treated females.

[2]Determined after ovarian biopsy which involves sampling of intraovarian eggs using a cathether inserted into the urogenital pore of the fish.

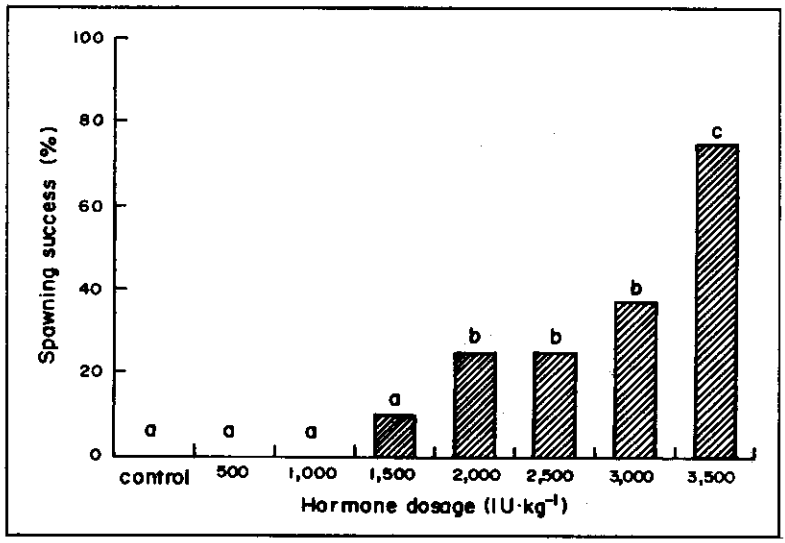

Fig. 1. Spawning success of tilapia injected with 500-3,500 IU·kg^{-1} hCG. Bars annotated with different letters are significantly different ($P<0.05$).

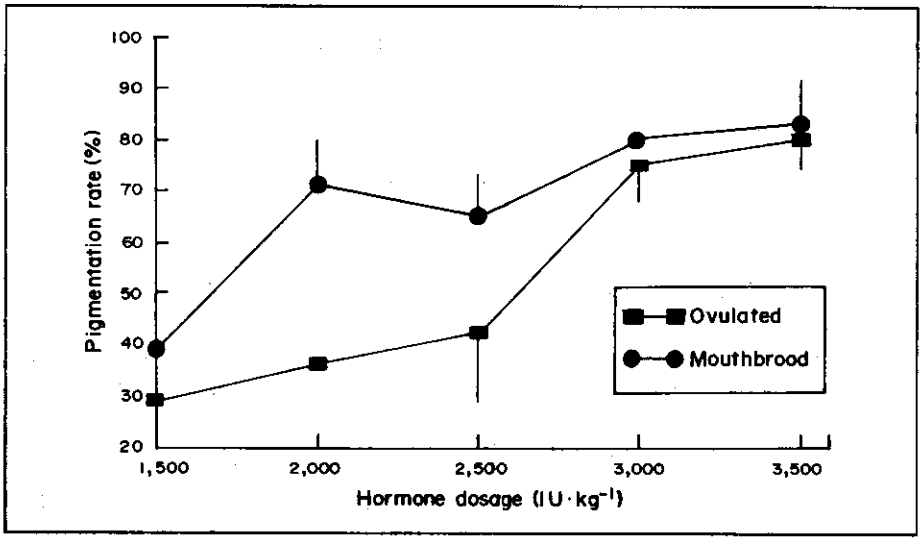

Fig. 2. Pigmentation rates in eggs 2 d after collection from ovulated or spawned females following injection of 1,500-3,500 IU·kg^{-1} hCG.

group, no females manifested any signs of ovulation or spawning, probably due to the lack of environmental and physiological cues that would trigger final oocyte maturation.

In Experiment 2, the difference in spawning success between control and treated groups was highly significant ($\chi^2=35.61$; $P<0.001$). Pigmentation rates in the treated group ranged from 74 to 78%.

Dadzie (1970) administered 250 IU·kg^{-1} hCG to *O. aureus* females and observed 100% spawning success 24 h after injection. However, with the small number of fish used (n=3) as well as the lack of information on the selection criteria of breeders prior to injection, it is difficult to compare his results with

our present findings. Itzkovich (1981) found dosages of 250, 500 and 5,000 IU·kg^{-1} hCG to be ineffective for the induction of spawning in *O. aureus*. Srisakultiew and Wee (1988) obtained partial success in *O. niloticus* using 250 and 500 IU·kg^{-1} hCG. In most of their experiments, natural spawning in the untreated group was equal or even greater than the hormone-treated group. More recently, Garcia et al. (in press) observed no significant effect of hCG treatment at 400 IU·kg^{-1} using randomly-selected females, compared to the control group. Previous results, although not directly comparable, imply that the absence of any significant spawning response in the treated groups must have been due to sub-optimal dosage of hCG used.

In gilthead sea bream, *Sparus aurata*, a single injection of hCG at 150 and 200 IU·kg^{-1} body weight induced 86% of the females to undergo final oocyte maturation and ovulation (Zohar et al. 1989). The most effective dosage observed from the present study is within the recommended hormone level for induction of ovulation or spawning in the African catfish (4,000 IU·kg^{-1}; Eding et al. 1982), Japanese flounder (2,600-8,400 IU·kg^{-1}; Hirose et al. 1979), silver carp (2,750 IU·kg^{-1}; Burlakov and Khapchaeva 1983) and rabbitfish (2,000 IU·kg^{-1}; Ayson 1991); but much greater in snapper (1,000 IU·kg^{-1}; Pankhurst and Carragher 1992), gilthead sea bream (400 IU·kg^{-1}; Eckstein et al. 1978; Gordin and Zohar 1978; Zohar and Gordin 1979) and grouper (600-1,000 IU·kg^{-1}; Tseng and Poon 1983). However, the dose is significantly less than that recommended by Kuo (1975) for mullet (60,000 IU·kg^{-1}).

Conclusions

HCG is a potential induced spawning agent in tilapia particularly when artificial fertilization of gametes is required, as is the case in chromosome manipulation studies. The effective dosages of the hormone ranges from 2,000 to 3,500 IU·kg^{-1} which could lead to 25-75% spawning success at a spawning latency time of 24 h. Pigmentation rates among ovulated and mouthbrooding females ranged from 58±22% and 75±8%, respectively.

It would be interesting to find out the spawning response of mature tilapia females to much higher dosages of 4,000-5,000 IU·kg^{-1} in order to verify the increasing trend of both spawning success and pigmentation rates observed in this study. The use of Chorulon® may find valuable application for research on chromosome manipulation where timed and predictable ovulation of the test fish is a prime requirement.

Acknowledgements

The authors thank Eduardo Saliganan for technical assistance. This paper is a contribution of the FAC/CLSU-UCS GMIT Project funded by a grant from the British Overseas Development Administration No. (R4803) to G.C. Mair and D.O.F. Skibinski. Intervet and Metrovet are acknowledged for providing the free samples of Chorulon® used in this study.

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Manuscript received 21 January 1994; accepted 9 September 1994.