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► **To cite this version:**

Claudine Weil, Alexis Fostier, Roland Billard. Induced spawning (ovulation and spermiation) in carp and related species. *Aquaculture of cyprinids*, INRA, pp.119-137, 1986, 2-85340-791-8. hal-02858278

**HAL Id: hal-02858278**

**<https://hal.inrae.fr/hal-02858278>**

Submitted on 8 Jun 2020

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## Induced spawning (ovulation and spermiation) in carp and related species

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### SUMMARY.

In fish farms, hormonal induced ovulation and spermiation are commonly performed in carps and related species by hypophysation which consists of direct stimulation of the gonads by administration of crude carp pituitary homogenates.

The use of synthetic hypothalamic hormones as luteinizing hormone releasing hormone analogues (LHRHa) seems promising. LHRHa administration is used routinely in China for different species of carps. However, LHRHa treatments are partially efficient in inducing ovulation in female common carp and furthermore, in chinese carps and tench, the latency of the ovulatory response is delayed when compared to hypophysation. The use of LHRHa treatment combined with pimozide, a dopamine blocking agent, improves these two points. These trials are still at an experimental stage.

This paper also reviews other techniques for inducing spawning and which are still at a research stage.

RESUME. Ovulation et spermiation induites chez la carpe et les espèces voisines.

La technique la plus couramment utilisée en pratique pour l'induction hormonale de l'ovulation et de la spermiation chez la carpe et les espèces voisines est l'hypophysation et consiste en la stimulation directe des gonades par des extraits bruts hypophysaires.

L'utilisation d'hormones synthétiques hypothalamiques induisant la décharge de l'hormone gonadotrope hypophysaire endogène tels des analogues du LHRH (luteinizing hormone releasing hormone LHRHa) semble prometteuse et est utilisée en routine en Chine pour différentes espèces de carpes. Cependant, d'une part ces hormones sont faiblement efficaces chez la carpe commune femelle et d'autre part elles entraînent une ovulation retardée par rapport à l'hypophysation chez les espèces telles que la carpe chinoise, la tanche. L'administration de LHRHa et de pimozide, facteur bloquant l'action de la dopamine (qui serait un inhibiteur de la décharge de GtH) améliore ces deux points. Ces essais sont encore au stade expérimental.

D'autres techniques qui sont encore au stade de la recherche sont également revues.

## INTRODUCTION

Induced spawning is used in carp species which can mature but cannot spawn in captivity (major indian and chinese carp) as well as for those which can spawn when environmental required conditions are provided (common carp). However, in this latter case, occurrence and success of spontaneous spawning are aleatory and the use of induced spawning techniques allow fish farmers to optimize broodstock management by increasing the quantity of produced fry.

Hormonally induced spawning is commonly used for carp and related species and the knowledge of endocrinology of reproduction is required to improve the use of these techniques. Besides, the success of these techniques can be improved by manipulating environmental conditions (temperature, photoperiod, water quality, social factors).

In the present paper we will review the different hormonal techniques used in carp and related species, including those which are routinely used as well as those which are still at an experimental stage. We will emphasize the hormonal changes that occur and the influence of environmental factors on their success.

## I - ENDOCRINOLOGY OF REPRODUCTION

In fish environmental stimuli are known to synchronize the rhythms of reproduction via the brain by neural processes. The transmission is then communicated by the endocrine system which is a link between these factors and the gonads.

It is well established that the pituitary controls the activity of the gonads (review of PICKFORD and ATZ 1957). In goldfish, hypophysectomy experiments have demonstrated the role of the pituitary gland in vitellogenesis and meiosis (YAMAZAKI 1965), in spermatogenesis (BILLARD et al. 1971) and in spawning behavior in female (STACEY 1976). In carp two pituitary hormones have been characterized and purified :

- a gonadotropic hormone (GtH) purified by BURZAWA-GERARD 1971, active on spermatogenesis (BILLARD et al. 1970) and on oocyte maturation (JALABERT et al. 1973) in goldfish via steroid synthesis. A GtH radioimmunoassay has been developed allowing the study of gonadotropin secretion during the sexual cycle in different cyprinid species.
- a second gonadotropin has been isolated (IDLER and NG 1979) and is thought to be necessary for completion of vitellogenesis although this fact has been questioned (BURZAWA-GERARD 1982).

Pituitary activity is regulated by the hypothalamus which is a part of the central nervous system. Functional evidence from lesioning studies has implicated the nucleus lateral tuberis (NLT) (PETER 1970) in a stimulatory regulation of GtH secretion via a gonadotropin releasing hormone (GnRH). GnRH activity has been demonstrated in crude hypothalamic extracts in carp both in vitro (BRETON et al. 1972a) and in vivo (BRETON and WEIL 1973). GtH release was also induced by mammalian GnRH (LHRH) in vivo in carp (BRETON and WEIL 1973, ANONYMOUS 1978) and in goldfish (CRIM et al. 1976) as well as by mammalian superactive analogue (PETER 1980). Recently fish GnRH has been isolated from chum salmon hypothalami identified and synthesized (SHERWOOD et al. 1983). This salmon GnRH has similar potency to native LHRH in vivo in goldfish in terms of magnitude and duration of GtH response (PETER et al. 1985).

Recently experiments of brain lesioning (PETER and PAULENCU 1980) have demonstrated the presence of a gonadotropin release-inhibiting factor (GRIF) in the anterior-ventral preoptic region. CHANG et PETER 1983a found that dopamine has GRIF activity in goldfish modulating spontaneous release of GtH as well as LHRHa-stimulated release of GtH.

Finally, sexual steroids, which are under the control of GtH, act locally on the gonad to trigger gametogenesis. In the present paper we will focus on the regulation of the final aspects of oogenesis and spermatogenesis. Oocyte maturation (resumption of meiosis and structural and biochemical transformations which develop simultaneously) is induced in vitro by carp pituitary extracts or carp gonadotropic hormone in goldfish (JALABERT et al. 1973, JALABERT 1976, EPLER et al. 1979) and in carp (EPLER 1978, EPLER et al. 1979, 1982a). These in vitro study techniques have shown that gonadotropic action of the pituitary is probably mediated by direct steroid action on oocyte maturation in goldfish and in carp. Progestins and particularly  $17\alpha$ -hydroxy,  $20\beta$ -dihydroprogesterone ( $17\alpha$   $20\beta$  OH P) are active in inducing maturation in goldfish (JALABERT et al. 1976) and in carp (EPLER et al. (1980). Corticosteroids are also active in goldfish (JALABERT et al. 1973, JALABERT 1976, EPLER et al. 1979) and in carp (EPLER et al. 1980). Since cortisol increases the potency of carp pituitary extracts in in vitro goldfish maturation (JALABERT 1976), and desoxycorticosterone that of  $17\alpha$  $20\beta$ OH P (EPLER et al. 1980) and that of carp hypophysial homogenate (EPLER et al. 1982b) in carp, it is evident that corticosteroids may have some synergistic action in the process of oocyte maturation. Other glands such as the thyroid may have a synergistic action in oocyte maturation as suggested by the results of EPLER and BIENIARZ (1983) in carp. Triiodothyronine (T<sub>3</sub>) influences positively the maturational effect of gonadotropic and steroid hormones. Ovulation consists in follicular rupture and expulsion of the mature oocyte from the follicle. In goldfish ovulation was induced in vivo by prostaglandins (STACEY and PANDEY 1975) and in vitro in carp (EPLER 1978, EPLER et al. 1985) as well as by adrenaline (EPLER 1978). The action of prostaglandins is temperature independent (EPLER et al. 1985) while in vivo ovulation is temperature dependant (HORVATH 1978).

Spermiation in carp and related species (thinning of the semen) is pituitary dependant (CLEMENS and GRANT 1965). In goldfish hypophysectomy and replacement therapy studies indicate that spermiation seems to depend on androgen via GtH. Different steroids are active : 11-ketotestosterone, testosterone, methyltestosterone, testosterone propionate, progesterone (reviewed in BILLARD et al. 1982).

## II - HORMONAL TECHNIQUES USED FOR INDUCING SPAWNING.

The use of hormones to induce fish spawning has been reviewed many times and recent reviews dealing with this topic include those of CHAUDHURI (1976) HARVEY and HOAR (1979) and LAM (1982).

### A - Selection of breeders

Induced spawning is always performed on mature animals and fully ripe animals are selected for treatments.

Ripe males are selected based upon the possibility of collecting a small quantity of sperm by hand stripping. For females different criteria have been adopted and most depend upon external characteristics such as the swelling, softness and elasticity of the abdomen, colour and state of swelling of the cloacal region and body girth. However these are only rough indications of the stage of development of the ovary and attempts have been

made and must be made to improve the estimation of egg development. This implies firstly the use of a technique for egg sampling and secondly the determination of criteria for egg maturity. Samples of eggs can be obtained by aspiration either by inserting a tubing into the ovary through the genital pore as described by CHEN et al. 1969 for chinese carp or by inserting a needle through the abdominal wall cavity as described by BIENARZ and EPLER 1976 in common carp. Criteria of egg maturity used in carp are : the colour of the oocytes, their size or the position of the nucleus and the nucleolus after clearing the egg as described by BRZUSKA and BIENIARZ 1977.

#### B - Administration of exogenous gonadotropins.

##### 1) Hypophysation.

Hypophysation is the traditional method of induced fish breeding and is widely practiced with the major indian carp, chinese carp and the common carp. In these species it consists in the injection of homoplastic or heteroplastic pituitary extracts. However pituitaries collected from marine catfish have been used for indian carps (VARGHESE et al. 1975).

Details of techniques employed in the collection, preservation of pituitaries and preparation of extracts have been presented in PICKFORD and ATZ 1957 and often reviewed (SHEHADEH 1975, CHAUDHURI 1976, HARVEY and HOAR 1980, MARCEL 1980, ROTHBARD 1981).

In summary the following dosages, expressed in mg of dry weight, are used:

- common carp : In females 1 pituitary/kg is injected in two injections (equivalent to about 3mg/kg). The first injection or priming consists in 1/10 of the dose, the remaining 9/10 are given in a second injection. For males, 2mg/kg are given in one injection at the time of the second injection of females.

- indian carp : the female is given a priming dose of 2-3mg/kg b.w. and after an interval of 4-6h a final dose of 5-8mg/kg b.w. At the time of the second injection males are given 2-3mg/kg b.w.

- chinese carp : 4 to 4.5mg/kg b.w. is given in two injections for females, 2mg/kg b.w. in one injection for males. A more precise determination of the dose can be made by estimating the gonadal status of the females. This is done by measuring the girth of the female in the deepest part of the body. A dose relative to the body girth is given and the diagram is reported in MARCEL 1980 and ROTHBARD 1981 .

#### Hormonal profiles and oocyte evolution

Results obtained on carp (ANONYMOUS 1978) and goldfish (STACEY et al. 1979a) have shown that the periovulatory GtH surge is very brief during spontaneous ovulation. Classical hypophysation induces GtH increase of longer duration i.e. 3-4 days (JALABERT et al. 1977, FOSTIER et al. 1979, BIENIARZ et al. 1980). Priming dose induces a significant GtH increase one hour after the injection and then GtH levels continue to increase for 3 hours and remain high during the next 12 hours (WEIL et al. 1980) . The massive pituitary dose (given as a second injection or as a single injection) induces higher GtH levels (250-400ng/ml) which can last 3-4 days according the case as described by BIENIARZ et al. 1980. In fact these authors recorded high levels for 3 days in spawning fish and for 4 days in unspawned ones. This fact suggests that, following hypophysation, circulating gonadotropin levels may depend on the physiological conditions of the ovaries and in the case of spawning the ovaries can take up gonadotropin for maturation process. Priming dose induces shifting of the germinal vesicle towards the micropile in responding and unresponsive fish whereas the second injection induces maturation and ovulation in responding fish and

resorption without maturation in the non responding ones (JALABERT et al. 1977).

Although ovaries are ready to spawn they are able to respond to a GtH stimulation since an increase in plasma estradiol ( $17\beta$  E2) is noticed when compared to levels before injection (FOSTIER et al. 1979, WEIL et al. 1980, KIME and DOLBEN 1985). Serial samplings have shown that  $17\beta$  E2 are low during the first 3 hours following priming and then begin to increase 5 hours after the injection. Maximal values are attained 12 hours later (WEIL et al. 1980). However mean  $17\beta$  E2 levels are not increased following the second injection (WEIL et al. 1980, KIME and DOLBEN 1985). This increase in  $17\beta$  E2 might be due to the presence of vitellogenic oocytes since at the time of ovulation oocytes of all stages are present (BIENIARZ et al. 1977). The increase in  $17\beta$  E2 levels following priming is preceded by an increase in testosterone which is consistent with a precursor product relationship. The second injection of pituitary extract did not lead to a massive increase in testosterone related to the increase in GtH but greater amounts of glucuronides were observed (KIME and DOLBEN 1985).

Concerning progestins KIME and DOLBEN (1985) observed detectable levels of  $17\alpha$  hydroxyprogesterone ( $17\alpha$ OH P) before injection and an increase after hypophysation. Peak height varied from 1.6 to 13,3ng/ml and dropped rapidly after ovulation. On the other hand they observed no detectable levels of  $17\alpha$   $20\beta$  OH P except in one fish. Levels were 1,08ng/ml at the time of ovulation and 0,11 and 1,14ng/ml respectively 6 and 3 hours before ovulation. We must emphasize the fact that the treatment of hypophysation induced only partial ovulation in all the fish. In a work conducted in our laboratory blood samples were taken every 3 hours for 30 hours on 7 females submitted to hypophysation (100  $\mu$ g/kg of partially purified carp GtH). Two fish have completely ovulated, 2 partially and the remaining three have not ovulated. (fig. 1).

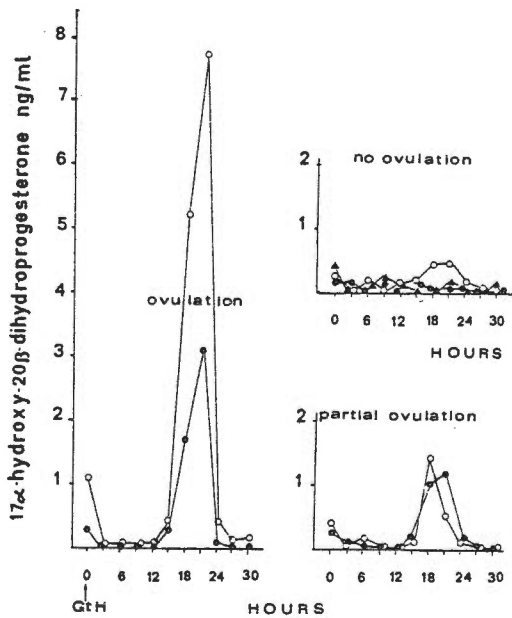


Figure 1 : Plasma  $17\alpha$   $20\beta$  OH P in female carp following hypophysation.

In the non ovulated fish,  $17\alpha$   $20\beta$  OH P levels were low varying between undetectable value and 0,5ng/ml. In the partially ovulated fish, a low rise in  $17\alpha$   $20\beta$  OH P was observed between 18 and 21 hours, followed by a decrease by 24 hours when ovulation was monitored. On the other hand higher levels were monitored in ovulating fish but the highest value was 7ng/ml, value much lower than that one indicated by LEVAVI-ZERMONSKY and YARON (summary this volume). These authors report a dramatic (111ng/ml) but transient increase followed by GV breakdown, corroborating the idea that  $17\alpha$   $20\beta$  OH P is involved in oocyte maturation. Furthermore during spontaneous ovulation transient increase of different progestins (progesterone,  $17\alpha$  OH P,  $17\alpha$   $20\beta$  OH P) have been recently described in goldfish (KAGAWA et al. 1983). Since these increases are transitory, higher values of  $17\alpha$   $20\beta$  OH P may have been attained between the sampling periods in our experiment.

#### Hormonal profiles and spermiation

In the literature, no data are available on circulating GtH and steroid levels following hypophysation. In a work conducted in our laboratory, (figure 2) plasma GtH, androgens and 11-ketotestosterone were monitored every 3 hours for a 24 hour period following a single injection of partially purified carp gonadotropin (100 $\mu$ g/kg). Collectable milt volume as well as spermatocrit were measured before the injection and 12 and 24 hours after the injection.

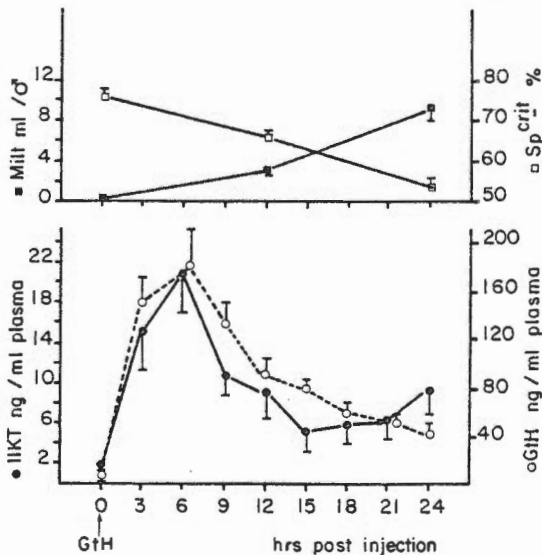


Figure 2 : Hormonal changes and collected milt volume after hypophysation.  
 $\bar{x} \pm s.e.$   $n = 8$

The treatment induced a large increase in GtH levels (64ng/ml) 3 and 6 hours after the injection. The levels decreased gradually from 9 to 24 hours to reach 40ng/ml by 24 hours. These circulating levels are much higher than those described during spontaneous spawning (ANONYMOUS 1978). 11 ketotestosterone levels followed the same profile than GtH with an increase the first

6 hours followed by a decrease to reach by 24 hours levels which are higher than those observed just before the injection and those previously described for androgens (about 3ng/ml) in a study conducted all the year round with males held in a concrete tank and which released a small quantity of milt (WEIL 1981). After hypophysation collectable milt volume increased considerably during the 24 hour experimental period (from 0,190ml to 9,5ml/♂) while spermatocrit was declining from 75 % to 53 % indicating a sperm dilution. This increase in GtH and 11-ketotestosterone confirms the role of GtH via steroid synthesis in spermiation (see above). These data and works from KIME and MANNING (summary this volume) and from TAKASHIMA et al. 1984 indicate that in carp 11-Ketotestosterone is closely associated with male reproductive development.

The technique of hypophysation has certain disadvantages as :

- its relatively high cost due to the unpredictable supply of pituitary and the necessity to maintain water temperature above 18°C.

- its partial success. Only about 70 % of the animals ovulate and unspawned fish undergo oocyte atresia leading to an increase in mortality even under ideal conditions (good oxygenation of the water and food supply).

2) Use of mammalian hormones.

HCG (Human Chorionic gonadotropin) and Synahorin (mammalian pituitary extract) can be reliably supplied and attempts have been made to use them instead of hypophysation. HCG efficiency depends on the species. It's effective in inducing ovulation in goldfish (YAMAMOTO and YAMAZAKI 1967). Chinese studies report that it is weakly effective in grass carp and mud carp but more efficient in silver carp and bighead. However it has harmful effects on unspawned fish leading to the death of the animals. In indian carp, the relatively low efficiency of HCG and Synahorin can be improved by using them in combination with a lower dose of pituitary extract (CHAUDHURI 1976).

C - Discharge of endogenous gonadotropin hormone.

1) Administration of gonadotropin releasing hormone (GnRH).

As mentioned above LHRH or LHRH analogues (LHRHa) evoke the release of endogenous pituitary gonadotropin supplies. A seasonal pituitary sensitivity to GnRH have been demonstrated but at the time of inducing spawning the sensitivity is maximal in carp (WEIL et al. 1975) and in goldfish (SOKOLOWSKA et al. 1985).

#### Action in female

In goldfish, spontaneous ovulation does not occur at 12°C when fish are held in aquaria without vegetation. Relatively high doses of LHRH given in one injection administered either by intraperitoneal (1µg/g) or by intracranial injection (200ng/g) were partially effective in inducing ovulation (respectively 50 % and 33 % of ovulation, LAM et al. 1975 -1976). A weak percentage of ovulation was also recorded with the total dose of 200ng/g of a potent LHRHa when given in two injections 12 hrs apart at 12 -14°C (CHANG et PETER 1983) or 3 hrs apart at 18 -20°C (SOKOLOWSKA et al. 1984). Sustained GnRH release allowed by the implantation of pellets containing 25µg or 125µg of LHRHa were also partially effective (20 - 50 % of ovulation SOKOLOWSKA et al. 1984). Both treatments with LHRHa induced high plasma GtH levels which were in the range of those reached during spontaneous ovulation but were of longer duration.

In carp, nine daily LHRH intrahypophyseal injections at 1µg/kg stimulated oocyte maturation (germinal vesicle migration and breakdown) at 18 - 20°C but the same dosage given by intracardiac or intraventricular injection produced any effect (SOKOLOWSKA et al. 1978). Germinal vesicle



migration but no ovulation was obtained after 5 daily intracardiac LHRH injections at 1 µg/kg (SOKOLOWSKA 1982) or two intracardiac injections at 3 µg/kg given 3 hrs apart (WEIL et al. 1980).

Single dose of LHRHa, 1 µg/kg given by intracardiac injection in females held in aquaria or spawning ponds (BILLARD et al. 1984) or 50 g given intraperitoneally in females held in concrete tank (BILLARD et al. 1983a) were also ineffective in inducing ovulation. Repeated doses, 5 µg/Kg plus 50 µg/Kg given 6 hrs apart, given by intraperitoneal injection in females held in aquaria with or without vegetation were ineffective in inducing ovulation while they were partially effective in females held in cages maintained in a pond. The authors think that the response was facilitated by the occurrence of circadian changes in dissolved O<sub>2</sub>, CO<sub>2</sub> and pH, changes similar to those observed in spawning ponds. This point will be discussed in a next paragraph. In this latter experiment plasma GtH levels were similar in ovulated and non ovulated females but were higher than those of females held in aquaria. However measured levels were lower than those circulating during spontaneous ovulation. This lack of efficiency of LHRH in carp was also reported by KOURIL et al. 1983 for doses ranging from 10 to 200 µg/kg. The same authors report successful results for tench at a dose of 100-200 µg/kg of LHRH and 10-40 µg of LHRHa and for grass carp at a dose of 200 µg of LHRH and 20 µg/Kg of LHRHa. In China LHRH analogue is routinely used to induce spawning of different species of chinese carp. Trials performed in 24 fish farms and conducted on 500 animals are reported in ANONYMOUS 1977a -1977b. In summary the recommended doses for the different species held at temperature lying within the normal range (20 -28°C) are the following. For black carp a single dose of 10 µg/kg is efficient ; effectiveness is increased when injection of LHRHa is followed by that of 1 -2mg of pituitary. In grass carp 5 - 10 µg/kg of LHRHa in one injection is prescribed while in bighead a priming dose of 1 - 2 µg/kg followed by 8-9 µg/kg 12 hrs later for matured fish or 24 hours later for less matured fish, is suggested. For silver carp 5 - 10 µg/kg of LHRHa in one injection is recommended for first time spawners or 10-25 µg in two injections for repeated spawners (10 µg/kg + 0,1 - 1 mg CAMP/kg in two injections spaced 12h apart enhances effectiveness).

From the literature it is noteworthy that there is a prolonged latency in the ovulatory response to LHRH or LHRHa, in goldfish, tench, chinese carp when compared with hypophysation or HCG treatment. This treatment presents the advantage of decreasing the rate of mortality in chinese carp (ANONYMOUS 1977b, ROTTMANN and SHIREMAN 1985).

Recent promising works from BIENIARZ et al. 1985 report that salmon GnRH analogue is very active in inducing ovulation in common carp as compared to LHRHa.

#### Action in male

Chinese studies (ANONYMOUS 1977a) report that LHRHa is very effective in inducing spermiation in carp. Injection of 10-30 µg induces milt release in the black carp after 12 - 24h. The effect is remarkable in spotted silver carp which shed motile spermatozoa within 6 1/2 hrs following the injection.

In common carp LHRH and LHRHa analogue induce also an increase in collectable milt volume. One injection of LHRH at the dosage of 50 µg/kg is more effective than at the dosage of 10 µg/kg while with LHRHa both dosages have the same effect (BILLARD et al. 1983b). Following an injection of 50 µg/kg collected milt volume is increased the first two days and then begin to decrease by day 3 for reaching basal levels on day 5 (BILLARD et al. 1983a). TAKASHIMA et al. 1984 report that smaller dosage of the analogue administered by 5 daily injections of 1 µg/kg does not stimulate the increase of collectable milt volume while collected milt volume is increased with 5

daily injections of 10µg/kg. The same authors demonstrated that the efficiency of LHRH administration depends on the solvent used (affecting the kind of release of LHRH) since spermiation was not stimulated by 5 daily injections of 10µg/kg of LHRHa dissolved in propylene glycol. In this latter case, plasma GtH and 11-ketotestosterone were not modified compared to control. On the other side, following the injections of 10µg/kg of LHRHa dissolved in saline an increase in 11-ketotestosterone levels was monitored as long as spermiation was stimulated i.e. from 24 h after the first injection until 24 hrs following the fifth.

3) Release of an inhibition influencing spontaneous and GnRH induced GtH release.

PETER and CRIM 1978 and PETER and PAULENCU 1980 have suggested the presence of a gonadotropin release inhibitory factor (GRIF) in goldfish on the basis of brain lesioning experiments. On the basis of *in vivo* studies on goldfish CHANG et PETER (1982, 1983a) demonstrated that dopamine has GtH release inhibitory activity by acting directly on gonadotrophs to inhibit spontaneous secretion of GtH and to block the action of GnRH. Removal of the dopamine inhibition of GtH release by pimozide treatment, a dopamine antagonist, potentiates the ability of LHRHa to stimulate GtH release and ovulation in female goldfish at 12-14°C (CHANG et PETER 1983b) as well as 18 - 20°C (SOKOLOWSKA et al. 1984). Indeed the low percentage of ovulation (between 25% - 50 %) obtained at 12 -14°C following LHRHa injection and at 18 - 20°C following LHRHa treatment (injection or pellet implantation) is improved by action of pimozide and can reach 80-90 %. The authors studied the timing of administration of pimozide and LHRHa allowing to optimize the rate of ovulation. Best results were obtained when pimozide is given prior to the injection of LHRHa or with the first of two injections of LHRHa or following the pellet implantation. These observations indicates that prior release from dopamine inhibition, greatly potentiates the ovulatory response to LHRHa as well as GtH release since higher GtH levels were obtained in this case. A transient increase in 17α 20β OH P and 17α OH P was observed in such treated fish before ovulation (PETER et al. 1984).

In female common carp, experiments of brain lesioning (PETER, SOKOLOWSKA, BILLARD and CRIM unpublished) suggest that a GRIF is also present. Preliminary promising results were obtained with combined treatment with pimozide (10mg/kg) and LHRHa (50 µg/kg) 6 hours later (BILLARD et al. 1983). This combination induced ovulation in some females (about 50 %) while only oocyte maturation was induced by LHRHa alone. Furthermore, promising recent results on the success of ovulation have been obtained in female chinese carp with pimozide + LHRHa and in female common carp with reserpine (a catecholamine depletor) + LHRHa (LIN et al. 1986). On the other hand, in male common carp, a combination of LHRHa + pimozide had only a slight effect on the stimulation of spermiation when compared to LHRHa treatment alone (BILLARD et al. 1983a).

4) Action of factors abating the negative feed back effects of endogenous sex steroids on hypothalamo-hypophysial axis.

Antioestrogens such as clomiphene citrate (clomid) or tamoxifen (ICI 46474) induce gonadotropin release in carp (BRETON et al. 1975) and goldfish (BILLARD and PETER 1977). BRETON et al. 1975, comparing different dosages (0,1mg -1mg - 10mg/kg) found that the dosage of 1mg/kg was the best to induce high GtH levels similar to those present during ovulation in goldfish and carp. The use of clomid (10mg/kg) was successful in inducing ovulation in goldfish (PANDEY and HOAR 1972, PANDEY et al 1973). In carp KUMAR and CHANDRASEKHAR 1980 accelerated spawning (4 months) by tri-weekly injections of clomid (25 and 50 µg/animal) for a period of 3 months during the prepatory period. On mature animals three daily injections of 1mg/kg failed to induce ovulation (BIENIARZ et al. 1979) while two successive injections of higher dosage (10mg/kg) were successful in overcoming the blockade induced

by indomethacine (an inhibitor of prostaglandin synthesis) treatment (KAPUR and TOOR 1979).

In cyprinid this technique has been used only at a research level.

#### D - Action of factors acting on oocyte maturation.

As mentioned above corticosteroids as well as progestins and particularly  $17\alpha 20\beta$  OH P have a role in inducing in vitro oocyte maturation in goldfish and carp. Corticosteroids and progestins were tested for induction of ovulation activity in vivo. In goldfish trials were successful with deoxycorticosterone (DOC), cortisone and corticosterone ( $100\mu\text{g/g}$ ) (KHOO 1974) while only partial ovulation was obtained with deoxycortisol (LAM and PANDEY cited in LAM 1982). Metopirone, an inhibitor of  $11\text{-hydroxylase}$  induced ovulation in goldfish (PANDEY et al 1977). With regard to progestogens, progesterone ( $100\mu\text{g/g}$ ) was effective (KHOO 1974) while  $17\alpha 20\beta$  OH P ( $12\mu\text{g/g}$  daily for 5 days) was ineffective in goldfish. (PANDEY et al cited in LAM et al. 1978). In carp, neither  $17\alpha 20\beta$  OH P ( $2\text{mg/kg}$ ) nor DOC ( $1\text{mg/Kg}$ ) alone or in combination, induced ovulation at  $13\text{-}15^\circ\text{C}$  (JALABERT et al. 1977).  $17\alpha 20\beta$  OH P gave positive results when administered 1 day after priming with a low dose of carp pituitary extract ( $0.6\text{mg/kg}$ ). At such temperatures classical hypophysation yielded worse results since only partial ovulation followed by resorption was observed. Priming with a low dose of pituitary extract induced migration of the GV toward the periphery of the oocyte, indicating that responsiveness to  $17\alpha 20\beta$  OH P seems dependent on GV migration. However BIENIARZ et al. 1985 obtained in vitro oocyte maturation only in some females although ovarian fragments were in the same stage i.e. with oocytes before migration of the GV. This indicates that oocytes in the same stage as determined by position of the nucleus have variable responsiveness to the same hormonal treatment.

Although thyroid hormones act synergistically with gonadotropin hormone to induce vitellogenesis (HURLBURT 1977) and T<sub>3</sub> influences the maturational response to gonadotropic and steroid treatments (EPLER and BIENIARZ 1983), they have not yet been tested in vivo.

These techniques are not used in fish farm.

#### E - Influence of factors promoting ovulation by action on follicular contraction and rupture.

As mentioned above prostaglandins and catecholamines can trigger ovulation in vitro. In vivo only the role of prostaglandins (PG) was investigated.

Indomethacine, an inhibitor of prostaglandins biosynthesis, blocks the ovulatory response of goldfish to a combination of HCG and  $20^\circ\text{C}$  warmed water while a single IP injection of PGE<sub>1</sub>, PGE<sub>2</sub> or PGE<sub>2</sub> $\alpha$  ( $5\mu\text{g/g}$ ) restores the response (STACEY and PANDEY 1975). In carp KAPUR and TOOR (1979) demonstrated that a single injection of indomethacine ( $10\mu\text{g/g}$ ) blocked ovulation for 12 days in females held in conditions in which control fish ovulated spontaneously. The role of prostaglandins mediated by and stimulation of GtH secretion is unlikely since PETER and BILLARD (1976) found that injection of PGF<sub>2</sub> $\alpha$  or PGE<sub>2</sub> but not PGE<sub>1</sub> into the third ventricle of the brain decreased serum GtH in the female. A direct action on follicular rupture via smooth muscle contraction seems more likely (STACEY and PANDEY 1975, KAGAWA and NAGAHAMA 1981) for goldfish (EPLER 1978, EPLER et al. 1985) for carp. Furthermore prostaglandins have a role in spawning behavior in goldfish (STACEY, 1976). Thus it may be possible to use prostaglandins to induce ovulation as well as spawning behavior in fish with mature oocytes.

III - INFLUENCE OF ENVIRONMENTAL AND SOCIAL FACTORS ON SPONTANEOUS AND HORMONAL INDUCED SPAWNING.

It is well established that in cyprinids water temperature plays an important role in gametogenesis development (review of PETER and CRIM 1979). In common carp, sexual maturity is reached earlier in warm climates than in temperate conditions and GUPTA 1975 obtained a precocious maturity in carp held in warm water aquaria (23°C). Male and female tench maintained all year in a heated pond (6°C above ambient temperature) showed an accelerated spermatogenesis and vitellogenesis compared to the control (BRETON et al. 1980a et b). However daylength may interfere with temperature since in an indian carp a constant short daylength retarded the maturity of gonads in female (VERGHESE 1970) and in male of common carp (WEIL 1981 - showed in BILLARD et al. 1982).

In practice the influence of environmental factors on induction of a rematuration of the gonad after hormonal induced spawning is used in common carp, chinese carp and indian carp (respectively HORVATH 1980a ; LIN 1982 and FREEZE and CRAWFORD 1983 ; BHOWNICK et al 1977). The combination of high water temperature and good feeding induces within 2 months the maturation of the gonad and a second spawning may be induced with hormonal treatment when temperature required for ovulation is provided.

With respect to the end of gametogenesis i.e. spermiation and ovulation, different factors may improve the spontaneous process. In goldfish spermiation is promoted a progressive rise in temperature which is in correlation with an increase in plasma GtH (GILLET et al. 1977) ; ovulation occurs at 20-21°C (YAMAMOTO et al. 1966., STACEY et al. 1979b) in presence of absence of aquatic vegetation while it occurs at 12°C only in the presence of vegetation (STACEY et al 1979b). Furthermore ovulation in goldfish is highly synchronized with photoperiod (STACEY et al 1979b). Social factors may act as well. In female goldfish YAMAZAKI 1965 and YAMAMOTO et al. 1966 indicate that the presence of sexually active males induce ovulation. For males KYLE et al. 1985 report that the presence of a receptive female or stimulus pairs of spawning goldfish induce an increase in expressible milt volumes and plasma GtH. NIKITINA and GODOVICH 1983 have demonstrated that the dynamics of sex hormones in the blood of male common carp depend on the presence or absence of spawning in the females placed with them. In practice in China it is possible to induce spawning of female mud carp by the presence of active female spawners (injected with pituitary extract) when they are held in a common spawning pond (LIN 1982).

Environmental factors modulate the response to hormonal treatment. Temperature is an important factor for :

- the injection interval
- the latency of the ovulatory and milting response as demonstrated in female and male common and chinese carp (HORVATH 1978b) and in female goldfish (STACEY et al. 1979b) following pituitary or HCG treatment.
- the time of overripening of ovulated oocytes. It depends on carp species. (HORVATH 1978b)
- the success of spawning i.e. the maturation of breeders.

Oxygen concentration also conditions the success of hypophysation (HORVATH and PETERI 1980). These authors demonstrated that both, water temperature and the amount of dissolved oxygen, strongly influence the maturation of spawners. If one of these parameters is lower than required the adverse effect cannot be counter balanced by the higher level of the other factor.

Time of injection (since there is evidence of a possible circadian rhythm of oocyte sensitivity, BIENIARZ et al., summary this volume) as well as the presence of vegetation, or a precise photoperiod, or spawning fish may be also important to ensure a maximal efficiency of hormonal treatment.

IV - CONCLUSION

The hormonal treatment most commonly used for inducing spawning in common carp, chinese and major indian carp is hypophysation. This technique presents some disadvantages such as the unpredictability of pituitary supply, the problem of standardization of the pituitary extracts and its partial effectiveness (around 70 %).

The use of GnRH promoting endogenous GtH release seems promising. LHRH analogs have different advantages : they are commercially supplied and they don't evoke antibody formation. On the other hand their efficiency on ovulation varies with the species and the latency to ovulatory response is delayed compared to hypophysation. Removal of the dopamine inhibition improves these two points as demonstrated by LIN 1986 and studies, still at an experimental stage, are made in this field. Furthermore, the use of salmon GnRH analogues alone seems promising and further studies must be done in this direction.

It is evident from the results reported above that the development of a new hormonal treatment for inducing spawning as well as the improvement of hypophysation technique implies the knowledge of criteria determining the appropriate time of its administration and this particularly in female.

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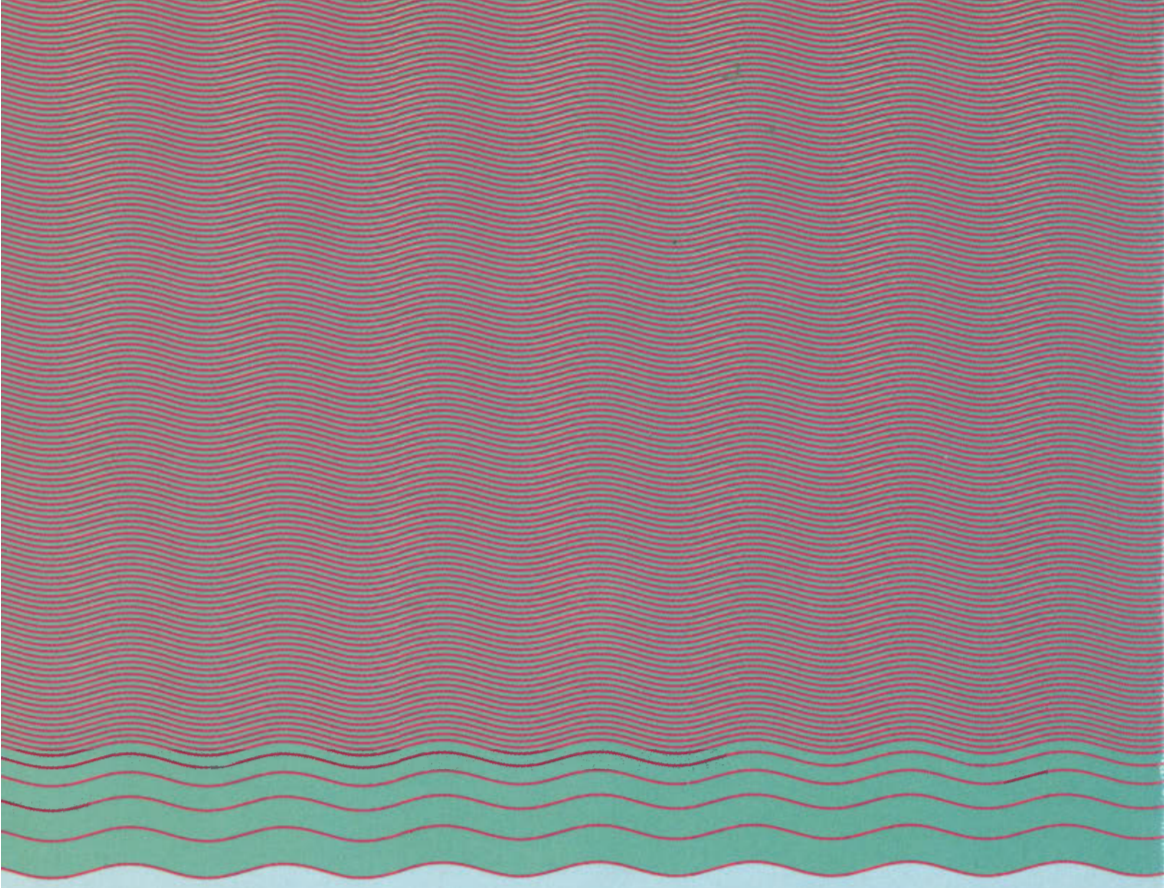
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**Cyprinids** constitute the largest group of cultured fish in the world and include species such as carp whose domestication dates from ancient times. The ability to adapt to a wide range of temperatures and high fluctuations in water oxygen content as well as to diverse modes of feeding in various biotopes has made this species the most highly produced in the world : 2 million tons of cyprinids out of a total of 3.5 million tons of fish, including all species. This book reviews the actual state of knowledge and of management techniques in the fields of nutrition, reproduction, genetics, management of larvae, growth and rearing, pathology, economics and processing of cyprinids.

***Les Cyprinidés** constituent le groupe de poissons le plus largement élevé dans le monde et comptent des espèces comme la carpe dont la domestication est très ancienne. Ses caractéristiques d'élevage : tolérance à une large gamme de températures, de fortes fluctuations d'oxygène dissous, modes d'alimentation diversifiés à partir des différentes niches du réseau trophique, font de cette espèce la première production de l'aquaculture mondiale (2 millions de tonnes de cyprinidés sur 3,5 millions de tonnes de poissons, toutes espèces confondues). Ce volume présente l'état actuel des connaissances et des techniques d'élevage en matière de nutrition, reproduction, génétique, élevage larvaire, croissance et élevage, pathologie, économie et transformation.*



ISBN 2-85340-791-8



HYDROBIOLOGIE ET AQUACULTURE

**Aquaculture of Cyprinids**  
***L'aquaculture des Cyprinidés***

R. BILLARD, J. MARCEL  
Editeurs

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# Aquaculture of Cyprinids

## *L'aquaculture des Cyprinidés*

Actes du Colloque International sur l'Aquaculture de la Carpe et des espèces voisines  
tenu à Evry (France) du 2 au 5 septembre 1985

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INRA, Département Hydrobiologie

F-78350 Jouy-en-Josas

*For sale / En vente*

INRA, Service des Publications

Route de St Cyr, F-78000 Versailles

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ISBN : 2-85340-791-8