Repeated administration of different hormonal preparations for artificial propagation and their effects on reproduction, survival and blood biochemistry profiles of female tench (*Tinca tinca* L.)

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ABSTRACT: The aim of the study was to compare physiological statuses of stripped and not stripped tench females with ovulation induced by GnRHa or carp pituitary extract (CPE). The comparison was based on selected biochemical stress indices (cortisol, glucose), condition (TP, TGA), and cell membrane characteristics (ALT, AST, CK). In the first and in the second year, 37 and 29 tench females were monitored, respectively. No differences in the values of biochemical parameters in either period were found between the two groups of stripped female tench whose ovulation was induced by GnRHa and CPE, respectively. No significant differences in TP, TGA, ALT, AST and CK values were found between the groups of stripped and not stripped females with ovulation induced by GnRHa or CPE. Different values were found in stress indices, specifically in glucose concentrations. A significant increase ($P < 0.01$) in glucose concentrations was found in tench females immediately after stripping and, in not stripped females, about 48 hrs after ovulation induction. Both artificial stripping and the inability to release eggs are important stress factors for female tench.

Keywords: tench; artificial propagation; hormonal stimulation; haematology; biochemistry profile

The reproduction of the majority of cyprinid fish used in aquaculture usually relies on hormonally induced stripping. In carp, Asian herbivorous species and a number of other fish species, the method based on injecting two separate doses of carp pituitary extract (CPE) has been used for decades. The use of carp pituitary in artificial propagation of tench was first described by Pokorný (1974). Based on his own experiments, Kouřil (1987) proposed to use a single dose of carp pituitary extract (CPE) in tench and to decrease the originally recommended higher doses to 2 mg/kg. For Asian herbivorous fish

Supported by Czech Research Projects (MSM No. 6007665809) Biological, Environmental and Breeding Aspects of Fishery and (ME 853) Kontakt.

species and salmon, artificial propagation methods based on the use of gonadotropin-releasing hormone (GnRH) for ovulation induction have been developed (Cooperative team, 1977; Donaldson et al., 1981). A possibility of using mammalian GnRH instead of CPE for artificial propagation has also been confirmed in female tench (Kouřil and Barth, 1981; Kouřil et al., 1986). The authors ascertained that the administration of mammalian GnRH increased the number of ovulating females compared with the use of CPE. The fertility of females injected with GnRH was also higher. It was found later that threshold doses of certain synthetic analogues of mammalian and salmon GnRH that would induce ovulation in female tench were significantly lower (Kouřil et al., 1989, 1991). Similar review studies of hormonally induced reproduction in female tench were published by Kouřil and Hamáčková (1996) and Kouřil (1998).

To compare and evaluate the effects of ovulation induction in female tench using GnRHa and CPE, the results of biochemical examinations of blood plasma were also used.

The aims of the present study were:

– to induce ovulation and perform artificial propagation of female tench by repeated GnRH analogue administration in two consecutive spawning seasons
– to evaluate the effects of repeated administration of different hormonal preparations on weight gain and mortality of breeding fish, on success in achieving ovulation, artificial propagation and fecundity compared with females that were repeatedly given CPE
– to compare the values of selected blood plasma biochemical parameters between the two above groups of female tench
– to compare selected blood plasma biochemical parameters between stripped and not stripped tench females with ovulation induced by either GnRHa or CPE

MATERIAL AND METHODS

The fish used in the experiments came from pond broodstocks of the Research Institute of Fish Culture and Hydrobiology in Vodňany. At the beginning of long-term monitoring, breeding tench were individually cryogenically marked. The fish were maintained in small experimental ponds 0.08 ha in size. In spring before spawning, the fish were collected, males and females were separated and released to flow-through troughs. Before the first-year propagation, the fish were randomly divided into two groups of 19 females each. The mean weight of individual CPE-injected and GnRHa-injected fish was 776.7 ± 19.63 and 816.7 ± 67.20, respectively. In the latter group, ovulation was induced by the intramuscular administration of 10 μg/kg of synthetic superactive analogue of gonadotropin-releasing hormone /(D-Ala^6) GnRHProNHEt/, in the former group by the administration of 2 mg/kg dehydrated CPE. The average water temperature from administration to ovulation was 22.15°C in the first year and 22.25°C in the second year.

To assess the physiological conditions of production tench females in the present study, selected blood plasma biochemical indices divided into the following groups were used (Masopust, 2000):

– stress indices (cortisol, glucose);
– condition indices (total proteins – TP, triacyl glycerol – TGA);
– tests of permeability and integrity of cell membranes (alanine aminotransferase – ALT, asparate aminotransferase – AST, creatine kinase – CK).

In the first year, blood samples of tench females (N = 37) were taken on 20 June about 0 to 6 hours after stripping. The fish were kept in troughs with flowing pond water. Water temperature was about 21°C. The next year, the tench were stripped on 16 June and blood sampling was performed on 17 June, i.e. about 24 hrs after stripping and around 48 hrs following the induction of ovulation. Blood samples were taken from 29 female tenches. At the time of blood sampling, water temperature was 22°C, and the fish were kept in troughs with flowing pond water. The fish were anesthetized with 2-phenoxyethanol before blood samples were taken (Hamáčková et al., 2004).

Blood (approximately 1 ml) was sampled from a. et v. caudalis per fish. Heparin sodium salt (50 i.u. per 1 ml blood) was used as anticoagulant (Svobodová et al., 1991). The radioimmunoanalytic method (Immunotech commercial kit) was used for the determination of blood plasma cortisol concentrations. Glucose concentrations, total proteins (TP), triacyl glycerol (TGA), transaminase (ALT, AST) and creatine kinase (CK) were determined using the automatic analyzer COBAS MIRA (F. Hoffmann, La-Roche and Co., Switzerland). Because the CK activity values were very high, blood plasma was diluted 5 to 10 times with physiological solution.

Table 1. Results of the first reproduction season

Preparation	Females		Relative weight of stripped eggs (%)	Latency period (°h)
	injected (N)	ovulated (%)		
GnRHa	17	63.2	6.19	674 ± 5
CPE	18	44.4	4.11	491 ± 34

Table 2. Results of the second reproduction season

Preparation	Females		Relative weight of stripped eggs (%)	Latency period (°h)
	injected (N)	ovulated (%)		
GnRHa	13	53.8	4.10	593 ± 5
CPE	16	37.5	3.40	531 ± 34

The results were statistically analyzed by means of one-way ANOVA.

RESULTS

In the first year of artificial stripping using GnRHa, 63.2% of female tench ovulated and the relative weight of stripped eggs was 6.19%. The mean absolute fecundity (with only stripped fish included) was 81 314 eggs per female. The relative fecundity (with only stripped fish included) was 96 528 eggs per kg. Ovulation in female tench treated with CPE was achieved in 44.4% of the cases and the relative weight of stripped eggs was 4.11%. The mean absolute fecundity in this group was 51 965 eggs per female, and the mean relative fertility was 68 062 eggs per kg. The latency period (between injection and ovulation) was 674 ± 5 °h and 491 ± 34 °h in fish injected with GnRHa and pituitary, respectively (Table 1).

Of the females injected with CPE and GnRH analogue in the first year, 84.2% and 72.2%, respectively, survived until the next year's spawning. The mean weight of females treated in the first year with CPE and GnRHa was 1 042.5 ± 150.4 and 998.8 ± 188.1, respectively. In the second spawning season, 37.5% fish injected with pituitary and 53.8% fish injected with GnRH analogue successfully ovulated and were stripped. The relative weight of stripped eggs, however, was lower by 4.1% and 3.4% after the administration of CPE and GnRHa, respectively. Both the relative and the absolute fecundity figures were also lower in both cases. In the second experimental year, the latency period was 531 ± 86 °h (fish injected with carp pituitary extract) and 593 ± 60 °h (fish injected with GnRHa) (Table 2).

Table 3 (year 1) and 4 (year 2) give the blood plasma biochemical profiles of studied female tench divided according to the method of ovulation induction into a group of (1) stripped female tench

Table 3. Comparison of blood plasma biochemical indices in stripped and not stripped tench females (year 1)

Index	GnRHa (stripped)	GnRHa (not stripped)	CPE (stripped)	CPE (not stripped)
	mean ± SD (n = 11)	mean ± SD (n = 6)	mean ± SD (n = 9)	mean ± SD (n = 9)
Fish body weight (g)	841.0 ± 126.67	774.8 ± 150.40	771.4 ± 121.35	779.2 ± 134.31
Glucose (mmol/dm^3)	8.80 ± 2.145^a	6.84 ± 2.299^{ab}	10.43 ± 3.831^a	5.93 ± 2.098^b
TP (g/dm^3)	36.8 ± 5.47^{ab}	42.17 ± 4.738^b	33.03 ± 5.440^a	34.92 ± 4.180^a
TGA (mmol/dm^3)	1.54 ± 0.184^a	1.53 ± 0.641^a	1.27 ± 0.252^{ab}	1.08 ± 0.296^b
ALT (μkat/dm^3)	0.25 ± 0.125	0.23 ± 0.132	0.19 ± 0.091	0.16 ± 0.059
AST (μkat/dm^3)	4.09 ± 2.237	5.15 ± 2.418	4.76 ± 0.964	4.63 ± 1.741
CK (μkat/dm^3)	87.7 ± 60.05	134.3 ± 88.11	102.2 ± 39.94	93.8 ± 54.85

The values with superscript^{a,b} express significant differences between the groups compared

with ovulation induced by GnRHa, and (2) stripped female tench with ovulation induced by CPE. It follows from the results that there were no differences in the biochemical indices of stress, condition or values characterizing the permeability and integrity of cell membranes between the groups of stripped female tench where GnRHa or CPE was used to induce ovulation. The same results were obtained in the first and in the second year of the experiment.

Another objective of the study was to compare the biochemical parameters of blood plasma between four groups of tench females, stripped and not stripped with ovulation induced by either GnRHa or CPE. The results of analyses and comparisons of these groups of female tench are shown in Table 3 (year 1) and 4 (year 2). The values of condition indices (TP, TGA) and of the cell membrane characteristics (ALT, AST, CK) in stripped and not stripped female tench following the application of either GnRHa or CPE immediately after stripping (year 1) and about 24 hrs after stripping (year 2) were practically comparable without any significant differences. Different values were, however, found in stress indices, i.e. in glucose and cortisol concentrations. In year 1 of female tench monitoring, higher blood plasma glucose levels were recorded immediately after stripping in stripped tench compared with not stripped females; in tench that had been administered CPE the difference was significant ($P < 0.01$) compared with fish treated with GnRHa. In year 2 when blood samples were collected about 24 hours after stripping, on the other hand, higher glucose and cortisol blood plasma concentrations were found in all not stripped female tench. The differences in glucose concentrations were significant in both the group of female tench treated with GnRHa ($P < 0.01$) and that treated with CPE ($P < 0.05$).

DISCUSSION

It follows from the results that the artificial spawning of female tench with repeated hormonally induced ovulation may significantly increase mortality in subsequent years. The assumption of higher mortality among females injected with CPE compared with females injected with GnRH analogue, however, was not confirmed. In fact, the results suggest that the opposite is true. Lower weight gains among tench females repeatedly administered the GnRH analogue compared with females that were administered the carp pituitary were found. In both investigated periods, the number of successfully spawning females was about 30 per cent higher in the group where ovulation had been induced by the GnRH analogue than in the group of females that had been administered CPE. The relative weight of stripped eggs (related to the weight of female tench before stripping) in two consecutive spawning seasons in females with artificially induced stripping showed a markedly decreasing trend. In the investigated period, the relative weight of stripped eggs was 1/4–1/2 lower in the group of females administered CPE than in the group of females administered the GnRH analogue.

Reproduction in fish is one of the factors that significantly influence the internal environment of the organism. For that reason, much attention is paid to the study of haematological and biochemical indices in the reproductive period. Changes in haematological indices in tench during their reproductive season were described by Einszporn-Orecka (1970) and Svobodová et al. (1978). Einszporn-Orecka (1970) reported a decrease in the values of haematological indices (erythrocyte count Er, haemoglobin count Hb, hematocrit value PCV) during the spawning season in breeding tench in lakes, and reported a marked decrease in the values in females. A similar character of changes in haematological indices (Er, Hb, PCV) in female and male tench after artificial propagation was described by Svobodová et al. (1978).

The present study reports the results of biochemical indices evaluating stress, condition and the characteristics of cell membranes in female tench after artificial propagation. No differences in biochemical profiles in the two years of investigation were found between the two groups of stripped female tench whose ovulation had been induced by GnRHa and CPE, respectively. In their study in female wels, Svobodová et al. (1997) found no differences in either haematological (Er, Hb, PCV, MCV, MCH, MCHC, leukocytes) or biochemical indices (TP, glucose, cortisol) when they used different hormonal substances (GnRH and CPE) to induce ovulation.

When ovulation was induced by GnRHa or CPE, only some females spawned while others failed to spawn. No significant differences in biochemical indices characterizing the condition and characteristics of cell membranes were found between the stripped and not stripped groups of females. Different values were recorded in stress indices,

Table 4. Comparison of blood plasma biochemical indices in stripped and not stripped tench females (year 2)

Index	GnRHa (stripped)	GnRHa (not stripped)	CPE (stripped)	CPE (not stripped)
	mean ± SD (n = 8)	mean ± SD (n = 5)	mean ± SD (n = 6)	mean ± SD (n = 10)
Fish body weight (g)	985.4 ± 223.10	1 020.2 ± 134.57	1 066.2 ± 109.70	1 056.8 ± 158.44
Cortisol (mmol/dm^3)	519.9 ± 172.67	622.6 ± 127.69	334.8 ± 228.28	517.3 ± 232.74
Glucose (mmol/dm^3)	9.09 ± 1.24^a	12.67 ± 2.751^b	7.10 ± 1.992^a	11.00 ± 3.969^b
TP (g/dm^3)	38.7 ± 5.92	33.6 ± 4.15	37.4 ± 4.14	35.0 ± 5.29
Tcg (mmol/dm^3)	2.45 ± 0.679	2.07 ± 0.385	2.16 ± 0.531	2.11 ± 0.315
ALT (μkat/dm^3)	0.15 ± 0.075	0.15 ± 0.065	0.16 ± 0.098	0.19 ± 0.058
AST (μkat/dm^3)	6.90 ± 1.154	6.39 ± 1.496	7.40 ± 2.073	6.83 ± 1.657
CK (μkat/dm^3)	205.0 ± 162.98	165.7 ± 27.41	201.3 ± 188.77	236.3 ± 133.93

The values with superscript^{a,b} express significant differences between the groups compared

specifically in glucose concentrations. A significant increase ($P < 0.01$) in glucose concentrations was observed in females immediately after stripping. This was a persisting response to the physiological and handling stress from the stripping period. Similar values were reported by Jeney and Jeney (1992) and Svobodová et al. (1997), Svoboda et al. (2001) in the blood plasma of female carp, wels and tench immediately after artificial spawning. In not stripped female tench, significantly higher ($P < 0.01$) concentrations of glucose were found about 48 hours after the ovulation induction compared with stripped females about 24 hours after stripping. This was probably in response to the stress produced by the inability to release eggs, by the eggs remaining in the body cavity, etc. The stress also manifested itself by a slight, insignificant decrease in total protein and triacyl glycerol concentrations in the blood plasma of unspawned females (Table 4). It follows from the above that both artificial stripping and the inability to release eggs are important stress factors for female tench. This conclusion underlines the importance of welfare.

Hardly any investigated parameter showed differences between the groups of females whose ovulation had been induced by GnRHa or by CPE. The use of GnRHa for the induction of ovulation in artificially spawned tench is preferable because it seems to produce better results in absolute and relative fertility.

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Received: 2006–05–17
Accepted after corrections: 2007–04–02

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