Aquaculture Research, 2004, **35,** 204–212

Effects of exogenous neurohormone, gonadotropin (GtH) and dopaminergic drugs on the serum GtH content and ovulatory responsiveness of wild catfish, *Silurus asotus* (Linnaeus, 1758)

H Sh Wen[1,2] & H R Lin[1]

[1]School of Life Science, Zhongshan University, Guangzhou, China

[2]Fisheries College, Ocean University of China, Qingdao, China

Correspondence: H Sh Wen, Fisheries College, Ocean University of China, Qingdao 266003, China. E-mail: wenhaishen@ouc.edu.cn.

Abstract

Wild female catfish *Silurus asotus* (Linnaeus, 1758) were injected with domperidone (DOM) alone, [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) alone once or twice, LHRH-A plus DOM once or twice simultaneously at 6-h intervals, LHRH-A plus carp pituitary extract (CPE) twice simultaneously at 6-h intervals and LHRH-A plus human chorionic gonadotropin (HCG) twice simultaneously 6 h apart respectively. The results indicated that injection of LHRH-A at a dosage of 0.01–0.02 μg g^{-1} body weight (BWt) alone induced a low but significant increase in serum gonadotropin (GtH) ($P < 0.05$) and resulted in a very low ovulation rate, while DOM at a dosage of 5 μg g^{-1} BWt alone did not induce an increase in the serum GtH levels and ovulation; in contrast, LHRH-A at a dosage of 0.01 μg g^{-1} BWt plus DOM at a dosage of 5 μg g^{-1} BWt (termed the Linpe technique) increased the serum GtH ($P < 0.05$) significantly and induced an ovulatory rate of 100%, while LHRH-A plus CPE or HCG resulted in an increase in the serum GtH ($P < 0.05$) and high ovulatory rate, although the latency period was longer when fish were given LHRH-A plus HCG or CPE.

Keywords: catfish, gonadotropin, ovulation, LHRH-A

Introduction

The dual neurohormonal regulation of secretion of gonadotropin (GtH) by gonadotropin-releasing hormone (GnRH) and dopamine (DA) acting as a release-inhibition factor have provided the basis for a highly effective technique for inducing ovulation on many cultured fishes (Peter 1982). This involves intraperitoneal or intramuscular injection of a superactive analogue of GnRH, specifically salmon [D-Arg^6, Pro^9-NET]-GnRH (sGnRH-A) or [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A), with a DA receptor antagonist, specifically pimozide (PIM) or domperidone (DOM), to remove the inhibitory influence of DA on pituitary GtH secretion (Peter, Lin & Van Der Kraak 1988; Lin, Zhou, Van Der Kraak & Peter 1991). This combination well stimulated the normal ovulatory surge of GtH in goldfish *Carassius auratus* (Linnaeus, 1758) (Chang & Peter 1983; Sokolowska, Peter & Nahorniak 1985a; Sokolowska, Peter, Nahorniak & Chang 1985b) and common carp *Cyprinus carpio* (Linnaeus, 1758) (Lin, Van Der Krrak, Zhou, Liang, Peter, Rivier & Vale 1988; Peter *et al.* 1988), Chinese loach *Paramisgurnus dabryanus* (Sauvage, 1878) (Lin, Peng, Lu, Zhou, Van Der Kraa & Peter 1985; Lin, Peng, Van Der Kraak, Peter & Breton 1986; Lin *et al.* 1988; Lin *et al.* 1991), African catfish *Clarias gariepinus* (Burchell, 1822) (De Leeuw, Resink, Rooyakkers & Goos 1985; Goos, Joy, De Leeuw, Van Oordt, Van Delft & Gielen 1987; Van Oordt & Goos 1987) and coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) (Van Der Kraak, Donadson & Chang 1986). This method of using a GnRH-A or LHRH-A with DA antagonist PIM or DOM for inducing ovulation of cultured fish had been termed the Linpe technique (Peter *et al.* 1988). The technique was especially formulated and got excellent responses with salmonids (Salmonidae), carps (Cyprinids), clarid catfishes (Clariidae), bullhead catfishes (Ictaluridae), bagrid catfishes (Bagridae) and other freshwater cultured

© 2004 Blackwell Publishing Ltd

fish (Lin & Peter 1996; Peter & Yu 1997; Wang, Lin & Goos 1998). However, the Linpe technique has not been tried on silurid catfishes (Siluridae) to induce ovulation and spawning.

The silurid catfish *Silurus asotus* (Linnaeus, 1758) is widely distributed throughout the freshwaters of China, Korea and Japan, and is a commercially valuable aquaculture fish in the north regions of China and Japan (Miwa, Yoshizaki, Naka, Nakatani, Sakai, Kobayashi & Takeuchi 2001). In China, the natural stocks of this species have decreased gradually in the recent years. At present, the aquaculture industry of *S. asotus* depends on the production of the fry using traditional methods of inducing ovulation and spawning by injection of carp pituitary extract (CPE), human chorionic gonadotropin (HCG), or LHRH-A. However, the effects of induced ovulation and spawning as well as the amount fry produced varies greatly among individuals. Studies on ovarian development (Wei & Huang 1997), steroid profile (Shirai, Suzuki, Toukairin & Wada 2001), regulation of reproduction (Miwa *et al.* 2001; Wen & Lin 2001) and artificial propagation (Pan, Guo, Tian, Wang, Tian, Yan & Zhou 1992; Liu, Pu, Hu & Luo 1998) were conducted in *S. asotus*, but there have been no published studies on the effects of exogenous hormone on the serum GtH content and ovulation responsiveness. The present study investigated the effect of different hormone protocols on serum GtH and ovulation, in an attempt to develop a reliable method of inducing ovulation in wild *S. asotus*.

Materials and methods

Experimental animals

Female wild catfish ($n = 145$), with body weights 118–560 g and body lengths 24–45 cm, were captured from Pearl River (Guangzhou, Guangdong Province, China) during the breeding period (from March to July). Before the experiments, the fish were held indoors in recirculating 250-L aquaria at 20–31 °C under a natural photoperiod.

Experimental design

LHRH-A was purchased from the Ningbo Fish Hormone Factory, Zhejiang Province, China. LHRH-A was dissolved in freshwater teleost physiological saline (Saline), while DOM was purchased from Janssen Pharmaceutica (Beerse, Belgium), suspended in 0.7% NaCl with 0.1% sodium metabisulphite (vehicle (Veh)) or in Saline. Control groups received Saline and/or Veh.

Sexually mature (preovulatory) females were selected for experiments on the basis of a soft and distended abdomen. Individual fish were identified by means of a fin clip and weighed 1–2 days before hormone or drug treatment. Experiment A was conducted on 10–15 June 1999 at a water temperature of 25–28 °C, while experiment B was carried out on 2–10 July 1999 at 28–31 °C. Ninety-six females were randomly divided into 12 groups for experiment A and 46 females were randomly divided into six groups for experiment B (samples are shown in Table 1). All injections were intramuscular near the base of the dorsal fin. Experimental designs and injections with the dose of hormone and drugs were shown as Table 1. The dosages of hormone and the drugs utilized are in micrograms (LHRH-A and DOM) or International Units (IU, HCG) or pituitary (CPE) per gram (kilogram) body weight ($\mu g\,g^{-1}$ BWt or $IU\,g^{-1}$ BWt or pituitary kg^{-1} BWt).

Sampled and determined

The occurrence of ovulation, indicated by ejection of a stream of eggs from the ovipore with slight pressure on the abdomen, was checked at the time of blood sampling. Ovulated eggs were obtained by manual stripping as is done in most catfishes and examined under the microscope with clearing fluid to check if indeed the eggs had undergone germinal vesicle breakdown (GVBD). Blood samples were taken serially from fish of each group at 4, 8, 12, 16, 20 and 24 h after injection (postinjection (PI)) by puncturing the caudal vasculature with a 25-gauge 1.3-cm needle attached to a 1.2-mL disposable syringe. Blood samples were allowed to clot on ice for several hours, and the serum was separated by centrifugation (15 000 rps) and stored at -25 °C. Serum and GtH levels were determined by radiommunoassay (RIA) using an antiserum directed against the -β subunit of carp GtH (cGtHβ) and cGtH for the assay standard and tracer.

Analysis of results and statistical analysis

GtH data were expressed as mean values ± SE and were analysed using Duncan's multiple range test to compare the levels of GtH at different time PI ($P < 0.05$).

Table 1 Dosages and protocol of hormones and drugs used in the experiments

Hormone protocol	Experiment A	Experiment B	Samples
Control groups	Saline	Saline	8+8*
Treated groups			
DOM	5 μg g^{-1} BWt	5 μg g^{-1} BWt	8+8*
LHRH-A	Single: 0.01 μg g^{-1} BWt		8
LHRH-A	Two: 1st: 0.003 μg g^{-1} BWt 2nd: 0.007 μg g^{-1} BWt		8
LHRH-A	Single: 0.02 μg g^{-1} BWt		8
	Two: 1st: 0.01 μg g^{-1} BWt	0.01 μg g^{-1} BWt	8+7*
	2nd: 0.01 μg g^{-1} BWt	0.01 μg g^{-1} BWt	
LHRH-A+DOM	Single: (0.01+5) μg g^{-1} BWt		8
	Two: 1st: (0.005+2.5) μg g^{-1} BWt		8
	2nd: (0.005+2.5) μg g^{-1} BWt		
LHRH-A+DOM	Single: (0.01+7) μg g^{-1} BWt	(0.01+7) μg g^{-1} BWt	8+7*
	Two: 1st: (0.005+3.5) μg g^{-1} BWt		8
	2nd: (0.005+3.5) μg g^{-1} BWt		
CPE+LHRH-A	Two: 1st: (0.5 pituitary kg^{-1}+0.003 μg g^{-1}) BWt	Same as experiment A	8+8*
	2nd: (5 pituitary kg^{-1}+0.007 μg g^{-1}) BWt		
HCG+LHRH-A	Two: 1st: (0.1 IU g^{-1}+0.003 μg g^{-1}) BWt	Same as experiment A	8+8*
	2nd: (2 IU g^{-1}+0.007 μg g^{-1}) BWt		

DOM, domperidone; BWt, body weight; LHRH-A, [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone; CPE, carp pituitary extract; HCG, human chorionic gonadotropin; IU, International Units.
Samples with '*' are expressed as fish of experimental A plus experimental B, and others are fish of experimental A.

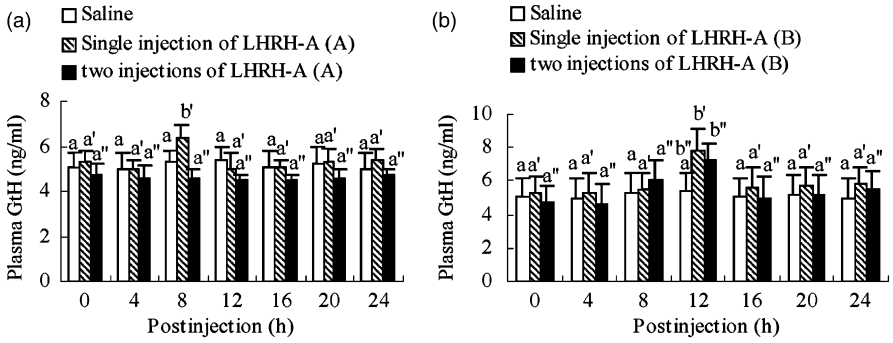

Figure 1 Serum gonadotropin (GtH) content induced by the injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) alone in wild female catfish in experiment A. Group of 'single injection of LHRH-A (A)' is injected once with LHRH-A at 0.01 μg g^{-1} body weight (BWt), group of 'two injections of LHRH-A (A)′ is injected twice with LHRH-A at 0.003 and 0.007 μg g^{-1} BWt, group of 'single injection of LHRH-A (B)′ is injected once with LHRH-A at 0.02 μg g^{-1} BWt, group of 'two injection of LHRH-A (B)′ is injected twice with LHRH-A at 0.01 and 0.01 μg g^{-1} BWt; control groups are injected with saline. GtH values are reported as mean ± SE, and groups with similar serum GtH levels are identified by the same superscript.

Results

Effects of LHRH-A alone on the plasma GtH content and ovulation in experiment A

The effects of one or two injections of LHRH-A alone on the serum GtH content and ovulatory rate are shown in Figs 1 and 2. At a dose of 0.01 μg g^{-1} BWt, a single injection of LHRH-A stimulated a low but significant serum GtH ($P < 0.05$) at 8 h PI. Two injections of LHRH-A (first: 0.003 μg g^{-1} BWt; second: 0.007 μg g^{-1} BWt) at 6-h intervals failed to induce a significant increase in serum GtH (Fig. 1a). Single (0.02 μg g^{-1} BWt) or two injections (first: 0.01 μg g^{-1} BWt; second: 0.01 μg g^{-1} BWt) of LHRH-A increased serum GtH ($P < 0.05$) at 12 h PI (Fig. 1b) significantly.

A single administration of 0.02 μg g^{-1} BWt LHRH-A resulted in an ovulatory rate of 37.5% within 12–16 h PI. However, two separate injections of LHRH-A

 © 2004 Blackwell Publishing Ltd,

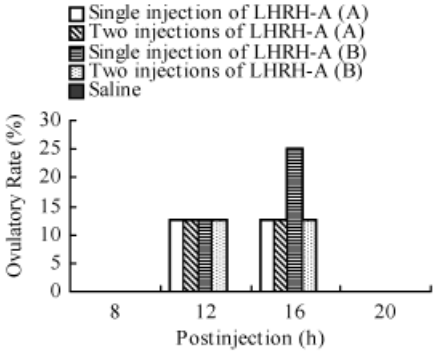

Figure 2 Ovulatory rate induced by once or twice injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) alone in wild female catfish in experiment A. Meaning of symbols as for Fig. 1.

at the same dose resulted in an ovulatory rate of 25.0% (Fig. 2). The ovulatory rate was similarly 25% when 0.01 μg g^{-1} BWt LHRH-A was given once or twice (Fig. 2).

Effect of LHRH-A plus DOM on the serum GtH content and the ovulation in experiment A

The effects of one or two injections of LHRH-A plus DOM on the plasma GtH content and ovulatory rate are shown in Figs 3 and 4. At a dose of 0.01 μg g^{-1} BWt LHRH-A plus 5 μg g^{-1} BWt DOM, a single simultaneous injection stimulated a significant increase in the serum GtH ($P < 0.05$) at 8 h PI (Fig. 3a); two simultaneous injections (first: 0.005 plus 2.5 μg g^{-1} BWt; second: 0.005 plus 2.5 μg g^{-1} BWt) at 6-h intervals failed to induce an increase in serum GtH ($P < 0.05$) (Fig. 3a). At a dose of 0.01 μg g^{-1} BWt LHRH-A plus 7 μg g^{-1} BWt DOM, a single simultaneous injection stimulated a significant increase in the serum GtH ($P < 0.05$) at 8 h PI (Fig. 3b). Two simultaneous injections (first: 0.005 plus 3.5 μg g^{-1} BWt; second: 0.005 plus 3.5 μg g^{-1} BWt) resulted in a siginificant increase in serum GtH at 12 h PI ($P < 0.05$) (Fig. 3b).

A single simultaneous administration of LHRH-A (0.01 μg g^{-1} BWt) plus DOM (5 μg g^{-1} BWt) resulted in an ovulatory rate of 100% within 8–12 h PI. However, two separate injections of LHRH-A plus DOM at the same dose of 0.005 plus 2.5 μg g^{-1} BWt resulted in an ovulatory rate of 75% within 8–16 h PI (Fig. 4). Single simultaneous injections of LHRH-A (0.01 μg g^{-1} BWt) plus DOM (7 μg g^{-1} BWt) resulted in an ovulatory rate of 100% within 8–12 h PI. Two separate injections of LHRH-A plus DOM at the same dose of 0.005 μg g^{-1} BWt plus 7 μg g^{-1} BWt resulted in an ovulatory rate of 100% within 8–16 h PI (Fig. 4).

Effect of single DOM alone, two LHRH-A plus CPE, two LHRH-A plus HCG on the serum GtH content and ovulation in experiment A

A single injection of DOM at a dosage of 5 μg g^{-1} BWt did not induce an increase in serum GtH (Fig. 5). Two

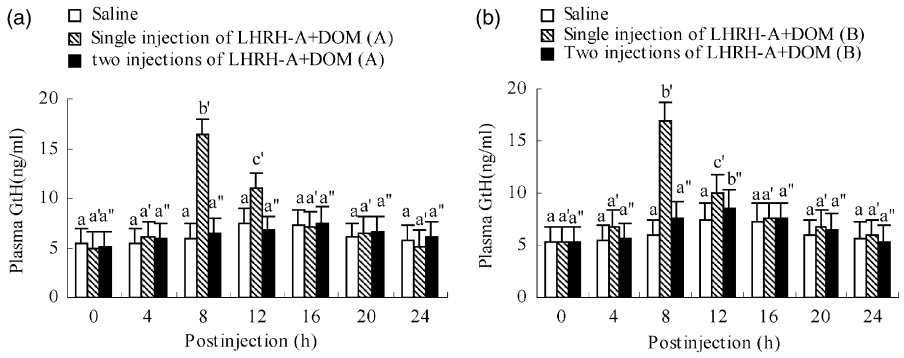

Figure 3 Serum gonadotropin (GtH) content induced by once or twice injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) plus domperidone (DOM) simultaneously in wild female catfish in experiment A. Group of 'single injection of LHRH-A+DOM (A)' is injected once simultaneously with LHRH-A plus DOM at 0.01 μg g^{-1} BWt LHRH-A plus 5 μg g^{-1} BWt DOM, group of 'two injections of LHRH-A+DOM (A)' is injected twice simultaneously with LHRH-A plus DOM at the same dosage of 0.005 μg g^{-1} BWt LHRH-A plus 2.5 μg g^{-1} BWt DOM. Group of 'single injection of LHRH-A+DOM (B)' is injected once simultaneously with LHRH-A plus DOM at 0.01 μg g^{-1} BWt LHRH-A plus 7 μg g^{-1} BWt DOM, group of 'two injections of LHRH-A+DOM (B)' is injected twice simultaneously with LHRH-A plus DOM at the same dosage of 0.005 μg g^{-1} BWt LHRH-A plus 3.5 μg g^{-1} BWt DOM; control groups are injected with saline. GtH values are reported as mean ± SE, and groups with similar serum GtH levels are identified by the same superscript.

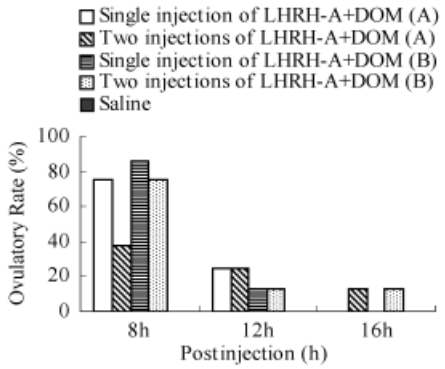

Figure 4 Ovulatory rate induced by once or twice injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) plus DOM simultaneously in wild female catfish in experiment A. Meanings of symbols as for Fig. 3.

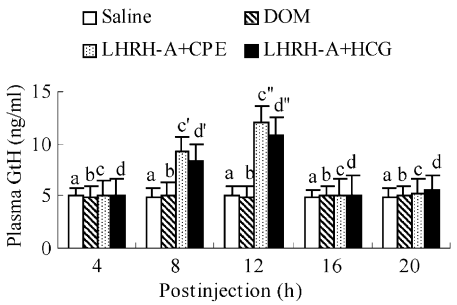

Figure 5 Serum gonadotropin (GtH) levels induced by single injection of domperidone (DOM) (5 μg g^{-1} body weight (BWt)) alone, single injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) (0.01 μg g^{-1} BWt) plus DOM (7 μg g^{-1} BWt) simultaneously, two injections of LHRH-A plus CPE (first: 0.003 μg g^{-1} BWt + 0.5 pituitary kg^{-1} BWt; second: 0.007 μg g^{-1} BWt + 5 pituitary kg^{-1} BWt) simultaneously, two injections of LHRH-A plus human chorionic gonadotropin (HCG) (first: 0.003 μg g^{-1} BWt + 0.1 IU g^{-1} BWt; second: 0.007 μg g^{-1} BWt + 2 IU g^{-1} BWt; IU, International Units) simultaneously in wild female catfish in experiment A. GtH values are reported as mean ± SE, and groups with similar serum GtH levels are identified by the same superscript.

simultaneous injections of CPE plus LHRH-A (first: 0.5 pituitary kg^{-1} BWt and 0.003 μg g^{-1} BWt; second: 5 pituitary kg^{-1} BWt and 0.007 μg g^{-1} BWt) at 6-h intervals induced a significant increase in serum GtH ($P < 0.05$) at 8 h PI (Fig. 5). Two simultaneous injections of LHRH-A plus HCG (first: 0.003 μg g^{-1} BWt plus 0.1 IU g^{-1} BWt; second: 0.007 μg g^{-1} BWt plus 2 IU g^{-1} BWt) at 6-h intervals resulted in a significant increase in serum GtH ($P < 0.05$) at 8 h PI, with the highest value at 12 h PI (Fig. 5).

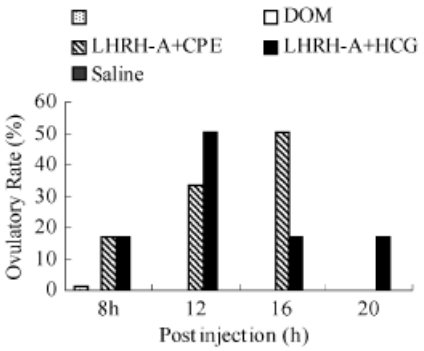

Figure 6 Ovulatory rate induced by single injection of domperidone (DOM) alone, single injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) plus DOM simultaneously, two injections of LHRH-A plus carp pituitary extract (CPE) simultaneously, two injections of LHRH-A plus human chorionic gonadotropin (HCG) simultaneously in wild female catfish in experiment A. Dosage of hormones and drugs as for Fig. 5.

A single injection of DOM alone was ineffective to induce ovulation. Two simultaneous injections of LHRH-A plus CPE resulted in an ovulatory rate of 100% within 8–16 h PI, while two simultaneous injections of LHRH-A plus HCG resulted in an ovulatory rate of 100% within 8–20 h PI (Fig. 6).

Effect of single injection of DOM alone, two injections of LHRH-A alone, single injection of LHRH-A plus DOM, two injections of LHRH-A plus CPE, two injections of LHRH-A plus HCG on the serum GtH levels and ovulation in experiment B

A single injection of DOM (5 μg g^{-1} BWt) alone did not stimulate a significant increase in serum GtH ($P < 0.05$). Two injections of LHRH-A at the same dosage of 0.01 μg g^{-1} BWt at 6-h intervals induced an increase in serum GtH (Fig. 7a). However, single simultaneous injections of LHRH-A (0.01 μg g^{-1} BWt) plus DOM (7 μg g^{-1} BWt) resulted in a dramatic increase in the serum GtH, and a peak value of GtH occurred at 8 h rather than 12 h PI (Fig. 7a). Two simultaneous injections of LHRH-A plus CPE (first: 0.003 μg g^{-1} BWt plus 0.5 pituitary kg^{-1} BWt; second: 0.007 μg g^{-1} BWt plus 5 pituitary kg^{-1} BWt) at 6-h intervals induced a significant increase in serum GtH ($P < 0.05$) and the highest value occurred at 8 h PI **(**Fig. 7b), while two simultaneous injections of

 © 2004 Blackwell Publishing Ltd,

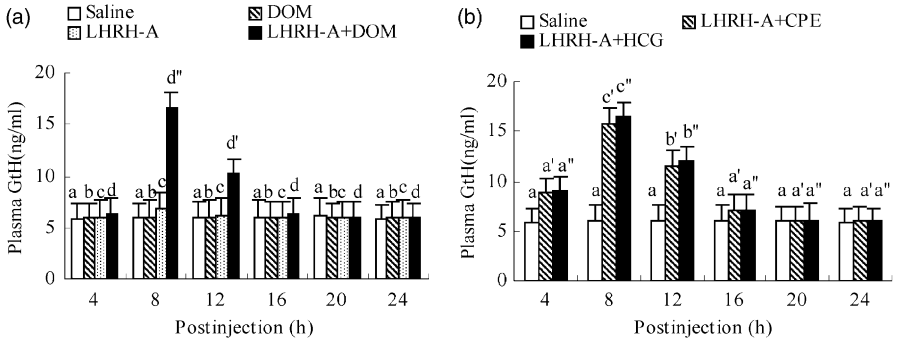

Figure 7 Serum gonadotropin (GtH) levels induced by single injection of domperidone (DOM) (5 μg g^{-1} body weight (BWt)) alone, single injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) (0.02 μg g^{-1} BWt) alone, single injection of LHRH-A (0.01 μg g^{-1} BWt) plus DOM (7 μg g^{-1} BWt) simultaneously, two injections of LHRH-A plus carp pituitary extract (CPE) (first: 0.003 μg g^{-1} BWt + 0.5 pituitary kg^{-1} BWt; second: 0.007 μg g^{-1} BWt + 5 pituitary kg^{-1} BWt) simultaneously, two injections of LHRH-A plus human chorionic gonadotropin (HCG) (first: 0.003 μg g^{-1} BWt + 0.1 IU g^{-1} BWt; second: 0.007 μg g^{-1} BWt + 2 IU g^{-1} BWt; IU, International Units) simultaneously in wild female catfish in experiment B. GtH values are reported as mean ± SE, and groups with similar serum GtH levels are identified by the same superscript.

LHRH-A plus HCG (first: 0.003 μg g^{-1} BWt plus 0.1 IU g^{-1} BWt; second: 0.007 μg g^{-1} BWt plus 2 IU g^{-1} BWt) at 6-h intervals resulted in a significant increase in serum GtH ($P < 0.05$), with the highest value of GtH at 8 h PI (Fig. 7b).

Two administrations of LHRH-A alone resulted in an ovulatory rate of 37.5% within 12–16 h PI, whereas a single simultaneous injection of LHRH-A plus DOM resulted in an ovulatory rate of 100% within 8–12 h PI (Fig. 8). Two simultaneous injections of LHRH-A plus CPE resulted in an ovulatory rate of 87.5% within 12–20 h PI, while two simultaneous injections of LHRH-A plus HCG resulted in an ovulatory rate of 100% within 12–24 h PI (Fig. 8).

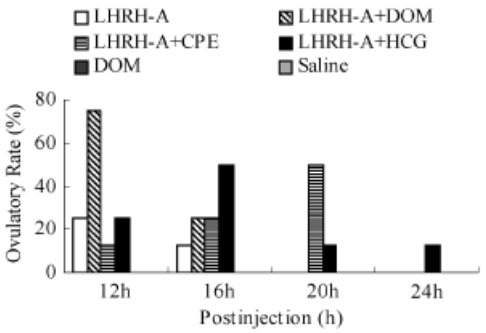

Figure 8 Ovulatory rate induced by single injection of domperidone (DOM) alone, single injection of [D-Ala^6, Pro^9 Net]-luteinizing hormone-releasing hormone (LHRH-A) alone, single injection of LHRH-A plus DOM simultaneously, two injections of LHRH-A plus carp pituitary extract (CPE) simultaneously, two injections of LHRH-A plus human chorionic gonadotropin (HCG) simultaneously in wild female catfish in experiment B. Dosage of hormones and drugs as for Fig. 7.

Discussion

Dose–response competitive inhibition for cGtH and serum GtH from catfish indicated a strong parallelism between the displacement by the standard cGtH and the GtH present in the samples. The results demonstrated that cGtH RIA was valid for catfish; indeed, heterogenetic RIA have been used successfully for GtH determination from many fish, including the European eel *Anguilla anguilla* (Linnaeus, 1758) (Dufour, Belle & Fontaine 1983), Chinese loach (Lin *et al.* 1985), African catfish (Goos, De Leeuw, Burawa-Gevard, Terlou & Richter 1986), bagrid catfishes *Mystus macropterus* (Bleeker, 1870) (Wang *et al.* 1998) and *S. asotus* (Wen & Lin 2001). This RIA was a useful tool for reproductive endocrine studies in some freshwater teleost.

In experiment A, two injections of LHRH-A alone somewhat stimulated an increase in the serum GtH. A single injection of LHRH-A (0.01 or 0.02 μg g^{-1} BWt) induced a low but significant increase in serum GtH, but was a relatively ineffective treatment for the induction of ovulation because of an ovulatory rate of 25%. The results were consistent with goldfish and loach. In female goldfish, a single or two injections of LHRH-A given 12 h apart increased the serum GtH concentrations, but did not stimulate GVBD or ovulation (Chang & Peter 1983), whereas injection of

LHRH-A alone at a dosage of 0.1 μg g^{-1} BWt increased serum GtH levels, but was relatively ineffective for inducing ovulation with an ovulatory rate of 25% in goldfish (Sokolowska, Peter, Nahorniak, Nahorniak & Chang 1984). In Chinese loach, injection of LHRH-A alone stimulated an increase in serum GtH, but failed to cause ovulation (Lin *et al.* 1985, 1986). Injection of LHRH-A or sGnRH alone was ineffective in inducing ovulation in common carp (Lin *et al.* 1988), but, in other cyprinids (Ngamvongchon, Pawaputance, Leelatra & Johnson 1988), it was found that two injections of LHRH-A were effective for induction of ovulation in silver carp *Hypophthalichthys molitri* (Cuvier et Valenciennes, 1844) and bighead carp *Aristichthys nobilis* (Richardson, 1845), resulting in an ovulatory rate of 100%.

In catfishes (Siluriformes), the responsiveness of plasma GtH levels and the ovulation to LHRH-A varied among species or individuals. In European catfish *Silurus glanis* (Linnaeus, 1758) (Brzuska & Adamek 1999), ovulation was stimulated by the injection of LHRH-A at a dosage of 20 μg kg^{-1}, with an ovulatory rate of 100%. In Gunther's walking catfish *Clarias macrocephalus* (Günther, 1864), it was also found that a single injection of LHRH-A induced ovulation within 16–18 h PI, with an ovulatory rate of 70% (Ngamvongchon *et al.* 1988), whereas Tan-Fermin, Pagador & Chavez (1997) did not induce ovulation by injection of LHRH-A at a dosage of 0.05 μg g^{-1} BWt at any phase of the reproductive cycle in the same catfish. In Asian catfish *Clarias batrachus* (Linnaeus, 1758), a single injection of LHRH-A at a dosage of 0.05 μg g^{-1} BWt did not induce ovulation.

In experiment B, two administrations of LHRH-A at the same dosage of 0.01 μg g^{-1} BWt induced an increase in plasma GtH, but with no significant difference, resulting in an ovulation rate of 37.5%. The results from experiments A and B indicated that there was a seasonal variation in responsiveness of plasma GtH and ovulation of *S. asotus* to LHRH-A. These seasonal changes may be due to the difference in GtH content of the pituitary, the ability of the pituitary to synthesize GtH or changes in GtH receptors for GnRH and DA or a combination of these and other unknown factors (De Leeuw *et al.* 1985). A similar result was observed in African catfish (Goos *et al.* 1987). LHRH-A treatment was found to be more effective in spring close to the breeding season in nature, possibly due to the high sensitivity of GtH cells to LHRH-A during that period.

Combination injection of LHRH-A and DOM enhanced serum GtH levels significantly in female catfish no matter what gonadal development stage, and the serum GtH surge occurred at 12 h PI (at 20–25 °C) and at 8 h PI (at 30 °C), whereas injection of DOM alone did not stimulate a significant increase in serum GtH and failed to induce ovulation. These present results indicate that GtH secretion in *S. asotus* is under the dual control of GnRH and DA release from the hypothalamus and DA can only inhibit GtH release induced by GnRH and not inhibit spontaneous (basal) GtH release. This result is consistent with the results in African catfish (De Leeuw, Goos & Van oordt 1987). The inhibitory actions of DA on GtH secretion can vary in potency between species. For example, in the goldfish, the carp and the catfish (Lin & Peter 1996), DA inhibition of GtH secretion was strong. Injection of a DA blocker PIM is considered to have antagonistic properties for both D_1 and D_2 receptors, DOM is considered to be a D_2 receptor antagonist (Van Asselt, Goos, Smit-Van Dijk, Speetjens & Van Oordt 1988), resulting in potentiation of the action of GnRH analogue, leading to a large release of GtH and ovulation. These results coincide with our experimental data in *S. asotus*. On the other hand, in bream *Parabramis pekinensis* (Basilewsky, 1855), the DA inhibitory tone on GtH secretion was not strong. Injection of a high dose of LHRH-A or sGnRH-A alone overcomes dopaminergic inhibition, and was effective in stimulating GtH release and ovulation (Lin & Peter 1996). DOM was of equal or somewhat greater potency than PIM in potentiating the action of releasing hormone agonists in the loach; however, DOM had a markedly greater effectiveness in common carp (Lin *et al.* 1988). Injection of LHRH-A or sGnRH-A alone stimulated only a modest increase in the serum GtH levels and was ineffective in inducing ovulation of goldfish and carps (Sokolowska *et al.* 1985a, b; Peter *et al.* 1988). The administration of PIM or DOM greatly potentiated the action of LHRH-A and sGnRH-A on GtH release, and combined injections of PIM or DOM and LHRH-A or sGnRH-A are highly effective in inducing ovulation and spawning in these species. A formulation of injection of sGnRH-A (or LHRH-A) and DOM (or PIM) was termed the Linpe technique and has proven particularly useful, with those species having synchronous or group synchronous follicular development and a large preovulatory surge of GtH (Peter & Yu 1997).

In recent years, the Linpe technique has been used gradually for catfishes to induce ovulation and supported excellent results. In European catfish, injection of Ovaprim (contains sGnRH-A and DOM) at a dosage of 0.33 mL kg^{-1} BWt resulted in 80% ovulating

 © 2004 Blackwell Publishing Ltd,

females (Brzuska & Adamek 1999). In Asian female catfish *Pangasius hypophthamlus* (Sauvage, 1878), an ovulatory rate of 88% was obtained when fish were injected with Ovaprim at a dosage of 0.3–0.6 mL kg^{-1} BWt. These ovulatory rates were lower than those observed in our experiments, perhaps due to a difference in water temperature, gonadal development stages, species sensitivity difference and other factors. To compare the Linpe technique to traditional methods, e.g. two injections of CPE plus LHRH-A or HCG plus LHRH-A, whose dosage and timing were chosen because they were commonly used in Chinese fry production units in catfishes (Pan *et al.* 1992; Wei & Huang 1997). The plasma GtH containing exogenous hormone in treatments of CPE plus LHRH-A and HCG plus LHRH-A was very similar to that observed with the Linpe technique and the ovulatory rate was close to that of the Linpe technique. However, the latency time within 8–24 h according to the traditional method was difficult to forecast and the duration was longer than that in the Linpe technique (within 8–12 h according to water temperature). In Gunther's walking catfish, injection of HCG at a dosage of 4000 IU kg^{-1} BWt or CPE at a dosage of 1 pituitary kg^{-1} BWt resulted in an ovulatory rate of 100% within 16–18 h (Ngamvongchon *et al.* 1988). In European catfish, injection of CPE at a dosage of 4 mg kg^{-1} BWt induced ovulatory rate of 60% (single injection) and 66.7% (two injections) respectively (Brzuska & Adamek 1999). In Japan, the HCG injection alone at a dosage of 10 μg g^{-1} BWt resulted in an average ovulatory rate of 93.3%, but ovulation times varied among individuals in *S. asotus* (Miwa *et al.* 2001). In Asian catfish, injection of HCG alone at a dosage of 2000 IU kg^{-1} BWt resulted in an ovulatory rate of 90%, but ovulatory time was difficult to predict accurately (Legendre, Slembrouck, Subagja & Kristanto 2000). In catfish, the ovulation rate induced by HCG or CPE alone was close to or somewhat lower than our results obtained using HCG plus LHRH-A or CPE plus LHRH-A. This may demonstrate that the administration of LHRH-A greatly potentiated the action of HCG or CPE effects of induction ovulation, but the mechanisms have not been expounded thoroughly.

In conclusion, the Linpe technique compared with the so-called traditional methods had a high predictability of time from injection to ovulation, decreased stress on broodstock because only a single injection was needed and absence of side effects on subsequent reproductive cycles of *S. asotus*.

Acknowledgments

This work was supported by Grants 39970586 from the National Natural Science Foundation of China and by Grants 980308 from the Natural Science Foundation of Guangdong Province.

References

Brzuska E. & Adamek J. (1999) Artificial spawning of European catfish, *Silurus glanis*: stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary exact. *Aquaculture Research* **30**, 59–64.

Chang J.P. & Peter R.E. (1983) Effects of pimozide and des Gly^{10} [D-Ala]luteinizing hormone-releasing hormone ethylamide on serum gonadotropin concentration, germinal vesicle migration and ovulation in female goldfish, *Carassiu auratus*. *General and Comparative Endocrinology* **52**, 30–37.

De Leeuw R., Resink J.W., Rooyakkers E.J.W. & Goos H.J.T. (1985) Pimozide modulates the luteinizing hormone–releasing hormone effect on gonagotropin release in the African catfish, *Clarias lazera*. *General and Comparative Endocrinology* **58**, 120–127.

De Leeuw R., Goos H.J.Th. & Van oordt P.G.W.J. (1987) The regulation of gonadotropin release by neurohormones and gonadal steroids in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 43–58.

Dufour S., Belle N.D.L. & Fontaine A.F. (1983) Development of a heterologous radioimmunaassay for eel (*Anguilla anguilla*) gonadotropin. *General and Comparative Endocrinology* **49**, 404–413.

Goos H.J.Th., De Leeuw R., Burawa-Gevard E., Terlou M. & Richter C.J.J. (1986) Purification of gonadotropin hormone from the pituitary of the African catfish, *Clarias gariepinus*, and the development of a homologous radioimmunoassay. *General and Comparative Endocrinology* **63**, 162–170.

Goos H.J.Th., Joy K.P., De Leeuw R., Van Oordt P.G.W.J., Van Delft A.M.L. & Gielen J.T. (1987) The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with antidopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 143–156.

Legendre M., Slembrouck J., Subagja J. & Kristanto A.H. (2000) Ovulation rate, latency period and ova viability after GnRH- or hCG-induced breeding in the Asian catfish *Pangasius hypophthalmus* (Siluriformes, Pangasiidae). *Aquaculture Living Resources* **13**, 145–151.

Lin H.R. & Peter R.E. (1996) Hormones and spawning in fish. *Asian Fisheries Science* **9**, 21–33.

Lin H.R., Peng C., Lu L.Z., Zhou X.J., Van Der Kraa G. & Peter R.E. (1985) Induction of ovulation in the loach *(Paramisgurnus dabryanus)* using pimozide and [D-Ala^6, Pro^9-N-ethylamide]-LHRH. *Aquaculture* **46**, 333–340.

Lin H.R., Peng C., Van Der Kraak G., Peter R.E. & Breton B. (1986) Effects of [D-Ala^6, Pro^9-N-ethylamide]-LHRH and catecholaminergic drugs on gonadtropin secretion and ovulation in the Chinese loach (*Paramisgurnus dabryanus*). *General and Comparative Endocrinology* **64**, 389–395.

Lin H.R., Van Der Krrak G., Zhou X.J., Liang J.Y., Peter R.E., Rivier J.E. & Vale W.W. (1988) Effects of [D-Arg^6, trp^7, Leu^8, Pro^{9}Net]-luteinizing hormone- releasing hormone (sGnRH-A) and [D-Ala^6, Pro^{9}Net]-luteinizing hormone-releasing hormone (LHRH-A), in combination with pimozide or domperidone, on gonadotropin release and ovulation in the Chinese loach and common carp. *General and Comparative Endocrinology* **69**, 31–40.

Lin H.R., Zhou X.J., Van Der Kraak G. & Peter R.E. (1991) Effects of gonadotropin-releasing hormone agonists and dopamine antagonists on gonadotropin secretion and ovulation in Chinese loach *(Paramisgurnus dabryanus*). *Aquaculture* **95**, 139–147.

Liu H.L., Pu H.Y., Hu Zh.W. & Luo X.N. (1998) Critical technique for artificial propagation of catfish (*Silurus asotus*). *Journal of Dalian Fisheries University* **13**, 1–8 (in Chinese).

Miwa M., Yoshizaki G., Naka H., Nakatani M., Sakai K., Kobayashi M. & Takeuchi T. (2001) Ovarian steroid synthesis during oocyte maturation and ovulation in Japanese catfish (*Silurus asotus*). *Aquaculture* **198**, 179–191.

Ngamvongchon S., Pawaputance O., Leelatra W. & Johnson W.E. (1988) Effectiveness of a LHRH analogue for the induced spawning of carp and catfish in northeast Thailand. *Aquaculture* **74**, 35–40.

Pan W.Zh., Guo J.Sh., Tian F.Sh., Wang Zh.B., Tian B.W., Yan J.Y. & Zhou P.Y. (1992) Artificial spawning of catfish (*Parasilurus asotus*). *Journal of Fisheries of China* **16**, 278–281 (in Chinese).

Peter R.E. (1982) Gonadotropin secretion during reproduction cycles in teleosts: influences of environmental factors. *General and Comparative Endocrinology* **45**, 294–305.

Peter R.E. & Yu K.L. (1997) Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Fish Biology and Fisheries* **7**, 173–197.

Peter R.E., Lin H.R. & Van Der Kraak G. (1988) Induced ovulation and spawning of cultured freshwater fish in Chain: advances in application of GnRH analogues and dopamine antagonists. *Aquaculture* **74**, 1–10.

Shirai N., Suzuki H., Toukairin S. & Wada S. (2001) Spawning and season affect lipid content and fatty acid composition of ovary and liver in Japanese catfish (*Silurus asotus*). *Comparative Biochemistry and Physiology Part B* **129**, 185–195.

Sokolowska M., Peter R.E., Nahorniak C.S., Nahorniak C.H. & Chang J.P. (1984) Induction of ovulation in goldfish, *Carassius auratus*, by pimozide and analogues of LH-RH. *Aquaculture* **36**, 71–83.

Sokolowska M., Peter R.E. & Nahorniak C.S. (1985a) The effects of different doses of pimozide and [D-Ala^6, Pro^9-ethylamide]-LHRH (LHRH-A) on gonadotropin releasing and ovulation in female goldfish. *Canadian Journal of Zoology* **63**, 1252–1256.

Sokolowska M., Peter R.E., Nahorniak C.S. & Chang J.P. (1985b) Seasonal effects of pimozide and des- Gly^{10} [D -Ala^6] LHRH ethylamide on gonadotropin secretion goldfish. *General and Comparative Endocrinology* **57**, 472–479.

Tan-Fermin J.D., Pagador R.R. & Chavez R.C. (1997) LHRHa and pimozide-induced spawning of Asian catfish *Clarias macrocephalus* (Gunther) at different time during an annual reproductive cycle. *Aquaculture* **148**, 323–331.

Van-Asselt L.A.C., Goos H.J.Th., Smit-Van Dijk W., Speetjens P.A.M. & Van Oordt P.G.W.J. (1988) Evidence for the involvement of D-2 receptors in the dopaminergic inhibition of gonadotropin release in the African catfish, *Clarias gariepinus. Aquaculture* **72**, 369–378.

Van Der Kraak G., Donadson E.M. & Chang J.P. (1986) Dopamine involvement in the regulation of gonadotropin secretion in coho salmon. *Canadian Journal of Zoology* **64**, 1245–1248.

Van Oordt P.G.W.J. & Goos H.J.Th. (1987) The African catfish, *Clarias gariepinus*, a model for the study of reproductive endocrinology in teleosts. *Aquaculture* **63**, 15–26.

Wang D.Sh., Lin H.R. & Goos H.J.Th. (1998) Studies on the regulation of gonadotropin secretion in the bagrid catfish, *Mystus macropterus. Acta Zoologica Sinica* **44**, 322–328 (in Chinese).

Wei G. & Huang L. (1997) Studies on the reproductive biology of *Silurus asotus. Journal of Fisheries of China* **21**, 225–232 (in Chinese).

Wen H.Sh. & Lin H.R. (2001) Effects of LHRH-A and domperidone on gonadotropin releasing in *Silurus asotus. Journal Fisheries of China* **25**, 393–397 (in Chinese).

 © 2004 Blackwell Publishing Ltd,