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Effect of GnRHa, pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish *Clarias gariepinus*

S.M. Sharaf*

Animal Production and Fish Resources Dept., Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

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Abstract

Nine groups each of four fish were injected with a single intramuscular dose of the following preparations: Physiological saline (0.9% NaCl) as a control group, 0.5 ml kg^{-1} Ovaprim, 20 and 40 μg kg^{-1} BW of GnRHa, 8 and 16 mL kg^{-1} pimozide tablets and the following combination of GnRHa with pimozide (GP): 20 μg + 4 mg, 30 μg + 8 mg and 40 μg + 16 mg kg^{-1} BW. The primary oocyte diameter (POD) before hormone administration ranged from 943.3 to 1071.0 μm. The latency periods (LP) were in the range of 9.0 to 12.0 h after injection. The highest ovulation ratio (OR) was observed in groups Ovaprim, GP(30 + 8) and GP(40 + 16). Other treatments were effective for ovulation, the ovulation ratio in Groups G(40) and GP(20 + 4) were significantly higher than G(20) treatment. The ovulation index (OI) was in the range 62 to 77% and showed significant differences among groups. There was no significant difference in fertilization ratio (FR) among Ovaprim, GP(30 + 8) and GP(40 + 16) groups, while there were significant difference between the previous group and G(20) and G(40) groups. Control, P8, P16 showed negative results in all the parameters LP, OED, OR, OI and FR. Levels of sex steroids were analyzed on 6 and 12 h after initiation of treatments. A significant increase in plasma E_2 with GP(30 + 8) injection was observed 6 and 12 h after injection, while there were no significant increase between all the other groups 6 h after injection. Treatments with GP(20 + 4) resulted in a significant increase in plasma T concentration in females compared with control after 6 h. In contrast, plasma T and E_2 concentrations were lower during the combined GP(20 + 4), GP(30 + 8) and GP(40 + 16) after 12 h than after 16 h of injection. The combined treatments (GnRHa + PIM) are better compared with Ovaprim which gave the same results, they have some advantages, such as reliable response and low cost. Ovaprim is more than 3 to 5-fold of the cost of (GnRH + PIM). Therefore, this method could be useful tool for commercial catfish breeders to ensure spawning success.

Keywords: *Clarias gariepinus*; GnRHa; Pimozide; Ovaprim; Sex steroids; Ovulation

1. Introduction

In nature, African catfish, *Clarias gariepinus*, has a discontinuous annual reproductive cycle with alternate periods of resting, pre-spawning and breeding, regulated by cyclically active gonadotrophes [1]. The breeding season correlates with periods of maximal rainfall and a pre-spawning LH surge takes place at least once during this period [2]. Spawning occurs usually during the scotophase, after rain in recently inundated marginal areas.

In captivity, catfish are kept under constant environmental conditions. Throughout the year, their pituitaries contain large and densely granulated gonadotrophes, storing large amounts of LH [3].

Under laboratory and fish-farming conditions, the natural cues are difficult to mimic. Over the last few

* Corresponding author. Tel.: +201224173750.
E-mail address: safaa_sharaf@agr.suez.edu.eg (S.M. Sharaf).

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decades, hormonal manipulations to induce final oocyte maturation and spawning have made possible the control of reproduction in cultured fishes and have contributed significantly to the expansion and diversification of the aquaculture industry [4].

Although *Clarias gariepinus* complete vitellogenesis within the first year of captivity, final maturation does not occur unless gravid females are induced to spawn by hormonal manipulation [5]. The introduction of GnRH analogues has proven to be efficient in inducing maturation and spawning in many fish species [6–13]. Likewise, an antidopaminergic drug, pimozide, has also been found to be highly effective for stimulating the spawning process of fishes mainly in cyprinids and catfishes [14,15]. These methods stimulate secretion of endogenous gonadotropin (Gth) [4,16].

Many fish exhibit this dopaminergic inhibitory action and, therefore, require the addition of a DA antagonist to facilitate the release of LH in response to GnRHa [17,18]. The GnRHa and domperidone are the most popular compounds for induction of ovulation and spermiation in various fish species [18–21].

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 [16,17,22]. Induction of spawning in fish using GnRHa together with DA, such as metoclopramide, domperidone (DOM) and pimozide, is known as the Linpe method [17]. The success of using GnRHa alone or in combination with DA has been described in several species, such as common carp (*Cyprinus carpio*) [23–26], catfish (*Heteropneustes fossilis*) [27], Indian major carps, such as rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) [28], nase (*Chondrostoma nasus*) [29] (Szabo, et al., 2002), pearl mullet (*Chalcalburnus tarichi*) [30,31], rainbow trout (*Oncorhynchus mykiss*) [14], lake trout (*Salvelinus namaycush*) [32] and sockeye salmon (*Oncorhynchus nekra*) [9]. The form of GnRHa, the type of DA, the species of fish and environmental factors may affect the ovulatory response [4]. For these reasons it is necessary to examine the response in each species under local conditions.

2. Materials and methods

2.1. Broodstock selection and maintenance

Experiments were conducted at the Animal and Fish Production Department, Suez Canal University, Ismailia, Egypt. Adult female catfish *Clarias gariepinus* (selected by certain external morphologic characteristics) were captured from fish farms [14]. Ovarian biopsies were taken, only fish having more than 60% of the oocytes with a migrating germinal vesicle were selected for ovulation experiment. Thirty-six female fish weighing 500 to 1000-g body weight (BW) were selected. Before injection fish were individually weighed and assigned to nine groups. No food was provided during the experiment.

Table 1
Substances and doses applied to stimulate ovulation in Clarias gariepinus females (n = 4).

Treatment groups	Substance	Dosage
Control	Saline (0.9%NaCl)	
Ovaprim	Ovaprim	
G(20)	GnRHa	20 μkg^{-1}
G(40)	GnRHa	40 μkg^{-1}
P8	PIM	8 mg
P16	PIM	16 mg
GP(20 + 4)	GnRH + PIM	20 μkg^{-1} + 4 mg
GP(30 + 8)	GnRH + PIM	30 μkg^{-1} + 8 mg
GP(40 + 16)	GnRH + PIM	40 μkg^{-1} + 16 mg

2.2. Hormones and experimental design

Nine groups of four fish each were injected a single intramuscular dose into the dorsal muscle above lateral line with different preparations as follows: Physiological saline (0.9% NaCl) was injected as control group, Ovaprim (Syndel Laboratory, Limited, Canada) 0.5 ml kg^{-1} which contains the synthetic GnRH analog and domperidone, 20 and 40 $\mu g\ kg^{-1}$ BW of GnRH {42 $\mu g\ kg^{-1}$ buserelin acetate (Pyr-His–Trp–Ser–Tyr-D-Ser-(Bu^t)-Leu–Arg–Pro ethylamide) and 10 mg benzyl alcohol), synthetic, Receptal, Intervet, Germany}, 8, 16 ml kg^{-1} pimozide tablets (Orap Forte, Cilag, Belgium) were powdered and then dissolved in physiological saline and dimethyl sulfoxide, respectively [33] and the following combination of GnRH with pimozide: 20 μg + 4 mg, 30 μg + 8 mg and 40 μg + 16 mg kg^{-1} BW (Table 1).

After injection fish were placed in well-aerated tanks with recirculated water and temperature of 28 ± 1 °C. The first examination for ovulation was carried out 6 h after injection and repeated every hour. So when ovulation was observed, the eggs were stripped manually after the latency period (mean time between injection and ovulation [23]) and weighed from each female separately, the weight being expressed as percentage of female BW. The number of eggs released was calculated following the gravimetric method [34]. Assessment of ovulation was carried out by determining the ovulation success (number of ovulated females/number of injected) ×100% and by ovulation index (OI) weight