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Fish physiology

INDUCED SPAWNING OF THE STRIPED MURREL *CHANNA STRIATUS* USING PITUITARY EXTRACTS, HUMAN CHORIONIC GONADOTROPIN, LUTEINIZING HORMONE RELEASING HORMONE ANALOGUE, AND OVAPRIM®

TARŁO ŻMIJOGŁOWA INDYJSKIEGO *CHANNA STRIATUS* WYWOŁANE SZTUCZNIE ZA POMOCĄ EKSTRAKTU Z PRZYSADKI MÓZGOWEJ, LUDZKIEJ GONADOTROPINY KOSMÓWKOWEJ, ANALOGU PODWZGÓRZOWEGO HORMONU UWALNIAJĄCEGO HORMON LUTEINIZUJĄCY I OVAPRIMU®

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Striated murrel *Channa striatus* were injected with natural hormones (pituitary extract and human chorionic gonadotropin) and synthetic hormones (luteinizing hormone releasing hormone analogue and ovaprim). When compared to the LHRHap and ovaprim, the latency period was long in pituitary- (24 h) and HCG-injected (26 h) fish. In the pituitary-injected *C. striatus* the percentage of fertilisation was the lowest (60–68%) but the duration of hatching was longest (39–43 h) followed by HCG- (36–38h), LHRHap- (34–36 h) and ovaprim-injected (21–23 h) individuals. In terms of fertilisation (95–98%) and hatching, ovaprim yielded better results. Ova reached the highest diameter (1.34–1.45 mm) in *C. striatus* injected with ovaprim, followed by HCG (1.22–1.30 mm) and pituitary (1.21–1.27 mm). The lowest ova diameter (1.07–1.09 mm) was observed in *C. striatus* injected with LHRHap.

INTRODUCTION

Murrels breed naturally during southwest moonsoon and northeast moonsoon in flooded rivers and ponds in India. Since monsoon failure often limits their seed production, Parameswaran and Murugesan (1976) attempted induced breeding by carp pituitary glands.

Hypophysation is a simple practical technique but suffers from the disadvantage that often gonadotropic potency of pituitary glands used is unknown and difficult to standardise. Hence alternative sources viz. human chorionic gonadotropin (HCG) (Mollah and Tan 1983; Zairin et al. 1992; Inyang and Hettiarachchi 1994) luteinizing hormone releasing hormone (Billard et al. 1984; De Leeuw et al. 1985; Fermin 1992) and Ovaprim (Alok et al. 1993; Francis 1996; Haniffa et al., 1996) have been attempted in air-breathing fishes. The aim of the present study is to use different hormones viz. pituitary extract, HCG, LHRHa + pimozide (LHRHap), and ovaprim and assess their efficiencies concerning latency period, spawning response, fertilisation rate, incubation period, and hatching in the striped murrel *Channa striatus*.

MATERIAL AND METHODS

Rectangular ponds (each 16 × 7.5 × 1.5 m) of CARE each partitioned into three breeding compartments were used for the induced breeding experiments. Water (dissolved oxygen: 6.1–6.8 ppm; CO_2 5.1–6 ppm; pH 7.9–8.1; salinity 1.01–1.04%; temperature 27–29°C) was pumped upto a level of 1 m depth from a nearby well within the campus. One-year-old *C. striatus* breeders were collected from the culture pond of CARE. Healthy males and females (480–770 g) were selected by external morphological characteristics and hand stripping (Billard et al. 1984). For each hormone three doses were chosen and for each dose three trials were made. Pituitary extracts were injected intramuscularly in the dorsolateral region in two instalments with an interval of 6 h whereas the other hormones [Human Chorionic Gonadotropin (HCG), Luteinizing Hormone Releasing Hormone analog (LHRHa) + Pimozide and Salmon Gonadotropin Releasing Hormone analogue (SgnRHa) marketed as Ovaprim by Glaxo India Limited, Mumbai] were given in a single dose. Control fish were given corresponding volumes of physiological saline solution. Immediately after administering the injections, the breeding sets were released into the breeding compartment provided with *Eichhornia crassipes*. Eggs were collected from each compartment and the percentage of fertilisation was estimated by examining a sample of at least 150 eggs from each compartment. Then the eggs were fixed in 1% buffered formalin and observed within 4 h (Tan Fermin 1991) under a microscope. The percentage of hatchability was determined from the total number of live eggs in each sample.

RESULTS AND DISCUSSION

The breeders showed aggressiveness after 10 h of injection irrespective of the type of the hormone. Each female paired with only a single male (Parameswaran and Murugesan 1976; Thakur 1976; Moitra et al. 1979) and the other male was rejected. Mating was preceded by an elaborate courtship. During spawning, the male bent its body close to the fe-

male and released its milt and the eggs were fertilised externally (Yaakob and Ali 1992). Both parents particularly the male guarded the juveniles (Devaraj 1973).

Spawning was complete in the medium (10 mg + 100 mg·kg^{-1}) and high dosages (10 mg + 150 mg·kg^{-1} body weight) of pituitary extracts whereas in the low dosage (10 mg + 5 mg·kg^{-1}) it was partial. A latency period of 23–24 h and a fertilisation of 60–70% were observed (Tab. 1). The latency period available in the literature is 6–25 h for *Channa punctatus* (cf. Banerji 1974), 22–25 h for *Heteropneustes fossilis* (cf. Kohli and Goswami 1987), 14 h (Rao et al. 1989) and 16–20 h (Munshi and Hughes 1991) for *Clarias gariepinus*. With regard to pituitary extract Parameswaran and Murugsesan (1976) reported 28–100% fertilisation in *C. striatus* and Kohli and Goswami (1987) noticed 45% fertilisation in *H. fossilis*. In the present study hatching was normal irrespective of dosages of pituitary extract. When compared to the effects of other hormones, the latency period was long in pituitary-injected fish whereas the fertilisation percentage was the least (60%). The duration for hatching was greater (39–43 h) when compared to HCG (36–38 h), LHRHap (34–36 h) and ovaprim-injected (21–23 h) individuals. ANOVA confirmed that pituitary exerted a significant effect ($p < 0.05$) on latency period when compared to HCG and LHRHap. With regard to fertilisation rate and incubation period, pituitary showed a significant difference ($p < 0.05$) with ovaprim only (Tab. 1).

C. striatus given a low dosage of HCG showed partial spawning. Incubation period was more or less same among different dosages. The latency period was high (26 h) for the fish injected with low dosage of (3000 IU) HCG. Francis (1996) too reported high latency period for *H. fossilis* and *Clarias batrachus* due to low potency of this hormone (Legendre 1986). Tukey test showed that HCG differed significantly ($p < 0.05$) from LHRHap with regard to latency period. A significant difference in fertilisation rate and incubation period was noticed with ovaprim only. LHRHap seems to be much more effective as an ovulating agent (Devauvchelle et al. 1988; Kestemont 1988). Fertilisation was more in high doses than low doses but the embryos began to die 24 h after fertilisation. After 32 h, dense bacterial growth covered fish eggs and mass mortality was noticed. Latency period was relatively shorter (18–20 h) for LHRHap ($p < 0.05$) when compared to other hormones (Tab. 1). Single injection of LHRHap resulted in successful induction of spawning in *H. fossilis* after 14–18 h (Alok et al. 1993) and in *C. batrachus* after 18–21 h (Manickam and Joy 1989). The combination of pimozide and LHRHa was highly effective in inducing ovulation (Peter et al. 1987; Bush and Steely 1990).

Table 1

Effects of different hormones on induced spawning in *Channa striatus*. Values are $\bar{x}$ ± SD (n = 3)
Values with different superscripts in column are significantly different (P < 0.05)*

Hormone	Female Weight (g)	Male Weight (g)	Dosage of hormone/kg 1st + 2nd	Latency period (h)	Spawning	Fertilisation (%)	Incubation period (h)	Ova diameter (mm)
								Control 0.77
Pituitary Extract	710	590	10 + 50 mg	24.3 ±0.3 [a]	Partial	60.0 ±5.0[a]	43.3 ±0.3[a]	1.21 ±0.02 [a]
	700	610	10 + 100 mg	22.5 ±0.1 [b]	Complete	70.3 ±3.0[b]	41.0 ±0.5[b]	1.23 ±0.01 [a]
	700	570	10 + 150 mg	23.0 ±0.2 [c]	Complete	68.6 ±2.0[b]	39.0 ±0.3[c]	1.27 ±0.01 [b]
HCG	625	550	3000 IU	26.0 ±0.5 [a]	Partial	65.3 ±7.0[a]	38.3 ±2.1[a]	1.22 ±0.01 [a]
	620	590	4000 IU	23.0 ±0.8 [b]	Complete	79.5 ±3.0 [b]	36.0 ±1.5[a]	1.28 ±0.03 [b]
	750	620	5000 IU	23.0 ±0.7 [b]	Complete	79.0 ±5.0[b]	36.5 ±1.0[a]	1.30 ±0.02 [b]
LHRHa +Pimozide	680	590	40 µg + 5 mg	20.0 ±1.0 [a]	Complete	75.3 ±2.0[a]	36.5 ±2.1 [a]	1.07 ±0.02 [a]
	600	540	50 µg + 5 mg	18.0 ±0.9[a]	Complete	84.0 ±2.0 [b]	34.0 ±1.0 [a]	1.09 ±0.03 [a]
	645	520	60 µg + 5 mg	19.3 ±0.8 [a]	Complete	80.3 ±2.0 [b]	Eggs died before hatching	1.09 ±0.01 [a]
Ovaprim	640	510	0.3 cm^3	Nil	Nil	Nil	Nil	1.34 ± 0.03 [a]
	680	525	0.5 cm^3	24.0 ± 0.9 [a]	Complete	98.0 ± 3.0 [a]	21.0 ± 1.4 [a]	1.41 ± 0.02 [b]
	765	580	0.7 cm^3	23.0 ± 0.9 [a]	Complete	95.3 ± 2.5 [a]	23.0 ± 2.0 [a]	1.45 ± 0.02 [b]

	Latency period	Fertilisation	Incubation period	Ova diameter :
Pituitary (P), HCG (H) LHRHap (L) and Ovaprim (O) vs.	$\mu_L \neq \mu_P = \mu_O = \mu_H$	$\mu_P = \mu_H = \mu_L \neq \mu_O$	$\mu_O \neq \mu_L = \mu_H = \mu_P$	$\mu_C \neq \mu_L \neq \mu_P = \mu_H \neq \mu_O$

* Data were analysed by one way ANOVA followed by Tukey multiple range test; ≠ P < 0.05; = P > 0.05

Spawning was complete for medium- and high doses of ovaprim-injected fish whereas low-dose injected fish did not respond. In terms of fertilisation and hatching, ovaprim yielded better results (Nandeesha et al. 1990, 1993; Alok et al. 1993). The highest percentage of fertilisation (95–98%) was observed in ovaprim-injected *C. striatus*. In mrigal injected with ovaprim, 90% fertilisation was observed by Azad and Shimray (1991). Statistical analysis confirmed that the latency period of fish injected with ovaprim differs significantly ($p < 0.05$) with LHRHap. With regard to fertilisation and incubation period, ovaprim differs significantly with other hormones (Tab. 1).

Mean egg diameter of all fish which ovulated in the four experiments ranged from 1.07 to 1.45 mm. Germinal vesicle broke down and an increase in the size of oocytes due to hydration indicated the changes in the nucleus and cytoplasm during final maturation (Goetz 1983; Guraya 1986). The lowest ova diameter (1.07–1.09 mm) was observed in the test fish injected with LHRHap. The highest ova diameter was (1.34 to 1.45 mm) in *C. striatus* injected with ovaprim followed by HCG (1.22–1.30 mm) and pituitary-injected (1.21–1.27 mm) individuals (Tab. 1). The early action of steroidogenesis through hormonal influence might have resulted in increased ova diameter. Due to early steroidogenesis, the batch of oocytes might not have obtained sufficient yolk and hence resulted in reduced diameter of ova.

CONCLUSIONS

Spawning of the striated murrel *C. striatus* can be induced by injection of natural as well as synthetic hormones. Female fish usually pairs with a single male. The latency period was the highest in pituitary-injected fish but fertilisation percentage was the least. The synthetic hormone ovaprim could be recommended for induced spawning in murrel since it produced better results in terms of fertilisation and hatching.

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TARŁO ŻMIJOGŁOWÓW INDYJSKICH *CHANNA STRIATUS* WYWOŁANE SZTUCZNIE ZA POMOCĄ EKSTRAKTU Z PRZYSADKI MÓZGOWEJ, LUDZKIEJ GONADOTROPINY KOSMÓWKOWEJ, ANALOGU PODWZGÓRZOWEGO HORMONU UWALNIAJĄCEGO HORMON LUTEINIZUJĄCY I OVAPRIMU®

STRESZCZENIE

Żmijogłowom indyjskim, *Channa striatus* wstrzyknięto naturalne (ekstrakt z przysadki mózgowej, HGC – ludzką gonadotropinę kosmówkową) oraz syntetyczne hormony (LHRHap – analog podwzgórzowego hormonu uwalniającego hormon luteinizujący i ovaprim®). Czas reakcji – w porównaniu do LHRHap i ovaprimu – był względnie długi w przypadku stosowania ekstraktu z przysadki (24 h) i ludzkiej gonadotropiny kosmówkowej (26 h). U ryb, którym wstrzyknięto ekstrakt przysadki, procent zapłodnienia był najniższy (60–68%). Dla odmiany, wylęganie u potomstwa tych ryb trwało najdłużej (39–43 h). Na kolejnych miejscach były ryby poddane działaniu HCG (36–38 h), LHRHap (34–36 h) i ovaprimu (21–23 h). Pod względem zapłodnienia i wylęgania ovaprim dał lepsze wyniki. Największą średnicę (1,34–1,45 mm) osiągnęły jaja *C. striatus* stymulowanych ovaprimem. Nieco mniejsze były jaja w przypadku ryb pod działaniem HCG (1,22–1,30 mm) i przysadki (1,21–1,27 mm). Najmniejsza średnica jaj (1,07–1,09 mm) była obserwowana u *C. striatus*, którym wstrzyknięto LHRHap.

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