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Profiles of plasma gonadotropin and 17β-estradiol in the common carp, *Cyprinus carpio* L., as related to spawning induced by hypophysation or LH-RH treatment

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Summary. Plasma gonadotropin (GTH) and 17β-estradiol (17β-E_2) levels and oocyte stage have been studied in the common carp, *Cypinus carpio* L., after classical « hypophysation » (two intraperitoneal pituitary injections of 0.3 mg/kg and 2.7 mg/kg at a 12-hr interval) or LH-RH treatment (two intracardiac injections of 3 μg/kg at a 3-hr interval). After the first pituitary injection of LH-RH treatment, GTH increase was followed by a significant increase of 17β-E_2 7 hrs after the injection. The higher GTH levels after the second pituitary injection did not increase that secretion. After classical « hypophysation », one-half of the females ovulated. There was no difference between spawning and non-spawning fishes in relation to hormonal parameters. LH-RH treatment only induced a shifting of the nucleus.

Introduction.

Few results are available on endocrine compounds in carp during the last stages of the reproductive cycle and ovulation. At ovulation time, gonadotropin (GTH) levels are high (Billard *et al.*, 1978 ; The Fish reproductive Physiology Research Group *et al.*, 1978) and estrogen levels are low (Eleftheriou, Norman and Summerfelt, 1968).

In temperate climates, carp spawning is usually initiated by injections of crude pituitary extracts. The most empirically efficient procedure is to give two injections, the first containing 10 p. 100 of the total dose and the second 90 p. 100 (Antalfi and Tölg, 1975). This classical so-called « hypophysation » has been tentatively replaced by LH-RH injections which induce ovarian maturation (Sokolowska, Popek and Bieniarz, 1978), or even ovulation (Conference on Application of Hormones to Economic Fish, 1975), in carp and ovulation in the ayu (Hirose and Ishida, 1974), goldfish (Lam *et al.*, 1975, 1976), plaice and goby (Aida *et al.*, 1978). Using these methods,

10 to 60 p. 100 of non-spawning females can generally be observed in a stock. Incomplete vitellogenesis could explain these results, the oocytes and/or follicles not being ready to ovulate. Moreover, vitellogenic estrogens could have a negative effect on gonadotropic secretion and even a direct negative effect on the ovary, as reported *in vitro* in trout by Jalabert (1975). Thus, the variability of the ovarian capacity to secrete estradiol after « hypophysation » of LH-RH treatment might express the variability in the spawning response.

LH-RH is able to stimulate gonadotropic hormone production in cyprinids (Breton and Weil, 1973 ; Weil, Breton and Reinaud, 1975 ; Crim, Peter and Billard, 1976) and salmonids (Crim and Cluett, 1974 ; Weil *et al.*, 1978). Exogenous gonadotropin injections increase estradiol production during vitellogenesis in trout (Billard *et al.*, 1978) and at the end of the cycle in carp (Fostier, Breton and Jalabert, 1979), but at that final stage in trout, Van Bohemen and Lambert (1979) did not detect aromatization enzymes in the ovary. In the dog-fish, an anti-gonadotropic serum has no effect on the plasma estradiol concentration (Sumpter *et al.*, 1978). Initiating tilapia spawning with warm water stimulates androgen and corticosteroid production but not that of estradiol (Katz and Eckstein, 1974). In carp, Horvath *et al.* (1978) could not determine the action of « hypophysation » on plasma estradiol levels, perhaps because of the high variability between the fish and the small size of the lots.

To confirm our previous results after using « hypophysation » to stimulate estradiol secretion in carp (Fostier, Breton and Jalabert, 1979), we repeated the same type of experiment using two different carp strains and analyzing the relations between the spawning response and the estradiol secretion. More accurate profiles of c-GTH and estradiol were obtained during the first 12 hours after the injection of a lower dose of pituitary extract or after the stimulation of endogenous gonadotropic production by LH-RH treatment. We used relatively low gonadotropin levels, only stimulating oocyte maturation but not ovulation. The actual safest LH-RH spawning treatment is LH-RH « priming », then an injection of pituitary extract 12 or 24 hrs later, according to the water temperature. The efficiency of the « priming » can be estimated by the stage of oocyte maturation reached before the second injection (Jalabert *et al.*, 1977).

In the present work, we have tried for the first time in fish to evaluate the time-lag of steroidogenic response after an experimental increase of the gonadotropin levels.

Material and methods.

Animals. — The experiments were conducted in May at the Warm Water Fish Hatchery in Szazhalombatta, Hungary. Two different carp strains (S and Z) were used. Before the experiment, females weighing 5 to 8 kg were kept outdoors in concrete tanks at 18 °C then transferred into raceways in the hatchery.

Classical « hypophysation » was performed at 22 °C. Total pituitary extracts were prepared in saline (3 mg/ml) from pituitary dried in acetone. A total dose of 3 mg per kg of body weight was injected intraperitoneally. A first injection, given at the onset of the experiment, included 10 p. 100 of the total dose ; the second one, given 12 hrs later, contained the remaining 90 p. 100. Hypophysations were repeated five times with 5 to 10 fish.

LH-RH treatment. — Intracardiac injections of LH-RH (3 μg/kg of body weight) (Hoescht OP-97) were given at the onset of the experiment and 3 hrs later. The experiment was performed at 22 °C with a group of 5 Z-strain carp.

Samples. — The fish were anesthetized with MS 222 (1/10 000). The blood was then sampled and the oocyte stages determined, as previously described by Jalabert *et al.* (1977).

Hormone measurement. — Gonadotropin (c-GTH) was measured by a double radioimmunoassay using a pure gonadotropin, as described by Breton, Kann and Burzawa-Gérard (1971). An antibody raised against this pure c-GTH was used at a dilution of $1/5.10^{-5}$.

Plasma 17β-estradiol levels were determined by a double radio immunoassay after LH-20 chromatography discarding the « estrone fraction », as described by Fostier *et al.* (1978). The antibody used, a gift from M. Terqui (Dray *et al.*, 1971), binds estradiol (100 p. 100), some estrone (11 p. 100), 16-ketoestradiol (9 p. 100) and epiestriol (8 p. 100) but no 17α-estradiol, estriol, 2-methoxyestrone, testosterone, 11-ketotestosterone or 17α-hydroxy-20β-dihydroprogesterone (0.5 p. 100). The intra and inter-assay coefficients of variation were 4 and 15 p. 100, respectively.

Statistical methods. — The mean of the independent samples was compared by the Mann-Whitney U-test (non-parametric method).

In a paired comparison, the significance of the mean differences was tested by Student's t-test.

The percentages were compared using the χ^2 test.

Results.

Classical « hypophysation ».

a) *Hormone levels.* Before injection, the GTH ($P < 0.001$) and E_2 ($P < 0.025$) levels of the two carp strains were different.They were low before spawning initiation, and no correlation was found between the two (table 1). Twelve hours after the first injection, an increase of the 17β-E_2 levels corresponded to the high quantities of exogenous GTH found in the plasma, but those levels did not increase after the second

TABLE 1

Plasma gonadotropin and 17β-estradiol levels in carp before spawning induction

Strain	c-GTH ng/ml			17β-E_2 ng/ml		
	n	mean	SD	n	mean	SD
S	33	3.76	1.56	32	1.02	0.64
Z	37	6.36	3.11	38	1.50	1.04

injection which gave higher GTH levels (fig. 1). The same hormonal pattern was obtained in both strains. The individual values showed no significant correlation between the two hormones at 12 and 24 hrs. Response sensitivity in each fish was evaluated using the ratio :

$$\frac{\text{estimated surface of } E_2 \text{ secretion between 0 and 12 hrs}}{\text{estimated surface of GTH secretion between 0 and 12 hrs}}.$$

No difference in sensitivity was detected between the two strains.

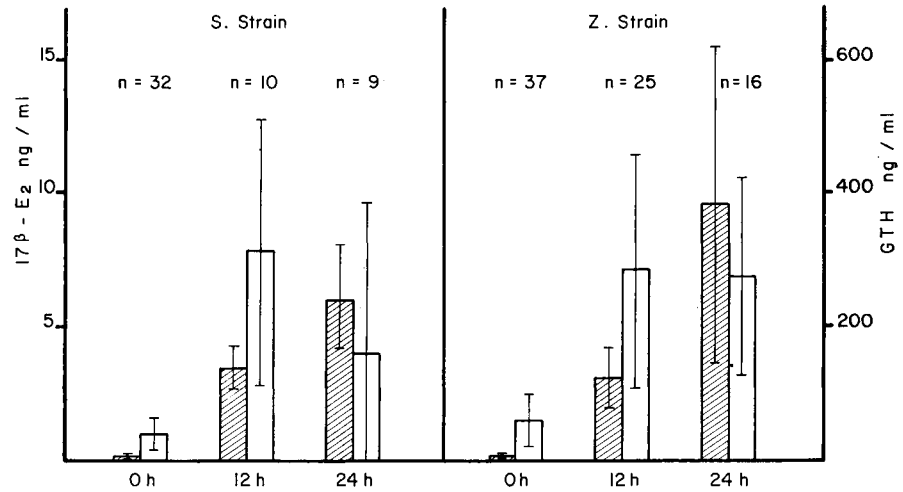

FIG. 1. — *GTH* (hatched bars) *and* 17β-E_2 (open bars) *profiles in two strains of carp plasma during classical « hypophysation » at* 22 °C. First injection at 0 hr : 0.3 mg pituitary/kg body weight ; second injection at 12 hrs : 2.7 mg pituitary/kg body weight. Blood samples were taken at 0, 12 and 24 hrs. n = number of observations. Means ± standard deviation.

b) *Spawning.* One-half of the group of females (n = 21), monitored for spawning, ovulated. We observed no qualitative difference in this respect between the two strains, and no differences could be found between the group of spawning females (n = 11) and the non-spawning one (n = 10), either in relation to the total exogenous GTH injected, the sensitivity of the response as described above, or the GTH and the 17β-E_2 at 0 hr.

Profiles of plasma 17β*-estradiol and c-GTH and oocyte stages after one pituitary injection or LH-RH treatment.*

a) *Hormone levels with pituitary extract injection.* GTH and 17β-E_2 levels were monitored in blood samples taken every 2 hrs during 12 hrs, in 5 females of the Z-strain, after the injection of 0.3 mg of pituitary extract for 1 kg of body weight (fig. 2). That dose was equivalent to the first injection of a classical « hypophysation » or so-called « priming ».

One hour after the injection, the c-GTH level increased in all the females ($P < 0.05$) as compared to the basic level (5.42 ± 1.36 ng/ml). This level continued to

rise for 3 hrs ($P < 0.001$) and then remained high during the next 12 hrs. On the other hand, 17β-E_2 was low during the first 3 hrs, began to increase in four females 5 hrs after the injection, and reached a significantly higher level ($P < 0.05$) than the basic one (3.0 ± 1.7 ng/ml) 7 hrs after the injection. Its maximal value was attained 12 hrs later in all the fish.

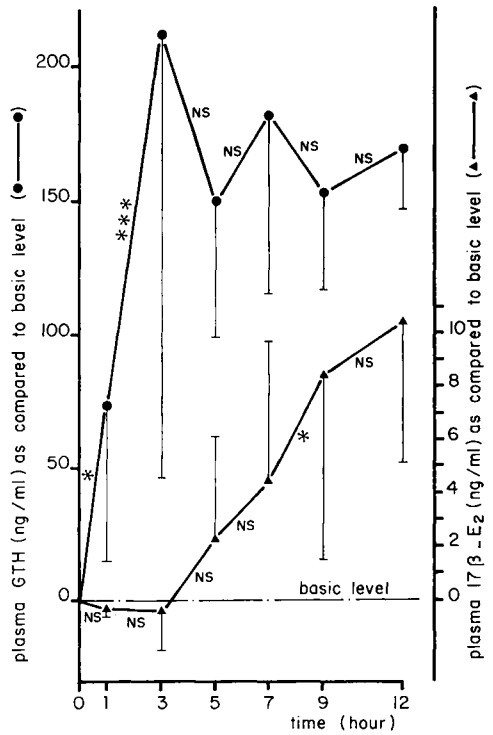

FIG. 2. — *Plasma GTH and 17β-E_2 profiles during 12 hrs after the first injection of classical « hypophysation » in 5 Z-strain carp.* Injection of 0.3 mg pituitary/kg body weight at 0 hr. Blood samples were taken at 0, 1, 3, 5, 7, 9, and 12 hrs. Basic level : GTH and 17β-E_2 levels at 0 hr. Each point represents the mean, the vertical lines the standard deviations. Differences between values are expressed as :

NS = non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

b) *Hormone levels with LH-RH treatment.* c-GTH and 17β-E_2 profiles were monitored for 12 hrs in 5 Z-strain females after LH-RH treatment (fig. 3). The level of c-GTH, observed 30 min after the first injection, increased ($P < 0.05$) in all the females, as compared to the basic level (5.42 ± 1.36 ng/ml). The second injection also stimulated c-GTH secretion since the values at 3 1/2 hrs were higher than those at 3 hrs.

Between 5 and 7 hrs, the c-GTH level decreased significantly ($P < 0.05$), and until 12 hrs was identical to the level at 3 hrs, just before the second injection. On the other hand, the 17β-E_2 level was low and steady during the first 5 hrs ; it then increased significantly ($P < 0.05$) between 5 and 7 hrs in 4 females to reach a value higher

($P < 0.05$) than the basic level (1.70 ± 1.22 ng/ml). During the next 12 hrs, it continued to augment ($P < 0.05$) in 4 of the females, while the fifth female showed a slower increase.

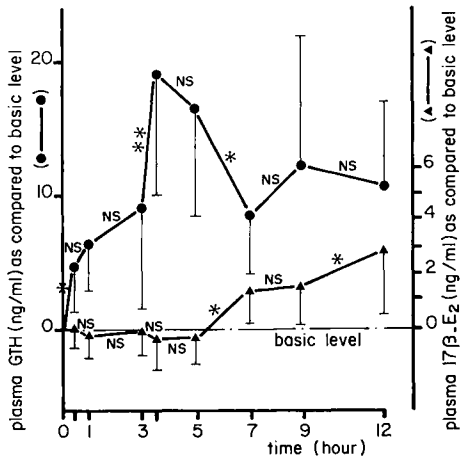

FIG. 3. — *Plasma GTH and 17β-E_2 profiles after two injections of LH-RH* (3 μg/kg body weight) *in 5 female Z-strain carp.* First injection at 0 hr, second injection 3 hrs later. Blood samples were taken at 0, 1/2, 1, 3, 3 1/2, 5, 7, 9 and 12 hrs. Basic level : GTH and 17β-E_2 levels at 0 hr. Each point represents the mean, the vertical lines the standard deviations. Differences between values are expressed as : NS = non-significant, * $P < 0.05$, ** $P < 0,01$, *** $P < 0.005$.

During those 12 hrs of sampling, the amount of circulating c-GTH was lower than that obtained after pituitary injection ($P < 0.001$). The estradiol levels with the two treatments only differed at 12 hrs, the observed value being higher after pituitary injection ($P < 0.02$).

Oocyte stages. — No spawning was noted 12 hrs after pituitary injection or LH-RH treatment, but the nucleus shifted towards the periphery (table 2). Before either treat-

TABLE 2

Percentage of eggs at different stages

	Stage 1	Stage 2	Stage 3	Stage 4
Before pituitary injection	37.5	58.8	1.8	1.9
12 hrs after pituitary injection	0	0	35.4	64.6
Before LH/RH treatment	24.4	72.8	2.4	0.4
12 hrs after LH-RH treatment	0.8	0	75.6	23.6

Stage 1 : nucleus at the center of the oocyte.
Stage 2 : nucleus starts peripheral migration but is still less than half way to the periphery.
Stage 3 : nucleus more than half way to the periphery.
Stage 4 : nucleus situated close to the micropyle.

ment, all the nuclei were at the center of the oocyte (stage 1) or starting peripheral migration (stage 2). Twelve hours later, all the nuclei had passed the middle of the oocyte radius (stage 3) or were near the micropyle (stage 4).

Discussion.

Estradiol and gonadotropin levels, though significantly different in the two strains analyzed, were low before spawning induction in both lots, when exogenous vitellogenesis seemed to be morphologically over. Higher levels were reported for 17β-E_2 by Eleftheriou *et al.* (1968) using a fluorescence assay and for estrogens by Horvath *et al.* (1978) using a gas chromatographic method. Higher estrogen values were also observed by Schreck and Hopwood (1974) using radioimmunoassay in another cyprinid, the goldfish, but the spawning time was not accurately recorded. According to the assay specificities claimed by those authors, the difference with our results may be related to the presence of estrone, which did not interfere with our assay. Different environmental conditions may also explain such results (Billard *et al.*, 1978).

The levels of exogenous c-GTH found after « hypophysation » were in the same range as those reported in other similar experiments (Jalabert *et al.*, 1977 ; Fostier, Breton and Jalabert, 1979 ; Bieniarz *et al.*, 1980). Steroidogenic structures can respond to c-GTH increase earlier, as observed by Fostier, Breton and Jalabert (1979). However, the stimulation is limited since the second pituitary injection did not increase the mean estradiol level. Furthermore, 19 fish out of 25 had lower E_2 levels 12 hrs after the second injection than they did 12 hrs after the first one. As total extracts were used, we could not determine whether the steroidogenic potentialities were exhausted or if an inhibitory factor was present.

After hypophysation, E_2 secretion was detected in all the females but no relation could be established between the estrogenic response and the spawning ability of an individual. However, during a natural cycle, the detected peaks of estradiol appeared during vitellogenesis and not during the spawning season when the c-GTH peaks were detected (Billard *et al.*, 1978). But we have no data on the period just before and after ovulation in natural conditions.

The LH-RH treatment we used (2 injections of 3 mg/kg at a 3-hr interval) induced oocyte maturation but not ovulation. Other authors (Sokolowska *et al.*, 1978), using doses of the same order (1 mg/kg/day for 9 days), also obtained more rapid oocyte maturation but not ovulation. Giving doses 600 to 1 500 times higher than ours induced ovulation in the ayu (Hirose and Hishida, 1974), the goldfish (Lam *et al.*, 1975, 1976), different species of carp (Conference on Application of Hormones to economic Fish, 1975), the Japanese medaka (Chan, 1977), the plaice and the goby (Aida *et al.*, 1978).

In vivo LH-RH stimulation of c-GTH has been previously demonstrated (Breton and Weil, 1973) to vary with animal maturity (Weil, Breton and Reinaud, 1975). For the first time, the present study reports steroidogenic stimulation in fish after LH-RH injection, although it has been shown in reptiles (Callard and Lance, 1977) and birds Sterling, Lea and Sharp, 1978).

The level of c-GTH, after only one injection of a relatively low dose of pituitary extract, remained high without significantly decreasing from 3 hrs to the end of sampling. That level was in the same range as the ovulatory surge in goldfish (Stacey, Cook and Peter, 1979), but the injection alone did not induce ovulation. The LH-RH treatment in our conditions induced lower c-GTH levels, but the main peak lasted about the same time (2 hrs) as the ovulatory surge in goldfish (Stacey, Cook and Peter, 1979). In our study, the total amount of c-GTH in the blood for 12 hrs after such a stimulation was 15 times lower than that caused by the first hypophysation injection. However, that amount was enough to induce estradiol synthesis in the ovary, and the plasma profiles of that hormone were similar after both treatments (pituitary extract or LH-RH injection) : between 5 and 7 hrs after the c-GTH level increased, the plasma E_2 rose. On the other hand, the higher 17β-E_2 level 12 hrs after hypophysation (as compared to the LH-RH treatment) might be related to the higher c-GTH level.

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Résumé. Les teneurs plasmatiques en hormone gonadotrope (GTH) et en œstradiol 17β (E_2-17β) ainsi que le stade des ovocytes ont été étudiés chez la Carpe commune *Cyprinus carpio* L., après « hypophysation » classique (2 injections intrapéritonéales d'hypophyses, 0,3 mg/kg et 2,7 mg/kg à 12 h d'intervalle) ou traitement au LH-RH (2 injections intracardiaques de 3 μg/kg à 3 h d'intervalle). Après la première injection d'hypophyses ou le traitement au LH-RH, on observe une augmentation de la GTH plasmatique suivie de celle de l'E_2-17β, 7 h après l'injection. La teneur élevée en GTH provoquée par la deuxième injection d'hypophyses n'entraîne pas une sécrétion accrue de l'E_2-17β.

Après « l'hypophysation » classique, la moitié des femelles ont ovulé mais leur caractéristique hormonale ne diffère pas significativement de celles qui n'ont pas ovulé.

Le traitement au LH-RH a provoqué uniquement la migration du noyau à la périphérie de l'ovocyte.

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