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Temperature effects along the reproductive axis during spawning induction of grass carp (*Ctenopharyngodon idella*)

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Abstract

For grass carp (*Ctenopharyngodon idella*) raised in the Ivory Coast (with water temperatures of 26–31 °C), induced spawning is obligatory for fry production. However, ovulation rates following hormonal treatment are often low. We hypothesized that high temperatures are an inhibiting factor for the reproductive axis (brain–pituitary–gonad) in these conditions. By in vivo and in vitro experiments, we tried to determine the thermosensitive steps during spawning induction. We compared gonadotropin and maturation-inducing steroid (MIS) profiles during a spawning induction at controlled temperatures of 24 and 28 °C in relation to ovulation success. We performed pituitary cell cultures and ovarian fragment incubations at controlled temperatures. The ovulation rate was lower at 28 °C (10%) than at 24 °C (36%). At the pituitary level, we found only minor thermal impacts on GnRH-stimulated LH release, but our data suggest an increase of the dopaminergic inhibition by high temperatures. The main effects were found at the ovary level, where ovary responsiveness to gonadotropin by MIS synthesis was disturbed, as well as oocyte responsiveness to MIS triggering final maturation, and probably ovulation. These results show the importance of regulating temperature during spawning induction to ensure a high rate of ovulation.
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Keywords: Steroids; Gonadotropin; GnRH; Dopamine; Temperature; Oocyte maturation; Spawning induction

1. Introduction

This work is part of a study aimed at optimizing artificial reproduction of grass carp (*Ctenopharyngodon idella*) under tropical conditions, namely in small-scale fish farming in the Ivory Coast. Grass carp are raised in earthen ponds, with water temperatures between 26 and 31 °C. Spawning induction is obligatory for fry production. Under these conditions, sexual cycles are disrupted (Glasser et al., 2003) and ovulation rates after pituitary extract injections are very low (less than 15%) from January to June. Artificial spawning can be achieved with acceptable success rates only from July, when the mean water temperature drops below 28 °C.

In fish, temperature is a major physiological regulator (Lam, 1983; Stacey, 1984), that can influence reproduction at different levels of the reproductive axis (brain–pituitary–gonad) (Stacey, 1984). Temperature has been regarded as the main determinant of reproduction in the cyprinid family (to which the grass carp belongs) (Billard et al., 1978). In grass carp, natural reproduction occurs from 17 to 26 °C (Krykhtin and Gorbach, 1982), and optimal temperatures for artificial reproduction lie between 22 and 26 °C (Bardach et al., 1972; Horvath et al., 1984). In Egypt, a drop in induced ovulation rates is observed above 27 °C (Rottman and Shireman, 1985), and no ovulation can be obtained above 30 °C (Zonneveld, 1984). Therefore, we hypothesized that temperature could be a major limiting factor for grass carp reproduction in tropical areas and this study aimed at determining the thermosensitive steps and mechanisms during spawning induction in this species.

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Endocrine mechanisms triggering final maturation and ovulation have been widely studied in cyprinids. Reproduction in teleosts is mainly under the control of gonadotropins. In cyprinids, a sharp peak of LH (luteinizing hormone) has been observed at the time of maturation and ovulation (Aida, 1988; Santos et al., 1986; Stacey et al., 1979). A first response of ovarian follicles to this gonadotropine surge is an extensive change in steroid secretion patterns. Steroid profiles during final maturation (resumption of oocyte meiosis) and ovulation have been described as a succession of peaks of testosterone, 17-α-progesterone and 17,20-β-dihydroxy-4-pregnen-3-one, often referred to as MIS (maturation inducing steroid) (Aida, 1988; Kagawa et al., 1983; Santos et al., 1986). Steroid synthesis in the ovarian follicle is the result of a cooperation between the different cell layers (granulosa and theca) enclosing the oocyte (Young et al., 1986). The MIS acts through a membrane receptor to trigger oocyte maturation, visualized by germinal vesicle breakdown. Hence, follicular maturational competence has several components, among which the aptitude of follicular cells to respond to gonadotropin by producing MIS, and the aptitude of oocytes to respond to MIS by maturing (Jalabert et al., 1977). Ovulation is the result of complex processes involving various factors in the whole follicle. However, these processes are probably triggered first by MIS (Goetz and Theofan, 1979).

Gonadotropins are released by the pituitary, mainly under the positive control of GnRH (gonadotropin-releasing hormone), directly secreted by hypothalamic neurons. The main central gonadotropin inhibitor is dopamine. Since final maturation and ovulation are the main blocking steps for fish reproduction in fish farming, many techniques have been developed to trigger them (Yaron, 1995; Zohar and Mylonas, 2001). One of the most common is the injection of a combination of GnRH and an antidopaminergic drug (Peter et al., 1988). Analogs of GnRH are more active than native GnRH forms for induced ovulation (Peter et al., 1987, 1988) and increase the duration and intensity of LH release (Weil et al., 1992).

To determine the potential effects of temperature on the reproductive axis, we compared spawning inductions at 24 °C (considered as optimal for grass carp) and 28 °C (mean temperature in the Ivory Coast, and probably above a thermal threshold for induced spawning) and assessed in vitro pituitary cell response to GnRH as well as in vitro ovarian response to pituitary extract at various temperatures.

2. Methods

2.1. Spawning induction

The experiment aimed at comparing the influence of conditioning at high (28 °C) or low (24 °C) temperatures on endocrinal profiles and ovulation in grass carp following spawning induction treatment. This study was performed at the Goslawice hatchery (Poland).

Female grass carp originating from heated ponds under natural photoperiod were kept at both experimental temperatures (24 and 28 °C) for a week before the normal spawning date (first week of June), in 1-m^3 tanks. Groups of 5–6 fish were randomly assigned to different injection protocols. The fish were weighed (mean body weight 7.9 ± 2.1 kg) and individually tagged at the beginning of the experiment. For each temperature, four injection protocols were performed:

- *control*: NaCl solution at 0.9%,
- *antidopaminergic*: pimozide (Sigma, St. Louis, MO), at 5 mg/kg body weight (BW),
- salmon GnRH analog (des-Gly^{10}, D-Arg^6-sGnRH-Ethylamide, Saxon Biochemical GmBH, Hanover), hereafter GnRH, at 20 μg/kg BW,
- a combination of pimozide (5 mg/kg BW) and GnRH (20 μg/kg BW).

Each treatment consisted of two injections, 24 h apart (a priming dose of 10% and a resolving dose of 90% of total dose). The experiment began at 06:00 local time. Ovarian biopsies by pipelle de Cornier (Laboratoire CCD, Paris) were performed before each injection, to assess germinal vesicle migration (central, beginning or end of migration) after clearing in Serra's solution. Blood was sampled just before the priming (T_0) and 6, 12, 24 (second injection), and 30 h after. Blood samples were centrifuged, and the plasmas were frozen at −20 °C. Ovulations were checked at each sampling time and every hour from 30 h after priming. Ovulations were considered as complete if stripped oocytes weighed more than 5% of female weight. All manipulations were performed under anesthesia by 2-phenoxyethanol (100 ppm).

2.2. Pituitary cell culture

The effect of temperature on LH release was assessed by grass carp pituitary cell culture at 24 and 28 °C. Cell culture was performed according to a protocol developed for rainbow trout (Weil et al., 1986). Four pituitaries sampled from the control fish (35 kg total weight) of the spawning experiment at 28 °C were used for this study. After dispersion, cells were put into 96-well microplates, at 50,000 cells/well, preincubated for 48 h at 32 °C, rinsed twice, and preincubated for 12 h at 24 or 28 °C. Then, for each temperature, a control and five concentrations of salmon GnRH (from 10^{-10} to 10^{-6} M) were compared after incubation for 3 and 12 h. The incubation medium was sampled after centrifugation of plates (200 g), and frozen at −20 °C until analysis.

2.3. In vitro ovary incubations

Thermal effects on ovarian MIS production and maturation were assessed by ovarian fragment incubation at various temperatures, in the presence of common carp pituitary extract. The extract was obtained by suspension of common carp acetone-dried pituitary powder in 0.9% NaCl, followed by centrifugation to eliminate cell fragments. The doses are expressed as dry weight of pituitary powder per saline volume. Three grass carps in the Ivory Coast were killed 12 h after an injection with a priming dose of 0.3 mg pituitary extract/kg BW. Their ovaries were removed, chopped, and fragments incubated at the rate of 500 mg for 1 ml incubation medium (Jalabert, 1976). Incubations were performed at 24, 28, and 32 °C, in triplicate on 24-well plates.

The kinetics of MIS concentration in the incubation medium were studied in response to 20 μg/ml of pituitary extract. The incubation duration varied from 2 to 24 h. The influence of pituitary extract dose on MIS secretion was studied with incubations of 8 h and with pituitary extract doses from 1.25 to 80 μg/ml. Incubations were performed in triplicate on 24-well plates.

The influence of pituitary extract dose on oocyte maturation (germinal vesicle break-down: GVBD) was assessed on ovaries from one female, with 500 mg of ovary incubated in 500 μl of incubation medium, since no maturation was obtained with 500 mg of ovary in 1 ml of medium. Maturation rate was determined after 12 h.

Incubation medium samples were frozen at −20 °C until assay.

2.4. Sample assays

LH assay was performed by ELISA (Kah et al., 1989), using an antibody raised against the common carp LH β-subunit (Breton et al., 1983), whose specificity in grass carp was assessed by serial dilutions of female plasma. After linearization by logit function, the dilution curve obtained was parallel to the standard curve of common carp LH, confirming the validity of the assay for grass carp.

Steroid assays were performed by RIA (Fostier et al., 1978, 1981). Incubation medium samples were assayed directly, whereas for plasma samples, steroids were extracted with cyclohexane:ethyl acetate (1:1) before measurement.

2.5. Statistical analyses

For spawning inductions, LH plasma levels were studied by a profile approach: the statistical analysis was performed by non-parametric tests on the variations of individual levels between two successive samplings. We used Kruskall–Wallis analysis of variance to assess factor influence, and if a significant difference appeared, then Mann–Whitney *U* tests to evaluate differences between groups. ANOVA was used for in vitro ovary incubations, with Scheffé post hoc tests. Significant differences were accepted with a risk of 5%, and data are presented as means ± standard deviation.

3. Results

3.1. Spawning induction

At the beginning of the experiment, estradiol, testosterone, and LH plasma levels were higher in females kept at 24 °C (Table 1). However, there was no difference between temperatures concerning the stage of germinal vesicle (GV) migration: females had 18% of oocytes with a central GV, 74% at the beginning and 8% at the end of GV migration.

There was a significant effect of temperature on ovulation rate following spawning induction (Table 2). The overall ovulation rates were 36% at 24 °C and 10% at 28 °C. There was also an interaction between temperature and the induction protocol, since GnRH alone was effective only at 24 °C. Seven ovulations out of 10 occurred before the second injection. There was no re-

Table 1
Plasma levels (estradiol, testosterone, LH) and germinal vesicle (GV) migration in females grass carp at the beginning of the spawning induction, after a week of thermal conditioning at 24 °C or 28 °C

	Temperature (°C)	
	24	28
Estradiol (ng/ml)	0.46 ± 0.37^{a}	0.35 ± 0.38^{b}
Testosterone (ng/ml)	4.49 ± 2.28^{a}	2.42 ± 1.73^{b}
LH (ng/ml)	8.1 ± 2.4^{a}	5.6 ± 1.9^{b}
GV migration		
Central	$19 \pm 9\%^{a}$	$17 \pm 11\%^{a}$
Beginning	$73 \pm 7\%^{a}$	$76 \pm 9\%^{a}$
End	$8 \pm 6\%^{a}$	$7 \pm 6\%^{a}$

Values are means ± standard deviation. Same superscripts indicate non-significant difference between temperatures.

Table 2
Grass carp ovulations according to temperature (24 and 28 °C) and induction protocol

	Control	Pim	GnRH	Pim + GnRH
24 °C	0/5	0/6	3/6	5/5
28 °C	0/5	0/6	0/6	2/4

Control: NaCl; Pim: pimozide at 5 mg/kg; GnRH: D-Arg^{6}-sGnRH-A at 20 μg/kg; Pim + GnRH: combination of pimozide (5 mg/kg) and D-Arg^{6}-sGnRH-A (20 μg/kg). Numbers indicate ovulated females/total female number in the group (due to some mortality during fish manipulations, numbers differ between groups).

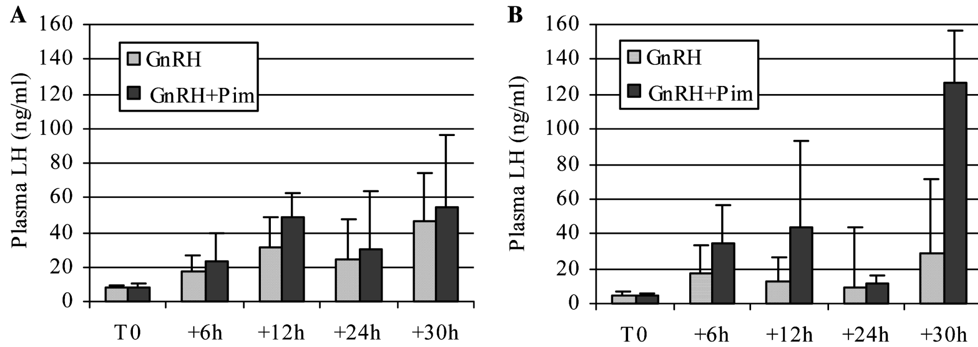

Fig. 1. Plasma LH levels during spawning induction, according to induction protocol and temperature. (A) 24 °C; (B) 28 °C (see Table 2 for induction protocols). A priming dose (10%) was injected at the beginning and the resolving dose (90%) was injected after 24 h.

lation between individual initial plasma levels or GV migration and the result of spawning induction.

For both control groups, plasma LH did not significantly vary according to temperature or sampling time and was always lower than 10 ng/ml. For females injected with pimozide alone, LH levels were also constantly low for the first 24 h. After the second injection, two females in each temperature group exhibited a rise in LH up to 30 ng/ml: there was no temperature effect either. We will thus focus on the results of the GnRH and GnRH + Pim groups (Figs. 1 and 2).

Six hours after the priming injection, there was no significant difference in plasma LH increase among the four groups (Fig. 1), but the pimozide effect seemed to be greater at 28 °C. From 6 to 12 h post-injection, there was a positive effect of low temperature and also of pimozide on LH levels. From 12 to 24 h, there was no difference between groups: all mean LH levels were decreasing. After the second injection, there was a strong positive effect of pimozide at 28 °C, but no significant difference between temperatures for the GnRH-treated groups.

In the control and pimozide treated groups, MIS plasma levels always stayed below 0.25 ng/ml, irrespective of temperature or sampling time. For the other groups, individual variability was great, as seen in the individual profiles (Fig. 2). At 24 °C a peak in MIS levels occurred 12 h after priming, whereas no such profile was observed at 28 °C. Only one fish in each 28 °C group exhibited a MIS peak 6 h after injection, whose magnitude was higher than that observed at 24 °C. At 24 °C, fish that had the highest LH levels also had the highest MIS levels after 12 h and were found to have ovulated at 24 h.

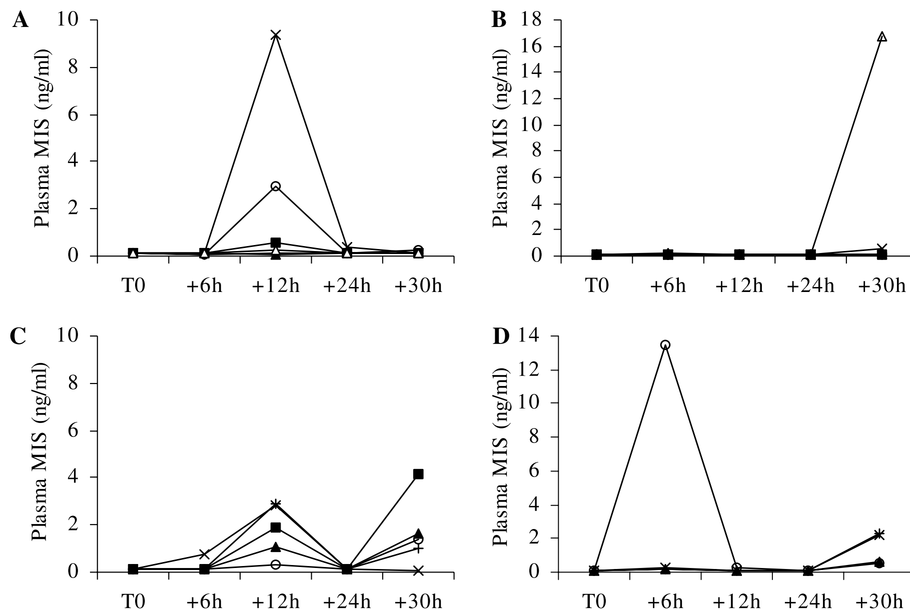

Fig. 2. Individual plasma levels of MIS during spawning induction, according to induction protocol and temperature. (A) GnRH at 24 °C; (B) GnRH at 28 °C; (C) GnRH + Pim at 24 °C; (D) GnRH + Pim at 28 °C (see Table 2 for induction protocols). A priming dose (10%) was injected at the beginning and the resolving dose (90%) was injected after 24 h.

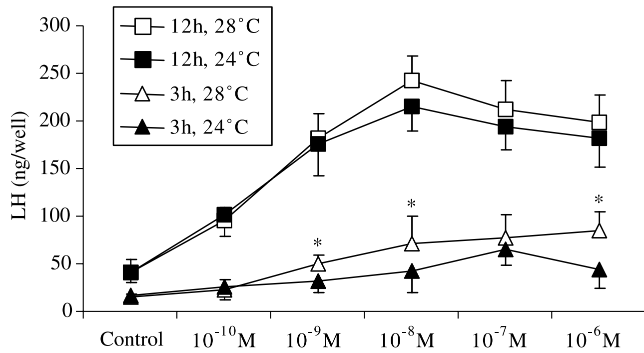

Fig. 3. LH release by pituitary cells incubated for 3 or 12 h with different sGnRH concentrations (M) at 24 and 28 °C. Values are means ± standard deviation, significant differences between temperatures are indicated by * (for the same incubation time).

3.2. Pituitary cell culture

Basal release (control) was higher after 12 h of incubation (41 ± 12 ng/well) compared to 3 h (16 ± 5 ng/well) and was not influenced by temperature (Fig. 3). After 3 h of incubation, pituitary cells sensitivity to GnRH stimulus was increased at 28 °C: GnRH significantly increased LH release (compared to basal) from a concentration of 10^{-9} M, whereas at 24 °C a hundred-fold higher dose was needed (Fig. 3). After 3 h incubation, LH release was increased at 28 °C compared to 24 °C for GnRH doses of 10^{-9}, 10^{-8}, and 10^{-6} M. After 12 h incubation, no further difference was observed between temperatures.

3.3. In vitro ovary incubations

Concerning the relation between pituitary extract dose and MIS secretion (Fig. 4), the main observed effect of high temperature was a decrease in ovary response at low doses: for a pituitary extract concentration of 10 μg/ml, secretion of MIS was inhibited at 32 °C compared to 24 and 28 °C. For higher concentrations, differences disappeared, mainly due to a large variability between females. The maximal secretion level was independent of temperature.

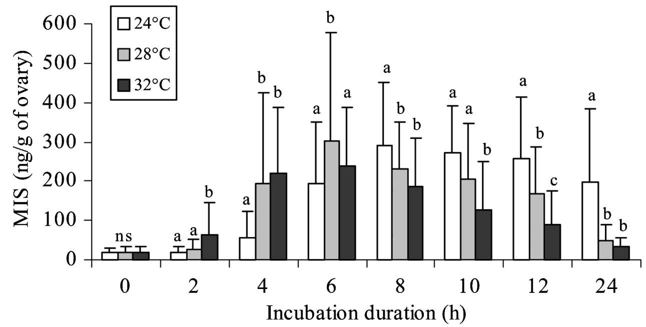

Fig. 5. MIS concentration during ovarian in vitro incubation, according to incubation duration and temperature. Values are means ± standard deviation, different letters indicate a significant difference between temperatures for a given time.

The kinetics of MIS concentration in the incubation medium differed between temperatures for the following characteristics (Fig. 5):

- The onset of MIS secretion: after 2 h, secretion was significant only at 32 °C and after 4 h it was still lower at 24 °C when compared to 28 and 32 °C.
- The time of maximum concentration: the peak value was reached after 4–6 h at 32 °C, after 6 h at 28 °C, and after 8 h at 24 °C.
- The maximum values: at 32 °C, maximum levels were lower to those at 24 and 28 °C (no significant difference was found between the two last ones).
- The decrease was accelerated at 28 and 32 °C when compared to 24 °C.

Concerning oocyte maturation, there was also a decrease of ovarian sensitivity to pituitary extract at high temperature (Fig. 6): the first maturations were observed for 2.5 μg/ml at 24 °C, 10 μg/ml at 28 °C, and 20 μg/ml at 32 °C. The maximum in vitro maturation rate was lower at 32 °C (20%) when compared to 24 and 28 °C (30–40%), even if the MIS concentrations in the incubation medium were similar between temperatures (51–62 ng/ml).

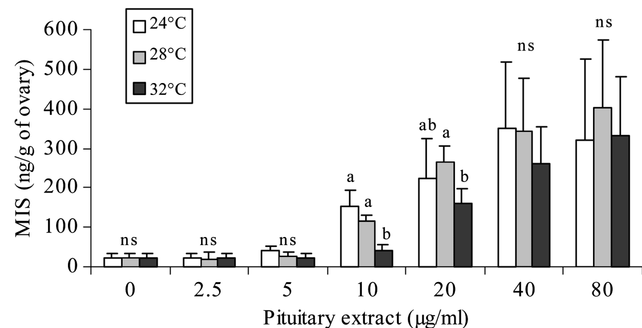

Fig. 4. In vitro ovarian MIS secretion according to pituitary extract dose and incubation temperature. Values are means ± standard deviation; different letters indicate a significant difference between temperatures for a given dose, ns: non-significant difference.

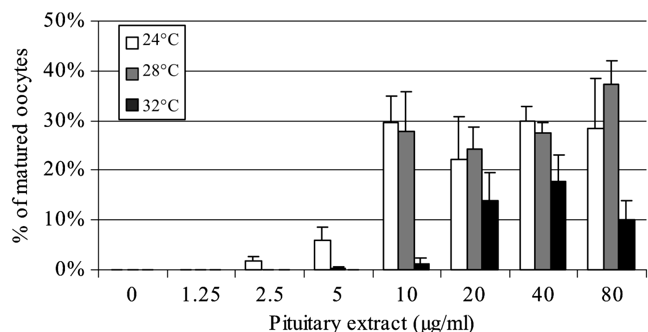

Fig. 6. In vitro maturation rate of oocytes, according to pituitary extract dose and incubation temperature. Values are means ± standard deviation.

4. Discussion

Temperatures of 24, 28, and 32 °C were chosen for the experiments: 24 °C is considered as optimal for grass carp artificial reproduction (Bardach et al., 1972; Horvath et al., 1984; Krykhtin and Gorbach, 1982), 28 °C as a threshold above which problems seem to occur (Horvath, 1986; Rottman and Shireman, 1985) and 32 °C as potentially blocking reproductive functions. We indeed observed a significant inhibition of ovulation at 28 °C. After the spawning induction, the difference in ovulation rates was greater than the difference observed in plasma LH levels, which suggests that the thermal inhibition mainly lies at the gonadal level. The hormonal profiles observed at 28 °C in Poland were similar to those observed in the Ivory Coast at the same temperature (data not shown), despite the different thermal and photoperiodic history of the fish.

We will first discuss the results on gonadotropin release regulation, and then on gonadal regulation, in relation with the ovulatory response.

4.1. Gonadotropin release

In cell culture, pituitary sensitivity to GnRH was increased at 28 °C after 3 h, but LH release was not affected by temperature after 12 h of incubation. These results are in accordance with those obtained in common carp, for which basal and GnRH-induced LH release by pituitary cells in perfusion are constant between 20 and 25 °C (Lin et al., 1996).

In vivo, 6 h after the priming injection, there was no thermal effect on GnRH-induced LH release, contrary to the in vitro results. However, the temporary effect observed in vitro could be masked in vivo by an interaction with the dopaminergic system (Peter et al., 1986), a longer sampling interval (6 h) or great individual variability. The potentiation of the GnRH effect by pimozide is higher at 28 °C, after the first and the second injection. After the second injection, pimozide amplified GnRH-induced LH release by a factor 6 at 28 °C, whereas no effect was seen at 24 °C. This could result from an increase of the dopaminergic inhibition at higher temperatures. In mature female goldfish, in vivo pituitary LH content is not increased by temperature (Sohn et al., 1998), suggesting that the increased LH release observed in our experiment was more likely to be caused by the suppression of dopaminergic inhibition than by an increase in pituitary LH content at high temperature. Such an increase of dopamine inhibition above the optimal spawning temperature has been hypothesized in arctic charr *Salvelinus alpinus* (Gillet et al., 1996) and can explain the lower ovulation rate in LHRH-A induced grass carp above 27 °C (Rottman and Shireman, 1985). At the opposite, some authors (Khan and Joy, 1990; Senthilkumaran and Joy, 1995) observed a decrease in hypothalamic dopamine content and/or turnover with increasing temperature in other teleosts, but they were under the optimal spawning temperature, which could explain the different pattern observed here, above the optimal temperature.

However, after the injections with pimozide alone, LH release was very low at both temperatures, confirming the strong dopaminergic inhibitory tone in this species as in many cyprinids (Lin et al., 1988). From 6 to 12 h after priming, mean LH levels were nearly constant at 28 °C and increasing at 24 °C. Several hypotheses can be raised: a difference in LH clearance rate (Cook and Peter, 1980a) or a difference in LH capture by the ovary (Cook and Peter, 1980b). A more rapid drain of LH pituitary content at 28 °C, preventing a prolonged release, could also explain this observation, as well as a temporary desensitization of gonodotroph cells to GnRH (Habibi, 1991). These processes are probably all involved in explaining the drop in LH observed at both temperatures from 12 to 24 h after priming. The second injection triggered a rise in plasma LH, particularly pronounced for the GnRH + pimozide group at 28 °C. Some females ovulated between 12 and 24 h after priming, but their pituitary responsiveness was not affected.

4.2. Ovarian response

The main effect of high temperature was a decrease in gonadal sensitivity for low doses of pituitary extract, while maximum secretion did not seem to be affected. MIS kinetics were influenced by high temperatures in several ways: MIS secretion appeared more precociously, the peak concentration occurred earlier, the maximum level was lower, and MIS levels decreased more rapidly. These last three characteristics are likely to be the result of an accelerated clearance of MIS at high temperature, a phenomenon already observed for androgen glucuronidation in goldfish testis (Kime, 1980). This stimulating effect of high temperatures on steroid metabolism has been demonstrated in several species (Fostier et al., 1983).

Oocyte maturation (GVBD) was inhibited at 32 °C despite similar MIS concentrations in the medium. In the goldfish, in vitro induction of oocyte maturation by the MIS was progressively inhibited by temperature rising above an optimum level around 13 °C (Gillet et al., 1977), suggesting an inhibitory effect of higher temperatures downstream of the MIS signal.

During spawning induction, MIS secretion was clearly inhibited at 28 °C, more than observed in vitro, and despite similar LH levels. However, in two 28 °C females the peak levels were higher than in 24 °C females. It thus seems that the inhibition lies in the receptivity of follicles to LH more than in MIS synthesis capacities. In common carp held at 20 °C, MIS

production capacities are established 6 h after priming injection, GV migration occurs between 6 and 12 h after priming, and the longer the interval after priming, the higher the GV breakdown rate (Kime et al., 1987). In our case, peak MIS production was advanced in vivo (6 h vs. 12 h at 24 °C), in accordance with in vitro results. The sequence or the speed of acquisition of maturational competence could be disrupted, or high temperatures could have an effect on other factors that influence maturational competence, such as FSH (Van der Kraak et al., 1992), IGF (Fostier et al., 1994; Maestro et al., 1997), GnRH (Habibi et al., 1988) or activins (Pang and Ge, 2002). From the few individuals that exhibited a MIS secretion it appears that MIS production is rather stimulated by high temperatures in vivo. Therefore, it can be hypothesized that the different steps of intrafollicular oocyte maturation could exhibit different thermal optima as suggested in the common carp by Epler et al. (1985), who found an optimum temperature of 15 °C for maturational competence acquisition and of 20–25 °C for oocyte maturation itself.

Relationships between individual profiles and ovulation success are difficult to establish: the time intervals between samplings were too long to determine the exact magnitude of peaks and this problem is likely to be increased at high temperature due to the accelerated kinetics. At 24 °C, all fish whose plasma LH or MIS levels exceeded a certain threshold ovulated, whereas at 28 °C some fish exhibited high levels of LH or MIS without subsequent ovulation. For both temperatures, there was no relation between hormonal levels and the fact that fish ovulated completely or only partially. In another similar experiment (Glasser et al., unpublished results), the partial ovulation rate was higher at 28 °C (75%) than at 24 °C (31%). These partial ovulations suggest a probable thermal inhibition of the ovulation process, as demonstrated in goldfish (Gillet et al., 1977) and rainbow trout (Pankhurst et al., 1996). However, in common carp, the effect of prostaglandin on ovulation seems to be independent of temperature between 20 and 25 °C (Epler et al., 1985). The disruption is thus more likely in the coupling between MIS and ovulation at high temperatures: if MIS production is too early compared to the rest of the maturation process, it could induce maturation without ovulation, like in rainbow trout (Jalabert et al., 1978).

5. Conclusions

Temperature effects have been demonstrated at different levels of the reproductive axis during spawning induction in grass carp. At the pituitary level, there is a temporary positive effect of high temperature on GnRH-induced LH release in vitro, which we did not observe in vivo, probably because of the long sampling interval or individual variability. At 28 °C, we observed a greater individual variability and accelerated kinetics of plasma LH levels. An increase of the dopaminergic inhibition was also probable.

At the gonadal level, thermal disruptions affect the acquisition of maturational competence, maturation and probably ovulation. The follicular ability to respond to gonadotropin by an appropriate MIS secretion is disrupted, since it is inhibited at 28 °C in most females despite normal LH levels. The oocyte receptivity to MIS is also disrupted, since in vitro maturation is inhibited even when MIS synthesis is not affected. Ovulation mechanisms are also probably disrupted, since maturations with partial ovulations or no ovulation were observed at 28 °C.

All these factors must contribute to the great decrease of induced ovulation rate at 28 °C, but the major effects probably lie at the gonadal level. To obtain a better insight into these phenomena, it would be interesting to determine the thermal regulation of the different steps (maturational competence acquisition, maturation and ovulation), which could have different thermal optima (Epler et al., 1985), and thus be differently affected by high temperatures. Knowing the kinetics of the competence acquisition at different temperatures (MIS production capacities acquisition, GV migration, receptivity to MIS, etc.) (Kime et al., 1987) could also aid the optimization of induction protocols in high temperature environments.

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