The present study correlates the profiles of certain ovarian steroid hormones with stages of oocyte maturation in common carp induced to spawn by classic hypophysation. Sixteen females were injected at midday with a calibrated homologous pituitary extract (cPE) containing 0.07 mg immunoreactive cGTH/kg body wt, as a priming dose. Ten of those females were injected 11 hr later with cPE containing 0.35 mg IR cGTH/kg body wt as an induction dose (Group P + IN). The other females were left without further treatment (Group P). An additional 6 fish were injected around midnight with the induction dose only (Group IN). A control group was injected with 0.7% saline (0.2 ml/kg) at midday and at midnight (Group S). Ovarian biopsies were taken at intervals and the maturational stages of individual females were recorded. Blood was sampled simultaneously with the biopsies. GTH, estradiol (E2) and 17a,20β-dihydroxy-4-pregnen-3-one (17,20-P) were determined by specific RIAs. Germinal vesicle breakdown (GVBD) and ovulation were observed only in Group P + IN 2-4 hr after the second injection. The change in circulating ovarian steroids, in response to hypophysation, was found to depend on the maturational stage of the oocytes. Fish with oocytes showing central and eccentric GV responded to the increased GTH level by elevating E2, but not 17,20-P in the circulation. However, fish with ovaries containing peripheral GV responded by an increase in circulating 17,20-P (111 ± 14.6 ng/ml) concomitantly with a transient decrease in E2. Only fish that showed such a shift in the steroidogenic profile exhibited GVBD in their oocytes and consequently ovulated. This finding is consonant with the hypothesis that 17,20-P is the maturational-inducing steroid in the carp. © 1986 Academic Press, Inc.

The relationship between ovarian events and steroids during the phase of final maturation and ovulation has been studied extensively mainly in salmonids. Female trouts with ovaries approaching final maturation show a decrease in circulating estradiol, which is followed by an increase in GTH and a change in its daily pattern of fluctuations. There is also a transient increase in androgen, and a dramatic increase in 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P), (Fostier et al., 1978; Scott and Baynes, 1982; Scott et al., 1982, 1983; Zohar et al., 1982). This increased secretion of GTH was attributed to the decline in plasma levels of estradiol and the subsequent removal of its inhibitory effect on the pituitary (Scott et al., 1983; Young et al., 1983a). The significance of the surge in circulating 17,20-P during the periovulatory phase in salmonids is associated with its function as a maturation-inducing steroid in these fish (reviewed by Nagahama, 1983; see also Zhao and Wright, 1985).

The evidence about hormonal fluctuations during the periovulatory phase and their association with ovarian events in cyprinids is not that complete. In carp exhibiting spawning behavior there is a 10-fold increase in circulating GTH above the basal level (Zhao et at., 1984). A decrease in circulating estradiol in monthly samples of spontaneously spawning carp before the onset of spawning was reported by Eleftheriou et al., (1968). Combined in vivo and in vitro studies of ovarian steroidogenesis in the carp show that ovaries containing midvitellogenic oocytes are producers of.
high estradiol and low testosterone. Low estradiol, high testosterone is characteristic of ovaries at the end of vitellogenesis (Manning and Kime, 1984). Also other incubation experiments with labeled precursors have indicated low estrogen synthesis in postvitellogenic ovaries of the carp (Colombo et al., 1982). However, administration of a whole pituitary extract to postvitellogenic carp was followed by a significant increase in both GTH and estradiol (Fostier et al., 1979; Weil et al., 1980). Similarly, in the goldfish there is a significant increase in circulating estradiol in postovulatory females (Stacey et al., 1983). These reports on increased estradiol level, in response to gonadotropic stimulation, may have indicated a periovulatory hormonal profile in cyprinids which differs fundamentally from that of salmonids which show a low estradiol level at this phase.

The progestogen 17,20-P is a most efficient agent inducing final maturation and ovulation in carp ovarian fragments (Epler, 1981a,b; Epler and Bieniarz, 1983). Furthermore, administration of this progestogen into primed female carp was more effective inducer of final oocyte maturation than whole pituitary extract, even when fish were maintained at a temperature which is normally too low for spawning induction (Jalabert et al., 1977). Also in the goldfish this progestogen is a very effective agent for inducing final maturation (Jalabert, 1976). Indeed, the circulating level of both 17,20-P and 17α hydroxyprogesterone increase considerably after two injections of LH-RH analog combined with the dopamine antagonist pimozide (Peter et al., 1984). These results would suggest that 17,20-P in the goldfish is associated, as in salmonids, with final oocyte maturation and ovulation. In spite of the fact that certain progestogens have been found to stimulate carp spawning, more research has been recommended “to establish whether these or equivalent steroids are indeed secreted at the time of ovulation in carp” (Colombo et al., 1982).

The present paper describes the fluctuations in both estradiol and 17,20-P levels in the circulation of carp induced to spawn by GTH-calibrated pituitary extract. These fluctuations are correlated, step by step, with stages of oocyte maturation in the corresponding fish.

MATERIALS AND METHODS

Fish and maintenance. The experiment was performed at the Gan Shmuel Fish Breeding Center, Israel. Female carp of “Dor 70” strain (Wohlfarth et al., 1980) were kept separate from males in earthen ponds as from the previous breeding season. Two days before the experiment 28 fish were transferred to male carp in earthen ponds as from the previous breeding season. Two days before the experiment 28 fish were transferred to polypropylene tanks (450 liter) with running well water at 22°C. Two females differing in size, or other external marks, were housed in each tank to allow individual identification.

Blood and ovarian sampling. Blood samples (1.5-2.0 ml) were taken in heparinized syringes from the caudal vessels of all fish at 0900, 1200, 1800, and 2300 hr on June 9, 1984 and at 0100, 0300, and 0800 hr of the next day. Lights were kept on until 2300 of June 9. To detect changes in the maturational stage of the oocytes, ovarian biopsies were taken together with the blood samples from about half of the fish in each group; the other half was biopsied at the consecutive sampling. At 1200 hr of June 9th no biopsy was taken. The ovary was biopsied by inserting a “Tygon” tubing, 4 mm internal diameter via the genital pore into the gonad. The ovarian sample (of about 30–40 oocytes) was cleared in ethanol:formalin 40%:acetic acid (6:3:1). Within 3 min the oocytes became translucent and remained so for an additional 6–7 min. The position of the germinal vesicle was determined and the number of oocytes at each stage was recorded.

Hypophysation procedures (Table I). Fish of groups P (priming only) and P + IN (priming and induction) were injected intramuscularly at 1200 with calibrated carp pituitary extract (cPE) containing 0.07 mg cGTH/kg body wt as measured by RIA. Fish of groups P + IN and IN (induction only) were injected at 2300 with the same pituitary extract at a dose containing 0.35 mg cGTH/kg. Control fish were injected with 0.7% saline, 0.2 ml/kg once at 1200 and again at 2300. Between 0800 and 0900 on June 10th fish were stripped, eggs were fertilized, and incubated in funnels according to Rothbard (1981).

Hormone Assays

Carp GTH. A double-antibody radioimmunoassay (RIA) was established using purified carp GTH (cGTH) kindly given by Dr. E. Burzawa-Gérard,
Hormones and Maturation in Carp

The RIA was performed using anti 17,20-P kindly given by Dr. A. P. Scott, Lowestoft, United Kingdom, according to the procedure detailed above for E₂. Cross-reaction of various steroids using this antiserum has been described by Scott et al. (1982). The possibility that substances other than 17α,20β-P occurring in carp plasma have interfered in the assay was tested by comparing direct determinations of the extracted progestogen with those performed after further isolation on TLC. The difference between the means of the two sets of determinations was insignificant, \( P > 0.3 \). Intraassay and interassay variances were 8.17 and 17.7%, respectively.

**RESULTS**

Changes in Hormone Levels during Spawning Induction

Levels of immunoreactive carp gonadotropin (IR-cGTH), estradiol, and 17,20-P did not change appreciably throughout the experiment in the saline-injected control fish. Levels of IR-cGTH and E₂ increased 45-fold and about 5-fold, respectively, in the two groups injected at noon with the priming dose (Fig. 1). The levels of these hormones decreased gradually in the fish that were not injected again (Group P). However, the primed fish injected again at 2300 with the induction dose (Group P + IN) had a high level (23 1.1 + 2.9 ng/ml) of IR-cGTH after only 2 hr. The level remained high until the end of the experiment. The level of E₂ in this group dropped from 8.68 ± 1.01 to 3.64 ± 0.98 ng/ml following the second injection. The level remained low until 0300 and then gradually

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Code</th>
<th>n</th>
<th>Body wt (kg) mean ± SEM</th>
<th>Hypophysation/kg bw</th>
<th>Volume of eggs ( ^{a} ) (liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saline 0.2 ml</td>
<td>Priming 0.07 mg</td>
</tr>
<tr>
<td>Saline control</td>
<td>S</td>
<td>6</td>
<td>3.08 ± 0.67</td>
<td>Twice</td>
<td>−</td>
</tr>
<tr>
<td>Priming only</td>
<td>P</td>
<td>6</td>
<td>5.08 ± 0.59</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Induction only</td>
<td>IN</td>
<td>6</td>
<td>4.20 ± 0.69</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Priming and induction</td>
<td>P + IN</td>
<td>10</td>
<td>4.51 ± 0.42</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^{a}\) The amount of extract injected per kg bw contained the equivalent cGTH as measured by RIA (Yaron et al., 1984).

\(^{b}\) The volume was recorded after rinsing and swelling for 1 hr.
Fig. 1. Concentration of selected hormones in the plasma of female carp following injection of saline (0.2 ml/kg; Group S); priming dose of homologous pituitary extract (cPE) containing 0.07 mg IR-cGTH/kg bw (Group P); induction dose of cPE containing 0.35 mg IR-cGTH/kg bw (Group In) or both doses at 11-hr interval (Group P + In). Time of injection is indicated by arrows. (A) Immuno-reactive carp GTH; (B) estradiol-17β (E₂); (C) 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P).
increased. Fish injected with the induction dose only (Group IN) had a high cGTH level, similar to that of Group P + IN. E₂ level in these fish increased and reached a level comparable to that of Group P and Group P + IN after the priming dose (Fig. 1B).

The level of 17,20-P remained low (0.5 ng/ml) after the priming dose. A surge of the progestogen (111.02 ± 14.59 ng/ml) occurred in one group only, namely the fish that had been primed previous to the induction dose (P + IN) (Fig. 1C). It should be emphasized that ovulation occurred only in fish of this group (Table 1). The level of 17,20-P increased only slightly in fish injected with the induction dose without prior priming (Fig. 1C).

Effects of Treatment on Oocytes

Maturation and Ovulation

Oocyte maturational stages of fish in the various groups are shown in Fig. 2. At the onset of the experiment, ovarian biopsies of all fish exhibited oocytes at Stage I [central germinal vesicle (GV)] and Stage II (eccentric GV) only. In the control group (Group S) oocyte stages did not change throughout the experiment. However, the injection of pituitary extract, at either the priming dose or the induction dose was followed by a migration of the GV toward the periphery within 2 to 6 hr (Fig. 2). Germinal vesicle breakdown (GVBD, Stage IV) and ovulation (Stage V) occurred only in fish primed with a low dose of cPE, and 11 hr later injected with the induction dose.
DISCUSSION

It was not surprising to find an increase in the circulatory level of IR-cGTH following the administration of a gonadotropin-containing homologous pituitary extract. However, the possibility that the observed effects were due also to hypophyseal agents other than GTH cannot be excluded. The circulating levels of cGTH increased, generally, in proportion to the injected dose of the extract. It should be emphasized, however, that the maximal level of IR-cGTH (231 ± 23 ng/ml) attained after induction in previously primed fish was not different from that attained after the induction injection only (234 ± 27 ng/ml), although the starting level in the former was about 80 ng/ml higher than in the latter. The maximal circulating IR-cGTH level in fish injected with the high dose of cPE (234 ± 27 ng/ml) was not much different from that measured in naturally spawning fish (256 ng/ml; Zhao et al., 1984).

The increase in circulating estradiol during the first 11 hr after administration of the priming dose (containing 0.07 mg cGTH/kg) was rather similar to that observed after administration of the main dose which was five times higher (Group IN, Fig. 1B). Apparently, postvitellogenic carp ovaries containing oocytes with central or eccentric GV's respond to a first surge of GTH, disregarding its level, by enhanced output of estradiol. Similar results were obtained in other strains of the common carp after administration of 0.3 mg/kg uncalibrated carp pituitary extract (Fostier et al., 1979; Weil et al., 1980). In the present work, after the second administration of cPE, the level of circulating E2 dropped. At the same time, the level of 17,20-P increased dramatically, and reached a peak of 111 ± 14.6 ng/ml (Fig. 1C). A drop in E2 level around the time of final maturation of oocytes and ovulation was previously found in the carp (Eleftheriou et al., 1968); the rainbow trout (Scott et al., 1978; Scott et al., 1980, 1983; Whitehead et al., 1983); the amago salmon (Kagawa et al., 1983a); the white sucker (Scott et al., 1984), and the pike (Simon-tacchi et al., 1982). However, no decrease in E2 prior to ovulation could be noted in the goldfish (Kagawa et al., 1983b).

The decrease in E2 level in the present work proved to be only transient; the level increased again within a few hours after ovulation. The source of the high E2 level after ovulation is unknown as yet. Apparently, the ovary contained at that time not only ovulated eggs and discharged follicles but also many small follicles which were not represented in the biopsy records. It is possible that the high level of E2 originates from the small follicles. This assumption is corroborated by the findings that high E2 levels are maintained in the circulation several weeks after natural spawning, when the ovaries contain only small, previtellogenic follicles (Yaron and Levavi-Zermonsky, 1986).

A rise in 17,20-P level around final maturation and ovulation was reported in the Atlantic and coho salmons (Wright et al., 1982); the rainbow trout (Wright et al., 1982); the ayu (Hirose et al., 1983); the goldfish (Kagawa et al., 1983b; Peter et al., 1984); and the African catfish (Lambert and van den Hurk, 1982). An increase in circulating levels of 17,20-P concomitantly with the decrease in E2 level during that phase was found, thus far in salmonids (Fostier and Jalabert, 1982; Scott et al., 1982; Scott and Baynes, 1982; Ueda et al., 1984; van der Kraak, et al., 1984), the white sucker (Scott et al., 1984), and the gilthead bream (Kadmon et al., 1985).

The change in the steroid pattern was attributed to the activation of 20β-hydroxysteroid dehydrogenase and the suppression of C21-C19 desmolase activity in the ovary (Scott and Baynes, 1982; Scott et al., 1983). Since the decrease in E2 level is coupled with an increase in androgen level, apparently steroid aromatase activity is also
lepressed at the same time (Zohar et al., 1982; Young et al., 1983).

In the carp, the second administration of pituitary extract resulted in a second and higher surge of circulating IR-cGTH. This was followed by an apparent shift in the main ovarian steroid in the circulation, namely from estradiol to 17,20-P (Fig. 1). In vitro studies of follicles of the amago salmon at various stages of vitellogenesis and maturation have indicated that addition of GTH to the incubation medium resulted in a considerable increase in the output of estradiol and that the sensitivity to GTH developed as vitellogenesis continued. During maturation, E2 output in response to GTH declined. Immature follicles just prior to maturation (October) responded to the presence of GTH by increased 17,20-P production (Kagawa et al., 1983a). From these results it was implied that also in the amago salmon a shift occurs in the biosynthetic pathway of the ovarian follicle from the secretion of predominantly estrogens during vitellogenesis to secretion of progestogens during the maturation phase (Young et al., 1983a,b). Long-term incubation experiments of whole follicles in the Atlantic salmon containing oocytes with migrating GV show that 17,20-P output is enhanced in the presence of GTH prior to and during GVBD. There is also an increase of E2 output, but this is independent of GTH (Zhao and Wright, 1985). Also in the carp a fundamental change has been reported in the steroidogenic capacity of the ovary, namely, from predominantly F2 production during vitellogenesis to predominantly testosterone at its end (Manning and Kime, 1984).

A considerable increase in 17α-hydroxyprogesterone (17-OH-P) was found to occur in the plasma of female carp following the administration of uncalibrated carp pituitary extract (Kime and Dolben, 1985). Since only one of the treated fish showed a marginal increase in circulating 17,20-P, these authors assumed that the maturation-inducing steroid in the carp is 17-OH-P. It is difficult to explain why the surge of 17,20-P was not detected in the carp induced to spawn by Kime and Dolben. However, it is most probable that such a surge is associated with an increase of its immediate precursor (17-OH-P) as reported in the goldfish (Peter et al., 1984).

The results of the present work indicate that postvitellogenic follicles retain their capacity to secrete estradiol in response to a surge of GTH. The shift to progestogen secretion, in response to GTH, may occur within a few hours. Augmented secretion of 17,20-P in response to a surge of GTH, occurred only in fish administered both the priming and the induction doses, and their oocytes had reached Stage III (peripheral GV) prior to the surge. Fish exposed to the same high dose of GTH, but their oocytes were at that time only at Stage I and II (Group IN), showed only a marginal increase in the level of the progestogen. Moreover, this marginal increase occurred only after a lag of 2 hr, during which 60% of the oocytes had already a peripheral GV (Fig. 2). It may be assumed, therefore, that the advance in the maturational stage from eccentric GV to peripheral GV is associated with the shift in the steroidogenic response of the follicle to GTH.

Based on experiments in carp, "priming" was defined "as the treatment which induces GV migration as a correlative phenomenon with the acquisition of oocytes responsiveness to 17,20-P" (Jalabert et al., 1977). The present results indicate that priming induces GV migration but also the acquisition of follicular responsiveness (by 17,20-P secretion) to a second surge of GTH.

In spite of the high level of circulating GTH in Group IN and the appearance of the progestogen in the circulation (Fig. 1), none of these fish ovulated at least until noon of the next day. This emphasizes the necessity of a priming dose for a successful induction of spawning in carp. It is possible
that priming is followed by increased production of GTH receptors in the follicular cells while high level of the trophic hormone results in their down regulation.

Maturation and subsequent ovulation can be induced in vitro by exposing carp ovarian fragments to various progestogens, corticosteroids, and androgens, but the most effective steroid is 17,20-P (Epler, 1981a). Injection of 17,20-P to carp previously primed with a low dose of homologous pituitary extract was followed by final maturation and ovulation even when kept at 13°C (Jalabert et al., 1977). These findings had led to the assumption that this progestogen may be the actual mediator of the gonadotropic effect on these processes in the carp ovary, as in that of the goldfish and salmonids. The results of the present work corroborate this assumption. Moreover, the present investigation shows that 17,20-P is actually increasing, in response to a GTH surge, a few hours before GVBD and ovulation take place in the carp.

ACKNOWLEDGMENTS

We thank Dr. E. Burzawa Gérard, Paris for the gift of standard cGTH; Dr. V. Lichtenberg, Hamburg for the gift of anti-cGTH used in the GTH RIA; Dr. A. P. Scott, Lowestoft for the anti-17, 20-P. Thanks are due to Mr. S. Rothbard and the team at Kibbutz Gan Shmuel for kindly providing the facilities of the Fish Breeding Center and the fish for the experiments. We also thank Mrs. Hassia Rubin-Kedem and Ms. Alissa Bogomolnaya for their enthusiastic help in various stages of the research, and finally Mrs. R. Suzeen for the graphic work.

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