

## Pituitary response to LH-RH at different stages of gametogenesis in the rainbow trout (*Salmo gairdneri*)

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**Summary.** Pituitary response to LH-RH has been studied in the male and female rainbow trout (*Salmo gairdneri*) at different stages of gametogenesis. The male was hardly sensitive at the onset of spermatogenesis, but a response occurred at the spermatid stage and continued during spermatogenesis and spermiation. We particularly studied the end of the female cycle. Response was low in immature fishes and in the early stages of oocyte maturation, but became stronger at vitelline maturation. The relationships of these responses to plasma and pituitary t-GTH levels are discussed.

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### Introduction.

Hypothalamic control of gonad activity (Peter, 1970), and especially of gonadotropin secretion *in vitro* (Breton *et al.*, 1971b), has been demonstrated in teleost cyprinid fish. Synthetic LH-RH causes gonadotropin hormone release *in vivo* in the carp (Breton *et al.*, 1973), trout (Crim and Cluett, 1974) and goldfish (Crim, Peter and Billard, 1976). However, the pituitary response *in vivo* to LH-RH differs, and depends on the time of the reproductive cycle in carp (Weil, Breton and Reinaud, 1975).

As it has been demonstrated that trout plasma gonadotropin levels vary in relation to the state of the gonads (Breton *et al.*, 1975 ; Crim, Watts and Evans, 1975), we wished to find out if pituitary response to LH-RH varied with the stage of gametogenesis in the male and female trout. In the female, we have particularly studied the final stage of gametogenesis.

### Material and methods.

Trials were conducted in 1976 on adult 2-year old males during their second reproductive cycle. The animals came from the Etrun fish farm in the Pas-de-Calais department. They were kept in rearing tanks under natural photoperiod and temper-

ature until the experimental dates. The 4 lots studied corresponded to 4 different stages of gametogenesis according to the criteria of Billard and Escaffre (1975). The various stages were determined by microscopic examination.

Lot 1, stage II : onset of gametogenesis ;

Lot 2, stage III : spermatocytes present and beginning of spermatid stage ;

Lot 3, stage V : all spermatogenetic stages present but no spermiation ;

Lot 4, stages VI and VII : onset of spermiation.

Experiments were carried out on the females in February 1977 using fishes from Denmark. The animals were 2-years old and in their first reproductive cycle. Five lots were studied :

Lot 1 : immature animals (I) having oocytes of less than 100  $\mu$  diameter ;

Lots 2-5 : animals presenting oocytes in different stages of maturation as defined by Jalabert *et al.* (1976). The stage was determined after examination of several oocytes obtained by abdominal massage.

Lot 2, stages (i) and (o) : end of vitellogenesis (i) and oocyte with migrating germinal vesicle (GV) (stage o) ;

Lot 3, stage 1 : oocyte with peripheral GV ;

Lot 4, stages 2 and 3 : maturing oocyte before GV breakdown (GVBD) (2) and after GVBD (3) ;

Lot 5, stage 4 : mature and ovulated oocytes.

The animals of the different lots were anesthetized with 0.5 ml/l phenoxyethanol and then kept under anesthesia by perfusion (0.3 ml/l) in the gills during the experiment. 1  $\mu$ g/kg LH-RH (Hoechst, Batch Op. R6) was injected into each experimental animal by intracardiac catheter ; a buffer was injected by the same method into controls. Blood samples were taken with a heparinized syringe before (basic level) and 2, 4, 6, 8, 15 and 20 min. after the LH-RH or buffer injection. t-GTH level in the samples was determined by radioimmunological assay (RIA) as previously described for c-GTH by Breton *et al.* (1971a). Antibody was raised against a pure t-GTH and used at a dilution of  $1/2.10^{-5}$ . The animals were killed 20 min. later and the pituitary excised and ground to determine t-GTH content by RIA. A piece of testis was fixed for quantitative analysis of germ cells.

LH-RH response was studied by characterizing the gonadotropin secretion of each fish by a single overall value, obtained by adding the t-GTH levels from 2 to min. after the basic level had been subtracted from each value. All fish having an overall value higher than that of the corresponding control lot were considered as responsive to LH-RH. We term this overall value « t-GTH secretion during 20 min. » in order to simplify.

*Statistical methods.* — Mean confidence intervals ( $P < 0.05$ ) were computed only when there were more than 5 animals in a lot. Samples were compared either by variance analysis or by the U-test of Mann-Whitney (non-parametric method).

## Results.

### *Pituitary and plasma t-GTH levels (table 1).*

**Males.** — Basic plasma t-GTH level was low at the onset of gametogenesis (stage II, lot 1) and increased when spermatocytes were present (stage III, lot 2 :  $P < 0.001$ ). It then remained constantly high until spermiation. Pituitary t-GTH level was low in the first stages (II and III, lots 1 and 2) and then increased to a maximum at spermiation (lot 4).

TABLE 1  
*Plasma (basic level) and pituitary t-GTH levels*

		Lots					
		1	2	3	4	5	
♂	Plasma ng/ml	(1) 1.3 ± 0.2 (24)	← *** → 4.1 ± 0.5 (24)	← NS → 3.3 ± 0.3 (24)	← NS → 3.6 ± 0.3 (20)		(a)
	Pituitary µg/mg	0.6 ± 0.1 (7)	← NS → 0.7 ± 0.2 (8)	← * → 1.6 ± 0.3 (8)	← *** → 7.6 ± 1.1 (7)		(b)
♀	Plasma ng/ml	3.2 ± 0.6 (16)	← NS → 3.8 ± 0.6 (18)	← NS → 5.1 ± 1.1 (12)	← ** → 10.3 ± 1.4 (21)	← NS → 10.1 ± 2.3 (15)	(a)
	Pituitary µg/mg	0.55 ± 0.4 (8)	← *** → 2.2 ± 0.4 (9)	← NS → 2.5 ± 1.6 (4)	← NS → 3.0 ± 0.8 (6)	← NS → 2.7 ± 0.9 (8)	(b)

(1) : mean ± s.e.

(a) : variance analysis.

(b) : Mann-Whitney U-test.

NS :  $P > 0.05$  ; \* :  $P < 0.05$  ; \*\* :  $P < 0.025$  ; \*\*\* :  $P < 0.001$ .

( ) : Number of animals. The mean basic level of t-GTH was computed using data from control and experimental animals. Mean pituitary t-GTH content was computed from data on control animals.

**Females.** — Basic plasma t-GTH, level was low in immature fish and at the end of vitellogenesis (lots 1 and 2), but increased progressively during oocyte maturation. The lots 3 and 4 (« oocyte with peripheral GV » and « maturing oocyte ») showed a significant difference ( $P < 0.025$ ). At ovulation (lot 5), the level was still high. Pituitary t-GTH was constantly high in lots 2 to 5 (« end on vitellogenesis » until « ovulation ») as compared to immature fish (lot 1 :  $P < 0.001$ ).

### *LH-RH response.*

**Males (fig. 1a).** — At onset of gametogenesis (stage II), 1 animal out of 8 showed a very low response. LH-RH sensitivity increased when spermatocytes were present

(stage III). At that stage, 2 animals out of 8 responded to LH-RH ; one of these showed a very high peak (90 ng/ml). When all the spermatogenetic stages were present (stage V), 3 out of 4 animals responded as compared to the controls. However, maximum t-GTH was about 6 ng/ml. At spermiation (stages VI and VII), 2 animals out of 5 showed sensitivity ; high t-GTH values were found at that stage in some control subjects. Due to the heterogeneity of response in control and experimental animals beginning at stage III, a significant difference ( $P < 0.05$ ) between the overall mean value of treated animals and controls only appeared at stage V.

*Females* (fig. 1b). — Because of the small number of animals studied, our results can only be considered as preliminary. Immature animals in the early stages of oocyte maturation showed a weak LH-RH response. Two out of 5 subjects responded at immature stage (I) ; 3 out of 5 at stages (i) and (o), and 1 out of 2 at stage (1). The maximum value found was 8 ng/ml. Large amounts of t-GTH were only secreted at the matured oocyte stage (lots 2 and 3) ; 4 subjects out of 7 responded and 2 of them had t-GTH peaks of 25 ng/ml and 13 ng/ml, respectively. On the other hand, there was no response at ovulation, and LH-RH even seems to have a depressive effect on t-GTH level.

We only found a significant difference in the overall mean between control and experimental animals at the matured oocyte stage ( $P < 0.037$ ) and the ovulated stage ( $P < 0.028$ ).

## Discussion.

Pituitary response to LH-RH varies during gametogenesis ; there is none in the early stages of gametogenesis, but it increases later. This confirms the results of Crim and Cluett (1974) who found that LH-RH didnot release t-GTH in seasonally imma-

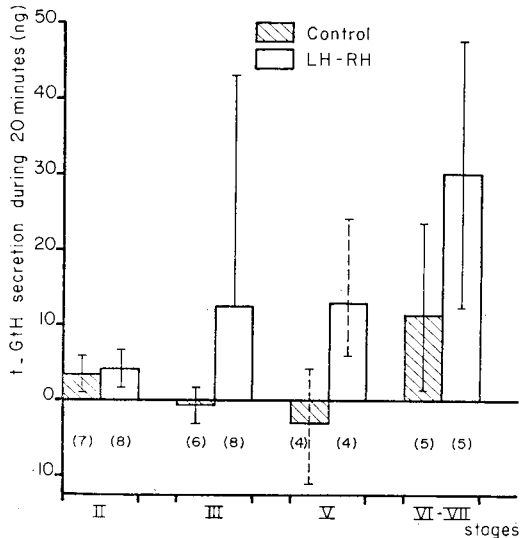


FIG. 1a.

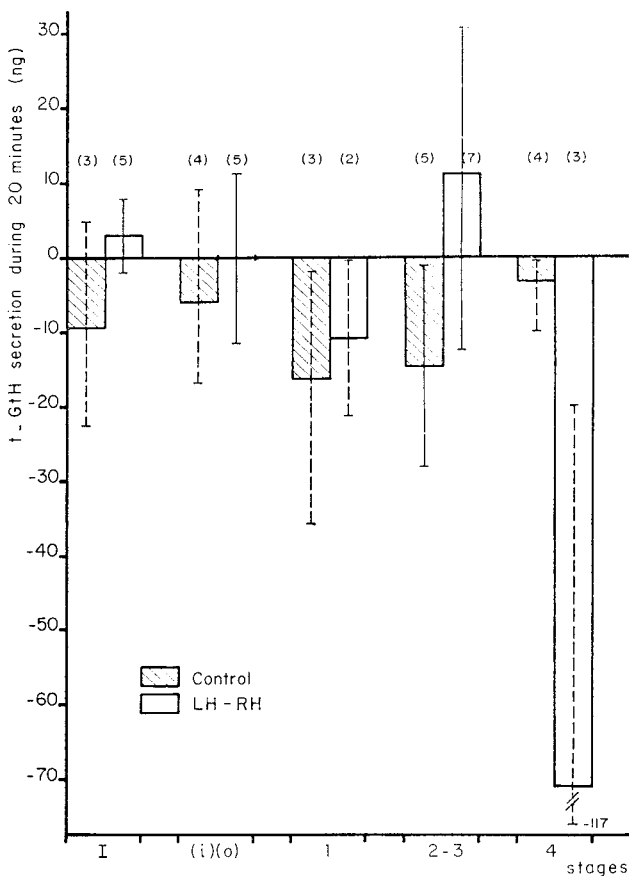


FIG. 1b.

FIG. 1a and 1b. — Response to LH-RH at different stages of gametogenesis.

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- mean and confidence limit at a threshold of  $P < 0.05$  when  $n \geq 5$  ;
  - mean and true high and low values when  $n < 5$  ;
  - ( ) number of animals.

ture animals. The appearance at stage III of pituitary sensitivity to LH-RH corresponds to an increase of plasma t-GTH.

In females, basic level of plasma t-GTH increases progressively during oocyte maturation. It should be noted that pituitary response to LH-RH varies in the same way until maturation when it is maximal. After ovulation, there is none. As pituitary t-GTH levels are identical from immature oocyte stages to ovulation, this difference in response may be due to the steroid environment. It is known now that estradiol  $17\beta$  level declines progressively until ovulation (Fostier *et al.*, 1978). Perhaps the ratio

between estradiol  $17\beta$  and  $17\alpha$ -hydroxy- $20\beta$ -dihydroprogesterone, the most potent steroid-inducer of trout oocyte maturation (Fostier, Jalabert and Terqui, 1973), modifies pituitary sensitivity to LH-RH.

After an LH-RH injection, the amounts of gonadotropin hormone released in trout plasma at reproductive time are low as compared to the carp (Weil *et al.*, 1975). The maximum values observed vary between 30 and 100 ng/ml in carp, and between 6 and 25 ng/ml in trout. At that stage, pituitary gonadotropin hormone content is 15 times higher in the carp than in the trout female and 7 times higher than in the trout male. The only elevated level in trout was observed in a male in mid spermatogenesis (beginning of spermatids).

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**Résumé.** La sensibilité hypophysaire au LH-RH a été étudiée chez la truite Arc-en-ciel mâle et femelle à différents stades de la gamétogenèse. Chez le mâle, elle est presque nulle au début de la spermatogenèse ; elle apparaît lorsque le stade spermatide est atteint, puis se maintient au cours de la spermatogenèse et de la spermiation. Chez la femelle, la fin du cycle a été plus particulièrement étudiée. La réponse est faible chez les poissons immatures et aux premiers stades de la maturation ovocytaire ; elle est la plus forte au moment de la maturation vitelline. Les relations de ces réponses avec les teneurs plasmatiques et hypophysaires en t-GTH sont discutées.

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