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Synthetic releasing hormones LH/FSH-RH and LH-RH : effect of intracerebral and intramuscular injections on female carp (*Cyprinus carpio* L.) maturation

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Summary. The influence of intracerebral and intramuscular injections of synthetic LH/FSH-RH on the maturation of female carp was investigated. The results indicate that intrahypophyseal injections of synthetic LH/FSH-RH at 1 μg/kg body weight stimulated ovarian maturation in female carp. Maturation was measured in terms of the percentage of oocytes with the nucleus shifted under the micropyle and of those after GVBD. Neither intramuscular injection of synthetic LH/FSH-RH or LH-RH at 1 μg/kg of body weight nor intraventricular injections of synthetic LH-RH had any effect on ovarian maturation in female carp.

Introduction.

The injection of fish hypothalamic extract as well as that of synthetic releasing hormones stimulates the release of gonadotropin from fish hypophysis (Breton *et al.*, 1972 ; Breton and Weil, 1973 ; Crim and Cluett, 1974 ; Hirose and Ishida, 1974 ; Weil *et al.*, 1975 ; Lam *et al.*, 1975 ; Crim *et al.*, 1976). Since experiments on mammals showed that the effect of synthetic LH-RH was more potent when given by intracerebral infusion than by intravenous or intramuscular route (Ben-Jonathan *et al.*, 1974), we used intraventricular and intrahypophyseal infusions in this study as well as intramuscular injections of synthetic releasing hormones LH/FSH-RH for comparison. In this paper, we report their effect on oocyte maturation in female carp in the final stage of maturity.

Material and methods.

Fifty-five 4-year old female carp weighing on an average 2.5 kg were used. They were given gonadotropin releasing hormone and 2 experiments (group I and group II) were carried out using 2 releasing hormone from different origin.

Group I, comprising 9 females given intramuscular injections and 16 given intrahypophyseal injections of synthetic LH/FSH-RH (Hoescht). The latter group was

divided into two subgroups, D_1 and D_2, according to the maturity stage of the oocytes before the first injection (2 Feb). Subgroup D_1 : 42 p. 100 oocytes with the nucleus shifted peripherally ; subgroup D_2 : 54 p. 100 (fig. 1). The control comprised 4 females receiving intramuscular and 4 given intrahypophyseal injections of NaCl physiological solution.

Group II, comprising 6 females given intramuscular and 8 given intraventricular injections of synthetic LH-RH (Lot 760003, Calbiochem, San Diego, Calif). The fishes were designated with a symbol, D. The control comprised 4 females receiving intramuscular and 4 intraventricular injections of the NaCl physiological solution.

Control females in both groups were designated with a symbol, K. Intracerebral injections were performed with a needle located in the hypophysis or in the third brain ventricle, according to the stereotaxic technique of Peter (1970). Hormones were given once a day for 9 consecutive days at a dose of 1 μg/kg of body weight. Water temperature during the time of experimentation ranged between 18 and 20 °C.

To investigate the effect of injections on ovarian maturation, the *in vivo* method of ovarian sampling was used (Bieniarz and Epler, 1976), and the maturity stage of an ovary was estimated on the basis of oocyte nucleus shift. A distinction was made among oocytes with the nucleus situated in the center or slightly shifted towards the periphery (stage 1 + 2), oocytes with the nucleus shifted under the micropyle (stage 3 + 4) and oocytes after germinal vesicle breakdown (GVBD). The degree of oocyte maturity was estimated before starting an experiment and prior to each successive injection.

Results.

Group I.

Intrahypophyseal injection (fig. 1). — As a result of 9 intrahypophyseal injections of synthetic LH/FSH-RH, the number of oocytes with the nucleus shifted peripherally (stage 3 + 4) increased in group D_1 from 42 p. 100 (2 Feb.-day of first injection) to 78 p. 100 + 4 p. 100 oocytes after GVBD ; in group D_2, from 54 p. 100 to 77 p. 100 + 22 p. 100 oocytes after GVBD ; in group K, from 57 p. 100 to 63 p. 100 at the end of the experiment.

Intramuscular injections (fig. 2). — The number of oocytes (stage 3 + 4) before the first injection (12 April) was 59 p. 100 in females of group K and 63 p. 100 in females

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FIG. 1. — *Intrahypophyseal injections of* 1 μg *synthetic LH/FSH-RH per kg.*

FIG. 2. — *Intramuscular injections of* 1 μg *synthetic LH/FSH-RH per kg.*

FIG. 3. — *Intraventricular injections of* 1 μg *synthetic LH-RH per kg.*

FIG. 4. — *Intramuscular injections of* 1 μg *synthetic LH-RH per kg.*

FIG.1

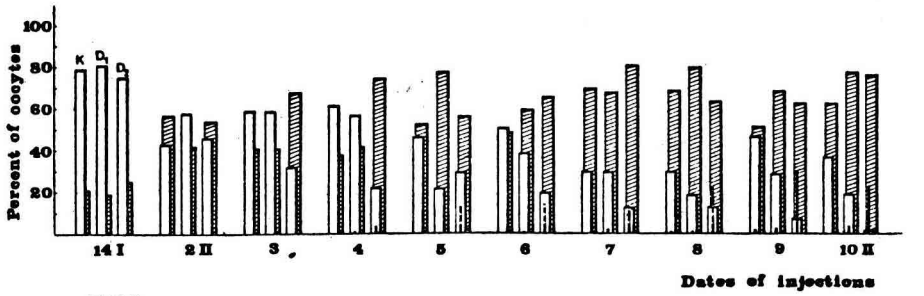


FIG.2

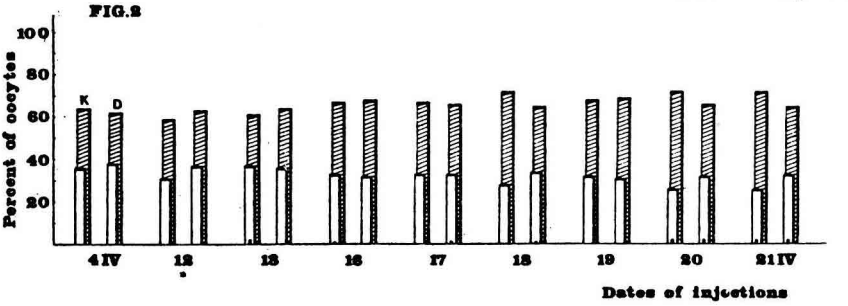


FIG.3

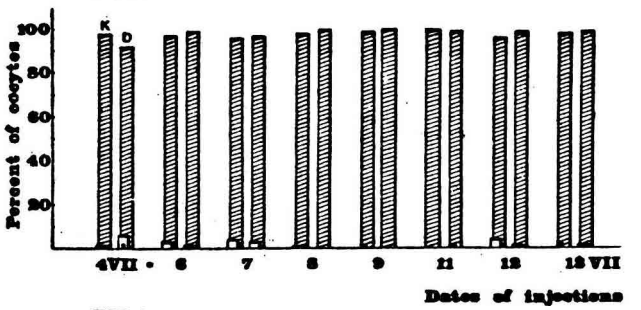
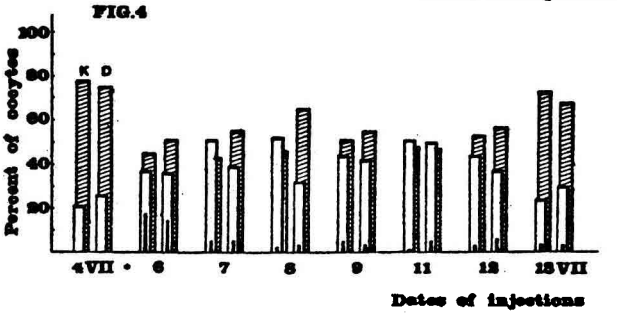


FIG.4



□ Stage 1-2
▨ Stage 3-4
| Oocytes after GVBD
• Day of first injection

of group D, while it was 72 p. 100 in group K and 65 p. 100 in group D after the last injection.

Group II.

Intraventricular infusions and intramuscular injections of synthetic LH-RH did not stimulate sexual maturity in female carp when measured in terms of the percentage of oocytes with the nucleus shifted under the micropyle and of those after GVBD (fig. 3 and 4).

Discussion.

Intrahypophyseal infusion of synthetic LH/FSH-RH at a dose of 1 μg/kg of body weight proved effective in accelerating oocyte maturity in female carp. A more potent effect, expressed in terms of a higher percentage of the oocytes with the nucleus shifted peripherally and of those after GVBD, was observed in a group of fishes which from the very beginning of the experiment had a greater number of mature oocytes (stage 3 + 4).

Hypophyseal sensitivity to an exogenic factor releasing gonadotropin in carp depends not only on external factors, but also on the condition of the hypophysis and gonad maturity (Weil *et al.*, 1975). Synthetic LH/FSH-RH given intramuscularly had no effect on stimulation of oocyte maturation, which suggests that when using a dose of 1 μg/kg of body weight, the site of hormone administration is an important factor.

Intraventricular and intramuscular injections of synthetic LH-RH had no effect on ovarian maturation of female carp. A dose of 1 μg/kg synthetic LH-RH seemed too low to cause an increase in the blood gonadotropic level high enough to have an effect on maturation which would be expressed by an elevated percentage of oocytes with the nucleus shifted towards the periphery. Perhaps infusion directly to the hypophysis would give a more pronounced effect, as in the case of hormone LH/FSH-RH, although hormone diffusion to other parts of the brain, as well as to the hypophysis cannot be excluded in the case of intraventricular injections.

Conclusions.

Intrahypophyseal injections of synthetic LH/FSH-RH at 1 μg/kg of body weight stimulated ovarian maturation in female carp. This was measured in terms of the percentage of oocytes with the nucleus shifted under the micropyle and of those after GVBD.

Intramuscular injections of synthetic LH/FSH-RH or LH/RH at 1 μg/kg of body weight as well as intraventricular injections of synthetic LH-RH had no effect on ovarian maturation in female carp.

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Résumé. Nous avons étudié l'influence de l'injection intracérébrale et intramusculaire de LH/FSH-RH synthétique sur la maturation de la carpe femelle. Les injections intrahypophysaires de 1 μg/kg de LH/FSH-RH stimulent la maturation ovarienne estimée par le pourcentage d'ovocyte dont le noyau a migré sous le micropyle et de ceux qui ont subi la GVBD. Les injections intramusculaires ou intraventriculaires des mêmes doses de LH/FSH-RH sont sans effet sur la maturation ovarienne.

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