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Short Communication

Effects of *in vivo* crude human chorionic gonadotropin on ovulation and spawning of the African catfish, *Clarias gariepinus* Burchell—1822

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Summary

The effects of increasing dosages of crude human chorionic gonadotropin (cHCG) on ovulation and spawning of *Clarias gariepinus* Burchell were examined. Four and 5 mg cHCG/100g body weight of fish gave the best response. The activation of the interrenals and stimulation of the ovary are discussed as a possible route of *in vivo* human chorionic gonadotropin administration on ovulation and spawning in catfish.

Introduction

Fingerling production is one of the most serious problems yet to be solved in the culture of African catfish (*Clarias gariepinus*). According to Eyo (1989), the long-term success on a year-round production and supply of fry and fingerlings depends on the capacity to control the entire life cycle of the fish through mastery of its reproductive biology, induced breeding, larval rearing and brood stock development and management. Aspects of the reproductive biology of *C. gariepinus* in the Anambra River have been studied (Eyo and Mgbenka 1992; Mgbenka and Eyo 1992; Rangoda 1988). On the other hand, the theory and practice of induced breeding involves induction of vitellogenesis, final oocyte maturation and ovulation in females and induction of spermiation in males followed by artificial fertilization on natural spawning. In this regard, pituitary homogenate and purified mammalian hormonal preparations such as luteinizing hormone–releasing hormone (LH–RH) and human chorionic gonadotropin (HCG), among others, are often employed in fin fish induced breeding. Considering the high cost of the aforementioned hormonal preparation and the inadequate supply thereof, this report deals with the utilization of local precipitated crude human chorionic gonadotropin (cHCG) in the induction of ovulation and spawning of *C. gariepinus*.

Materials and methods

Fish Collection and Management

Gravid female and mature male specimens of the catfish *C. gariepinus* caught from the Anambra River, Nigeria, in May 1993 were used. Live specimens were taken in F.A.O. fish transportation tanks to the laboratory. The brood stocks were acclimated to their new environment (glass aquaria—80 cm × 40 cm × 40 cm for 15 days) in groups containing four females and one male per tank at temperatures of 28 ± 2 °C and normal photoperiodic regimes (12 h light/12 h darkness).

The fish were given 3 ppm potassium tetraoxomanganate VII ($KMNO_4$) propylactic treatment and fed daily at 3% of their body weight with 35% crude protein pelleted fish feed. An environment relatively free of waste pollutants arising from uneaten pellets and fish excretion was maintained by changing the aquaria water every other day. Fortified procaine penicillin (Pronapen V. Pfizer) was added twice weekly to the aquaria water (35000 IU/L) as treatment against *Saprolegnia* growth on wounded skin and other skin infections. Aeration of the aquaria was achieved using an air blower (sweet water, Apopka FL, USA Model 5.41).

Extraction of Crude Human Chorionic Gonadotropin

Crude human chorionic gonadotropin (cHCG) was derived from pooled early pregnancy (9th to 14th week) urine collected from the maternity section of Bishop Shanahan Hospital, Nsukka. Alcohol precipitation was carried out according to the method of Bell et al. (1969). An aqueous solution of cHCG (2 g) was adjusted to pH 3.0 with formic acid. Absolute ethanol was added to a final concentration of 80%. The mixture was stirred under chilled conditions (4 °C) for 2 h, then allowed to settle overnight. The resulting precipitate was recovered by centrifugation at 8000 rev/min for 45 min using a B and T micro-angle centrifuge. The precipitated cHCG was freeze-dried in acetone, powdered and stored in vials kept under refrigeration at a temperature of 4 °C pending use.

In vivo administration of cHCG

A randomized block experimental plan was adopted. The experimental phase was divided into five groups (A–E). Each group had three replicates containing four females and one male. All aquaria were provided with palm fronds to act as spawning substrates. Only females with bulging abdomens and red swollen vents were used for the trials; fish were not fed 1 day prior to the start of the spawning experiment.

Group A (control) female catfish were given 0.6% normal saline, while 2, 3, 4, and 5 mg cHCG dissolved in 0.5 ml of 0.6% normal saline/100 g body weight of fish were administered to fish in groups B to E, respectively, as stimulatory (first) doses. The stimulatory doses were administered between 20 and 22 h, while the resolving (second) doses (same concentration as stimulatory doses), were given 12 h later. Males were given only the resolving doses. All injections were given intra-peritoneally. All aquaria were examined for ripe ova on the following morning. All injected female specimens, including those that had spawned, were stripped by the application of slight pressure on the abdomen, starting from the vent inward. Stripping of males

Table 1
Effects of *in vivo* crude human chorionic gonadotropin (cHCG) on ovulation and spawning of intact African catfish, *Clarias gariepinus* from Anambra River Basin, Nigeria

cHCG/100 g[1] body weight (mg)	Sex	% ripe ova[2]	Response of fish[3]
0+0	F	0	a No ripe ova.
0	M	—	small quantity of milt.
2+2	F	24.99+2.4	b No spawning, 50% ripe ova upon stripping.
2	M	—	large quantity of milt.
3+3	F	48.97±2.7	c No spawning, mixture ripe and unripe ova upon stripping.
3	M	—	large quantity of milt
4+4	F	78.43±1.7	d Profuse spawning, large number of ripe ova upon stripping.
4	M	—	large quantity of milt
5+5	F	79.92±1.4	e Profuse spawning, large number of ripe ova upon stripping.
5	M		large quantity of milt

[1] Given as stimulating and/or resolving dose, respectively.
[2] Values accompanied by different letters differ significantly at P = 0.05 according to Duncan's multiple range test.
[3] Based on observation of four females and one male per treatment, replicated three times. Mean body weights were 108.94±14.4, 110.67±8.2, 106.16±8.9, 110.74±8.8, 114.2±14.5 and 106.59±1.6, 111.91±12.7, 104.45±1.4, 106.50±4.5, 106.92±6.1 for females and males pre treatment group, respectively.

for milt was not possible. Thus, the whitish kidney-shaped testes were dissected and examined for milt. The statistical differences between treatment mean percentages were tested for their significance using Duncan's New Multiple Range test (Duncan 1995).

Results

The effects of *in vivo* cHCG administration on ovulation and spawning of the African catfish *C. gariepinus* are shown in Table 1. In the control group (A), no spawning and no ripe ova were recorded upon stripping. Group B did not spawn when stripped but yielded less than 5% ripe ova. No spawning was recorded for group C treatment, but treated fish gave a mixture of both ripe and unripe ova upon stripping. Group D and E treatments spawned profusely and yielded large numbers of ripe ova upon stripping. There was a significant difference (P = 0.05) in the effects of all the cHCG dosages except for 4 and 5 mg cHCG treatments. The male gave large quantities of whitish milt in all treatment groups except for group A. Milt was noticed to have clustered along the lobate convex edges of the testes.

Discussion

Table I indicates that cHCG was highly effective in *in vivo* induction of ovulation and spawning of *C. gariepinus*. Sundararaj and Goswami (1966) induced ovulation and spawning in *Heteropneutes fossilis* using purified HCG, 75–100 IU/fish.

Rangoda (1988) successfully induced ovulation and spawning of *C. lazera* and *C. anguillaris* using purified HCG. HCG administered to hypophysectomized gravid catfish acted directly upon the ovary or activated the interenals to produce corticosteroids which eventually brought about ovulation and spawning (Sundararaj and Goswami 1966). Hirose and Donaldson (1972) pointed out that the pathway of HCG-induced ovulation in *Oryzias latipes* is through the ovarian follicle itself. Similarly, Iwamatsu (1982) noted that in *O. latipes* the granulosa cells from large pre-ovulatory follicles promoted ovulation under *in vitro* conditions using purified HCG. The above cited literature agrees with the indisputable potency of HCG vis-à-vis ovulation and spawning. Conclusively, a stimulatory dose (4 mg cHCG) and a resolving dose (4 mg cHCG/100 g body weight of fish) is recommended for use in areas where purified HCG is difficult to obtain and expensive, while a single dose of 2 mg cHCG/male is necessary to obtain sufficient quantities of sperm. As the fish were held in groups which included both sexes, the possible role of enhancing substances emitted by treated animals has to be taken into consideration.

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