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Induced spawning of kutum, *Rutilus frisii kutum* (Kamenskii, 1901) using (D-Ala^6, Pro^9-NEt) GnRHa combined with domperidone

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Abstract

Kutum, *Rutilus frisii kutum* (Kamenskii, 1901), Cyprinidae is an endemic fish of the Caspian Sea. The Iranian Fisheries Organization (Shilat) produces up to 200 million fry (1–2 g b.w.) to restock the Caspian Sea population annually. These fish are produced by artificial breeding using carp pituitary extract (CPE). The objective of this study was to assay the effectiveness of a gonadotropin releasing hormone analogue (D-Ala^6, Pro^9-NEt GnRH) alone or in combination with the dopamine antagonist domperidone (DOM) on spawning success, latency period, ovulation index (OI), weight of stripped egg mass/weight of stripped egg mass+remnant ovaries, and fertilization success in kutum. Ninety fish were divided into nine groups and injected intraperitoneally as follows: 2 mg kg^{-1} b.w. of CPE as positive control, 20 μg GnRHa kg^{-1} b.w. in single injection, 5 μg+2.5 mg, 10 μg+5 mg and 20 μg+10 mg kg^{-1} b.w. of GnRHa+DOM in single or double injection (10–90%) 24 h apart. Propylene glycol injected fish were used as negative controls. The results showed that the highest doses of GnRHa and DOM in single injection lead to higher spawning success and latency periods in comparison with positive control ($P<0.05$), while no significant differences in the OI and fertilization success were found ($P>0.05$). Only 2/10 fish were ovulated in the group which received GnRHa 20 μg kg^{-1} b.w. alone suggesting dopaminergic tone on gonadotropin (GtH) secretion in this fish at the preovulation stage. Therefore, it can be concluded that like many other cyprinids, dopamine inhibitory tone is active in kutum and it is necessary to combine GnRHa with a dopamine antagonist for spawning induction.

Keywords: Kutum; *Rutilus frisii kutum* (Kamenskii, 1901); Spawning; GnRHa; Domperidone; Carp pituitary extract

1. Introduction

Kutum (*Rutilus frisii kutum* Kamenskii, 1901) live in the Caspian Sea near the coast, from the Terek River in the north to the southern part of the Caspian Sea. This species is a migratory anadromous fish spawning in rivers in March–April. It has a group synchronous, single spawning behaviour (Sharyati, 1993), spawning on aquatic weeds, gravelled and sandy substrates in rivers and lagoons (Abdoli, 1999). This is a very valuable commercial fish in the southern part of the Caspian Sea and has a great demand, due to its good taste and culinary customs of the local people, and is consumed all year round. The average annual catch of

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kutum in Iran was about 9600 tons in 1991–2001 (FAO, 2003).

To restock this valuable species in the Caspian Sea, the Iranian Fisheries Organization (Shilat) produces and releases up to 200 million fry (average weight 1 g) in to the Caspian Sea annually (www.shilat.com). Fry are produced by artificial breeding using the hypophysation technique to induce ovulation. Carp pituitary extract (CPE), the only agent used commonly to induce spawning in kutum, is expensive, not always readily available and with unpredictable potency (Drori et al., 1994). An alternative method for induced ovulation of many fishes is the use of different forms of gonadotropin releasing hormone agonists (GnRHa), which stimulate secretion of endogenous gonadotropin (GtH) (Zohar, 1989; Zohar and Mylonas, 2001). The addition of a dopamine receptor antagonist (DA) to potentiate the response to GnRHa depends on the presence of a dopaminergic inhibitory tone in the target species (Peter et al., 1988; Zohar, 1989).

Induction of spawning in fish using GnRHa together with DA, such as metoclopramide, domperidone (DOM) and pimozide, is known as the Linpe method (Peter et al., 1988). The success of using GnRHa alone or in combination with DA has been described in several species such as common carp (*Cyprinus carpio*) (Drori et al., 1994; Yaron, 1995; Kulikovsky et al., 1996; Arabaci et al., 2004), catfish (*Heteropneustes fossilis*) (Alok et al., 1993), Indian major carps such as rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) (Halder et al., 1991), nase (*Chondrostoma nasus*) (Szabo et al., 2002), pearl mullet (*Chalcalburnus tarichi*) (Arabaci and Sari, 2004), rainbow trout (*Oncorhynchus mykiss*) (Billard et al., 1984; Breton et al., 1990), lake trout (*Salvelinus namaycush*) (Erdahl and McClain, 1987) and sockeye salmon (*Oncorhynchus nerka*) (Slater et al., 1995). The form of GnRHa, the type of DA, the species of fish and environmental factors may affect the ovulatory response (Zohar and Mylonas, 2001). For these reasons it is necessary to examine the response in each species under local conditions.

The objective of the current study was to establish a protocol for spawning induction in kutum, employing GnRHa alone or in combination with DA, which will be based on spawning success, ovulation index (OI), weight of stripped egg mass/weight of stripped egg mass+remnant ovaries (Szabo et al., 2002), latency period and fertilization success.

2. Materials and methods

2.1. Fish stocks and maintenance

The experiments were conducted at Shahid Ansari Cyprinid Fish Complex, Rasht, Guilan, Iran. Kutum were captured from the Sefid Rood River inlets to the Caspian Sea during the spawning migration in April–May 2004 (water temperature 8–12 °C).

Ninety female fish weighing 400–1400 g body weight (b.w.) were selected for ripeness, based on the softness of their abdomens. Prior to injections, fish were individually weighted and marked by placing visible tags on the dorsal fin and randomly were divided into treatment groups.

2.2. Hormones and drugs

The GnRH agonist D-Ala^6, des-Gly^{10} mGnRHa Ethylamide and DOM were supplied as a kit by National Research Institute of Genetic Engineering and Biotechnology (NRIGEB), Tehran, Iran. The GnRHa was

Table 1
The effect of different hormone treatments on spawning success (%), latency period (h), ovulation index OI (%) and fertilization success (%) of kutum, *Rutilus frisii kutum* (Kamenskii, 1901)

Treatment ID	Treatment	Dosage		Spawning success (%)	Latency period (h)	OI (%)	Fertilization success (%)
		1st*	2nd*				
Negative control	Propylene glycol	–	–	10^{a}**	72^{b}	78^{b}	73^{a}
Positive control	CPE	2 mg	–	60^{b}	56±5^{a}	86±3^{b}	65±3^{a}
GnRHa only	GnRHa (G)	20	–	20^{a}	72±0^{b}	66±5^{a}	69±6^{a}
GD 1 (5+2.5)	GnRHa (G)+DOM (D)	5+2.5	–	20^{a}	48±0^{a}	62±4^{a}	70±6^{a}
GD 2 (5+2.5)	GnRHa (G)+DOM (D)	0.5+0.25	4.5+2.25	60^{b}	64±9^{b}	83±3^{b}	73±4^{a}
GD 1 (10+5)	GnRHa (G)+DOM (D)	10+5	–	60^{b}	72±0^{b}	79±3^{b}	77±6^{a}
GD 2 (10+5)	GnRHa (G)+DOM (D)	1+0.5	9+4.5	90^{c}	62±8^{b}	80±3^{b}	68±4^{a}
GD 1 (20+10)	GnRHa (G)+DOM (D)	20+10	–	100^{c}	67±8^{b}	83±4^{b}	67±6^{a}
GD 2 (20+10)	GnRHa (G)+DOM (D)	2+1	18+9	70^{bc}	62±10^{b}	80±4^{b}	64±4^{a}

*The first number indicates the dose of GnRHa (G) in μg kg^{-1} b.w. and the second of DOM (D) in mg kg^{-1} b.w.
**Mean (±S.E.M.) values with a different letter are significantly different ($P<0.05$).