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Claudine Weil, Odile Marcuzzi. Cultured pituitary cell GtH responses to GnRH at different stages of rainbow trout oogenesis and influence of steroid hormones. *General and Comparative Endocrinology*, 1990, 79, pp.483-491. 10.1016/0016-6480(90)90079-2 . hal-02713606

HAL Id: hal-02713606

<https://hal.inrae.fr/hal-02713606>

Submitted on 1 Jun 2020

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Cultured Pituitary Cell GtH Response to GnRH at Different Stages of Rainbow Trout Oogenesis and Influence of Steroid Hormones¹

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Accepted October 27, 1989

In rainbow trout, a variable *in vivo* pituitary sensitivity to GnRH has been previously observed, depending on the stage of oogenesis. The purpose of the present work was to study, *in vitro*, the role of oestradiol (E2) and 17 α -hydroxy,20 β -dihydroprogesterone (17 α 20 β P), respectively, involved in vitellogenesis and in oocyte maturation, upon this variability. The study was performed using primary cultures of whole pituitary cells from animals at different stages of oogenesis and subjected to increasing doses of salmon GnRH (sGnRH) after a 3-day pretreatment with control medium or medium supplemented with the steroid at levels corresponding to those circulating at the time of particular events of the sexual cycle (maturation and vitellogenesis). In control cultures, pituitary GtH responsiveness to sGnRH was maximal at ovulation, since at this time the gonadotrophs were able to respond to 10⁻⁹ M sGnRH, whereas during vitellogenesis and preovulatory stages, the minimal effective dose of sGnRH ranged between 10⁻⁶ and 10⁻⁸ M. We have demonstrated that 17 α 20 β P has a positive or negative effect by acting directly on pituitary cell responsiveness to sGnRH, depending on the stages at which it is applied; its effect is positive during early vitellogenic and preovulatory stages whereas it is negative at the time of ovulation. E2 also increased pituitary responsiveness to sGnRH when applied during early vitellogenesis at low doses, corresponding to circulating levels at the time of ovulation; higher levels of E2, corresponding to circulating levels found during the last stages of vitellogenesis, did not modify pituitary responsiveness but increased cell GtH content. Results obtained are discussed in relation to hormonal changes (gonadotropin and steroid hormones) observed by different authors during the sexual cycle. © 1990 Academic Press, Inc.

Pituitary response to GnRH throughout the sexual cycle in female and male rainbow trout has been previously studied *in vivo* (Weil *et al.*, 1978, Weil, 1981). A variable responsiveness to GnRH was recorded during the cycle with the highest responses when gonads were maturing and therefore when plasma gonadotropin (GtH) was increasing.

The object of the present work was to study, *in vitro*, the role of steroid hormones upon this variable response to GnRH in female trout. The *in vitro* system used consisted of primary cultures of whole pituitary

cells as previously set up (Weil *et al.*, 1986). At precise stages of oogenesis, the response of gonadotrophs present in the culture to various doses of GnRH were studied, as was the influence of the main steroids involved in particular events such as vitellogenesis (17 β -oestradiol) and oocyte maturation (17 α -hydroxy,20 β -dihydroprogesterone). The results obtained are discussed in relation to hormonal changes (GtH and steroid hormones) observed *in vivo* by different authors and to our previous results on GnRH sensitivity.

MATERIALS AND METHODS

Animals and Studied Stages of Oogenesis

Experiments were conducted on 3-year-old female

¹ Presented in part as a poster at the Third International Symposium on Reproductive Physiology of Fish, St. John's, Newfoundland, Canada, 2-7 August 1987.

rainbow trout coming from two different fish farms and kept at 12° in the laboratory in a recirculated water unit. Three experiments were carried out.

In Experiment 1, fish from two winter-spawning strains reared either in the Gournay/Aronde (Oise) or in the Cornec (Finistère) fish farm were used. On their arrival in the laboratory their oocyte stage was at the end of vitellogenesis. Every week, until ovulation, the oocyte stage was determined by examining a few follicles squeezed out by abdominal stripping as described by Jalabert and Fostier (1984). At ovulation time, ova were taken off the abdominal cavity and fish were kept until their use. The following stages were studied: a preovulatory stage characterized by oocytes with the germinal vesicle in a subperipheral position (SPGV) according to Jalabert and Fostier (1984), ovulation time (day of ovulation), two post-ovulatory stages (20 and 40 days following ovulation), and two vitellogenic stages, 3 and 7 months following ovulation corresponding respectively to endogenous and exogenous vitellogenesis as characterized by Breton *et al.* (1983).

In Experiment 2, fish from a spring-spawning strain reared in Gournay/Aronde fish farm were used 40 days after ovulation, corresponding to exogenous vitellogenesis (de Mones *et al.*, 1989).

Pituitary Response to GnRH in Control and Steroid Pretreated Cultures

Pituitary collection. At each defined stage of oogenesis, 15 to 30 animals were anaesthetized with phenoxethanol (0.3 ml/l) before decapitation and pituitary removal. Collected glands were stored in ice-cold Hanks' balanced salt solution supplemented with antibiotic-antimycotic mixture, as previously described (Weil *et al.*, 1986), until enzymatic dispersion.

Cell cultures. Cultures were set up as previously described (Weil *et al.*, 1986). Briefly, pituitary glands were chopped into small pieces and subjected to dispersion for 20 hr at 12° in 0.1% collagenase (Boehringer-Mannheim) in Hanks' balanced salt solution (GIBCO Laboratories) buffered with 20 mM Hepes and containing 9 mM NaHCO₃ and 3% BSA. Cells were harvested by centrifugation (200g for 10 min), washed twice with culture medium consisting of RPMI (GIBCO Laboratories) containing 20 mM Hepes, 9 mM NaHCO₃, 2% serum substitute, Ultrosor Steroid Free (IBF), and 1% antibiotic-antimycotic mixture (GIBCO Laboratories). Cell counting and cell viability tests were performed by using a Thoma hemocytometer. Ninety percent of living cells were obtained.

Salt solution and culture medium had an osmotic pressure of 300 mOsm/kg and a pH of 7.5, characteristic values of rainbow trout plasma.

Control and steroid pretreatments. Cells were di-

luted to 2.5×10^5 /ml either in culture medium (control wells) or in culture medium containing different steroids (steroid-pretreated wells). Two hundred fifty microliters of the suspension was plated into each well of 96-well plates (Nunc) at 18°. Cells were allowed to settle in control and steroid-pretreated wells for 3 days, the time required for cells to attach firmly to the plastic (Weil *et al.*, 1986).

The following steroids were tested:

—17 α -hydroxy, 20 β -dihydroprogesterone (17 α 20 β P) at 20 and 400 ng/ml, respectively, mean circulating levels at GVP (germinal vesicle peripheral) and maturation stages.

—17 β -oestradiol (E2) at 2, 8, 30, and 60 ng/ml, respectively, mean plasma levels at the periovulatory stage, beginning, full, and end of vitellogenesis.

Incubation with GnRH. On the third day of culture, control, and steroid-pretreated wells were washed twice with Ultrosor and antibiotic-antimycotic free RPMI and then subjected to increasing doses (0 to 10^{-6} M) of salmon GnRH (sGnRH) (Breton *et al.*, 1986), for 24 hr at 18°. Four to six replicates were done for each tested dose according to the experiment. At the end of the incubation period, media were collected and frozen at -20° until GtH measurement. Cells were washed twice with the salt solution and lysed by 0.1% Triton in RIA buffer for 4 hr at 18°. The cell lysates were collected and stored at -20° until GtH determination.

Data Analysis

Parameters recorded. For each stage of oogenesis, the following parameters were recorded at the term of the GnRH incubation period, in control and steroid pretreated cultures:

—basal GtH release: GtH levels in wells without GnRH stimulation (dose 0 of GnRH).

—GnRH-induced GtH release for each tested dose.

—pituitary GtH content in control and GnRH-stimulated wells.

These parameters were estimated using a radioimmunoassay as previously described for assaying plasma and pituitary trout GtH content (Breton *et al.*, 1983) and previously validated for GtH measurement in cells and culture medium (Weil *et al.*, 1986). They were expressed in nanograms of GtH per well.

Statistical analysis. Mean comparisons were performed by using an analysis of variance or a nonparametric test (Mann-Whitney *U* test). At each stage of oogenesis, the minimal effective dose of GnRH inducing GtH release higher than the basal level was determined by comparing mean GtH release using the Newman-Keuls test. The effects of steroid pretreatments were analyzed using a two-factor analysis of variance.

RESULTS

Pituitary Response to GnRH Throughout the Sexual Cycle

The experiment was carried out with females from the winter-spawning strains (Experiment 1).

During the preovulatory stages, when the germinal vesicle was in a subperipheral position (SPGV stage), basal GtH levels were low (4.68 ± 1.62 ng/well) as well as sGnRH-induced GtH release (Fig. 1). On the other hand, on the day of ovulation basal GtH release (17.80 ± 1.23 ng/well) and pituitary sensitivity to GnRH were high (Fig. 2); a low dose of GnRH (10^{-9} M) was effective in inducing a significant increase in GtH release and maximum values are 3 times higher than the basal level. Twenty and 40 days postovulation (Fig. 3), basal GtH release was still high (11.09 ± 1.91 ng/well and 16.03 ± 1.86 ng/well, respectively). At both stages, gonadotrophs were sensitive to GnRH, the minimal effective doses that increased GtH release were 10^{-7} and 10^{-9} M, respectively, with maximum increases of about 2 and 3.8 times. Three months fol-

FIG. 1. Response of pituitary cultures from preovulatory winter-spawning females (oocytes with subperipheral germinal vesicle) to various doses of sGnRH during a 24-hr incubation period after being maintained for 3 days in control medium (●) or in medium supplemented with $17\alpha 20\beta$ P, 20 ng/ml (□) and 400 ng/ml (○). For this and subsequent figures wells were initially placed with 6.2×10^4 cells. Values are $\bar{x} \pm \text{SEM}$ ($n = 4$).

FIG. 2. Response of pituitary cultures from ovulatory winter-spawning females to various doses of sGnRH during a 24-hr incubation period following a 3-day pretreatment in control medium (●) or in medium supplemented with $17\alpha 20\beta$ P, 20 ng/ml (□) and 400 ng/ml (○). Values are $\bar{x} \pm \text{SEM}$ ($n = 4$).

lowing ovulation, at the beginning of vitellogenesis (endogenous vitellogenesis; GSI%: 0.57 ± 0.07), gonadotrophs presented a weak (twofold increase) but significant response for GnRH doses ranging from 10^{-8} to 10^{-6} M, characterized by a kind of plateau (Fig. 4). When vitellogenesis was more advanced (7 months following ovulation, GSI%: 1.16 ± 0.14), gonadotrophs were still weakly responsive to GnRH since only the highest tested dose of sGnRH (10^{-6} M) caused a significant increase in GtH release (Fig. 5); the value reached is about 6 times higher than the mean GtH level present in the other wells.

Effect of Steroid Pretreatments on Pituitary Response to GnRH

17α-Hydroxy, 20β-dihydroprogesterone pretreatment. Experiments were per-

FIG. 3. Response of pituitary cultures from post-ovulatory winter-spawning females (20 (○) and 40 (●) days postovulation) to various doses of sGnRH, during a 24-hr incubation period. Cells were maintained for 3 days in control medium. Values are $\bar{x} \pm \text{SEM}$ ($n = 4$).

formed using fish from the winter-spawning strains (Experiment 1).

The pretreatment of pituitary cultures from fish at the preovulatory stage (SPGV) with the higher dose (400 ng/ml, plasma level of maturation stage) did not modify basal and sGnRH-induced GtH release (Fig. 1), although pretreatment with a lower dose (20 ng/ml, corresponding to the plasma level of the peripheral germinal vesicle stage) induced a significant increase in GtH release (Fig. 1). However, cell GtH content and basal GtH levels were unchanged (Table 1). At the time of ovulation, both doses of $17\alpha 20\beta\text{P}$ induced a decrease in pituitary responsiveness to GnRH, whereas basal GtH release and cell GtH content were not modified when compared to control wells.

The application of the higher dose (400 ng/ml) to cultures from fish at the endoge-

nous vitellogenesis stage (3 months following ovulation) increased the responsiveness to sGnRH ($P < 0.005$), when compared with untreated wells (Fig. 4) whereas basal GtH release and cell GtH content were unchanged (Table 1).

17β -Oestradiol pretreatment. The effect of 2 ng/ml E2 (mean plasma level at ovulation) was checked on pituitary cultures from the winter-spawning females 3 months following ovulation (GSI%: 0.57 ± 0.07 —Experiment 1). Following such a pretreatment, the minimal effective dose of GnRH was 10^{-9} M instead of 10^{-8} M and GtH release was not a plateau as observed in the steroid-unpretreated wells (interaction $P < 0.01$) (Fig. 4). Basal GtH release and cell GtH content were not significantly modified (Table 2).

Using fish from a spring-spawning strain (Experiment 2), doses of 8, 30, and 60 ng/ml E2 were added as a pretreatment to pituitary cultures set up with pituitary from early vitellogenic females (exogenous vitellogenesis, GSI%: 1.80 ± 0.30). The highest dose of E2 (60 ng/ml) induced an increase in cell GtH content (Table 2), when compared with control and the lowest E2 pretreatment. This same high dose of E2 induced an increase in the response to sGnRH in comparison with control and the 8-ng-pretreated wells, $P < 2.5\%$ (Fig. 6). However, when GnRH-induced GtH release is expressed as a percentage of total GtH (cell and medium GtH content), the increase is not significant (6 to 10% in both cases). GnRH-induced GtH release was intermediate in wells pretreated with 30 ng/ml E217 β while cell GtH content was unchanged compared to control and low-dose E2-pretreated cultures (Table 2).

DISCUSSION

In this work, cell GtH content, basal GtH release, and pituitary sensitivity to sGnRH were studied *in vitro* at different stages of oogenesis, using cultures of dispersed cells

FIG. 4. Response of pituitary cultures from winter-spawning females at an early stage of ovarian development (3 months following ovulation) to various doses of sGnRH during a 24-hr incubation period, following a 3-day pretreatment in control medium (●) or in medium supplemented with $17\alpha 20\beta P$, 400 ng/ml (○), or E2, 2 ng/ml (□). Values are $\bar{x} \pm \text{SEM}$ ($n = 4$).

from whole pituitaries maintained under standard conditions (Weil *et al.*, 1986). For each stage, the same number of total cells was plated and the same experimental protocol was followed with respect to steroid pretreatment and incubation with sGnRH.

Under such conditions, the cultured gonadotrophs have a basal GtH release (in the absence of sGnRH) increasing from ovarian recrudescence to ovulation, roughly with the same pattern as found *in vivo* for rainbow trout circulating levels (Billard *et al.*, 1978; Bromage *et al.*, 1982; Whitehead *et al.*, 1983; Scott and Sumpter, 1983; Lou *et al.*, 1984). The pattern of gonadotroph re-

FIG. 5. Response of pituitary cultures from vitellogenic winter-spawning females (7 months following ovulation) to various doses of sGnRH during a 24-hr incubation period. Values are $\bar{x} \pm \text{SEM}$ ($n = 6$).

FIG. 6. Response of pituitary cultures from vitellogenic spring-spawning females to various doses of sGnRH during a 24-hr incubation period following a 3-day pretreatment in control medium (●) or in medium supplemented with E2 at the doses of 8 ng/ml (○), 30 ng/ml (□), and 60 ng/ml (■). Values are $\bar{x} \pm \text{SEM}$ ($n = 5$).

sponsiveness to sGnRH throughout the cycle is in agreement with our *in vivo* results (Weil, 1981), maximal responses being recorded at the time of spawning and following it. However, the *in vitro* system is more suitable since various doses of sGnRH can be easily tested, whereas *in vivo*, only a single dose of LHRH (1 $\mu\text{g/kg}$ body wt) was

TABLE 1
EFFECT OF 17α -HYDROXY, 20β -DIHYDROTESTOSTERONE ($17\alpha 20\beta P$) ON BASAL GtH RELEASE AND CELL GtH CONTENT OF PITUITARY CULTURES SET UP WITH WINTER-SPAWNING STRAIN FEMALES AT DIFFERENT STAGES OF OVOGENESIS

Stage	Dose of $17\alpha 20\beta P$ (ng/ml)	Basal GtH release ^a	Cell GtH content ^a
Endogenous	0	2.98 ± 0.27	124.99 ± 7.91
vitellogenesis	400	3.42 ± 0.24^b	122.75 ± 6.16^b
Subperipheral	0	4.28 ± 1.62	74.69 ± 14.20
germinal	20	10.75 ± 2.40^b	178.52 ± 5.21^b
vesicle	400	7.46 ± 2.80^b	131.84 ± 12.37^b
Ovulation	0	17.80 ± 1.20	199.30 ± 16.88
	20	12.32 ± 5.09^b	122.60 ± 26.50^b
	400	16.27 ± 2.17^b	123.37 ± 10.93^b

^a Values are $\bar{x} \pm \text{SEM}$ and expressed as nanograms per well.

^b Value not significantly different from control.

TABLE 2
EFFECT OF 17 β -OESTRADIOL (E2) ON BASAL GtH RELEASE AND CELL GtH CONTENT OF PITUITARY CULTURES SET UP WITH FEMALES AT DIFFERENT STAGES OF OVOGENESIS

Stage	Strain	Dose of E2 (ng/ml)	Basal GtH release ^a	Cell GtH content ^a
Endogenous vitellogenesis	Winter-spawning	0	2.98 $\pm$ 0.27	124.99 $\pm$ 7.21
		2	1.95 $\pm$ 0.43 ^b	121.77 $\pm$ 10.54
Exogenous vitellogenesis	Spring-spawning	0	7.68 $\pm$ 0.64	234.69 $\pm$ 28.34
		8	7.38 $\pm$ 0.33 ^b	266.34 $\pm$ 29.43 ^b
		30	7.57 $\pm$ 1.02 ^b	308.54 $\pm$ 27.45 ^b
		60	7.26 $\pm$ 1.36 ^b	350.81 $\pm$ 28.79 ^{c,d}

^a Values are $\bar{x} \pm$ SEM and expressed as nanograms per well.

^b Value not significantly different from control.

^c Value significantly different from control ($P < 0.03$).

^d Value not significantly different from the 30 ng/ml dose.

tried per fish. In consequence, the *in vitro* system allows us to determine the minimal effective dose of sGnRH for each stage of oogenesis and to detect a response at low sensitivity. For example, *in vivo*, the response to LHRH only became detectable at maturation and was maintained on the day of ovulation and 3 and 5 days following it (Weil, 1981). *In vitro*, low responsiveness to sGnRH was recorded before oocyte maturation (SPGV) and during the early phase of vitellogenesis; the minimum effective dose of GnRH ranged between 10^{-6} and 10^{-8} M. At ovulation, responsiveness is higher, the gonadotrophs responding to 10^{-9} M sGnRH. The *in vivo* apparition of the response to LHRH during maturation is concomitant with the decrease in pituitary GnRH content (Breton *et al.*, 1986) when endogenous GnRH stimulates the increase in plasma GtH levels.

The negative influence of gonads or gonadal hormones on the hypothalamus-hypophyseal axis is now well established in different fish species as well as a positive influence, which is of special importance in the prepuberal fish (for review see Goos, 1987). Concerning adult female rainbow trout, a negative feedback exerted by ovaries on plasma circulating levels (Bommelaer *et al.*, 1981; Van Peutten *et al.*, 1981) and on pituitary GtH synthesis (Van

Peutten *et al.*, 1981) has been demonstrated by ovariectomy. The mechanisms involved are not precisely known in fish. Recent investigations indicate that gonads modulate GnRH binding in adult males (Crim *et al.*, 1987; De Leeuw *et al.*, 1987) and that in immature fish the administration of testosterone and oestradiol have a positive effect on mRNA levels for GtH subunits, β and α , respectively (Trinh *et al.*, 1986; Counis *et al.*, 1987). However, these cited works involved either castrated adult fish or intact injected immature fish and phenomena observed are the integration of multiple effects on the hypothalamus-hypophyseal axis. We have studied the direct action of steroids on pituitary, by measuring basal GtH release and sensitivity to sGnRH following steroid treatment of pituitary cultures. A "chronic" steroid treatment (steroid applied for 3 days during cell attachment and absent during GnRH action) rather than an "acute" one (steroid present only during GnRH incubation) was used, in order to ensure GnRH receptors modification (number and/or affinity). However, this choice is open to criticism since, in cultured rat pituitary cells, prolactin and not growth hormone cell proliferation is increased by oestradiol (Boockfor *et al.*, 1986). A single study in fish, conducted on dispersed pituitary cells collected from ju-

venile rainbow trout and maintained in suspension, indicated that 17α -methyltestosterone increased the number of immunoreactive gonadotrophs (Fahraeus-van Ree *et al.*, 1982) as well as the total amount of GtH. However, the authors do not specify the exact number of cells found and they worked on immature fish, in which it has now been well established that testosterone has a positive action on pituitary (for review, see Goos, 1987). The only work performed on adult fish, using cultured pituitary cells treated with steroids, was conducted on pooled male and female carps and the authors did not specify the effect of the steroid treatments on cell number (Ribeiro *et al.*, 1983).

The present work suggests that the same steroid can have either a positive or a negative effect by acting directly on pituitary cells. $17\alpha 20\beta$ P, the most probable maturation-inducing steroid in rainbow trout (Fostier *et al.*, 1973; Jalabert, 1976), has a positive effect at stages before ovulation and a negative one afterwards. When it is applied on pituitary cultures from fish at an early stage of ovarian development, at a dose corresponding to circulating levels at the time of maturation (400 ng/ml), it increased pituitary sensitivity to sGnRH. Furthermore, 20 ng/ml of this steroid, which is the mean circulating levels when the germinal vesicle is peripheral (PGV stage), increases the response to sGnRH of pituitary cells collected from fish with ovocytes at the stage just prior this one (SPGV stage). This increase in pituitary sensitivity to GnRH could explain the increase in plasma GtH observed between these two stages (Weil, 1981; Fostier and Jalabert, 1986). In contrast, during ovulation, $17\alpha 20\beta$ P at both tested doses (20 and 400 ng/ml) leads to a reduction in the response of the cells to sGnRH. This supports earlier evidence for its negative effect on plasma GtH levels, when injected to synchronize ovulation (Jalabert *et al.*, 1976).

We have shown that low levels of oestra-

diol, corresponding to mean circulating values at ovulation, increased responsiveness to GnRH when applied to pituitary cultures from females at an early stage of ovarian development. This could explain in part the high response to sGnRH observed at the time of ovulation. During early vitellogenesis, doses corresponding to circulating levels at the beginning and full vitellogenesis were without effect on basal GtH release, cell GtH content, and response to sGnRH. In contrast, doses of 60 ng/ml corresponding to circulating levels found at the last stage of vitellogenesis induced an increase in cell GtH content without modifying the relative response to sGnRH. This observation could explain in part the increase in pituitary GtH content observed *in vivo* at the end of vitellogenesis (Billard *et al.*, 1978; Weil, 1981).

The combined action of E2 and $17\alpha 20\beta$ P now needs to be investigated as does the effect of steroid pretreatment on the percentage of gonadotrophs present in the cultures and on GnRH receptor characteristics.

ACKNOWLEDGMENTS

We extend our thanks to Dr. A. Fostier for helpful discussions and critical reading of the manuscript. This work was supported by a grant from Ministère de l'Education Nationale-Formation par la Recherche dans les disciplines agronomiques, agroalimentaires et biotechnologiques.

REFERENCES

- Billard, R., Breton, B., Fostier, A., Jalabert, B., and Weil, C. (1978). Endocrine control of the teleost reproductive cycle and its relation to external factors: Salmonid and cyprinid models. In "Comparative Endocrinology" (P. J. Gaillard and H. H. Boer, Eds.), pp. 37-48. Elsevier, Amsterdam.
- Bommelaer, M. C., Billard R., and Breton, B. (1981). Changes in plasma gonadotropin after ovariectomy and estradiol supplementation at different stages at the end of the reproductive cycle in the rainbow trout (*Salmon gairdneri* R.). *Reprod. Nutr. Dev.* 21, 989-997.
- Boockfor, F. R., Hoeffler, J. P., and Frawley, L. S. (1986). Estradiol induces a shift in cultured cells

- that release prolactin or growth hormone. *Am. J. Physiol.* **250** (Endocrinol. Metab. 13), E103-E105.
- Breton, B., Fostier, A., Zohar, Y., Le Bail, P. Y., and Billard, R. (1983). Gonadotropine glycoproteique maturante et oestradiol-17 β pendant le cycle reproducteur chez la truite fario (*Salmo trutta*) femelle. *Gen. Comp. Endocrinol.* **49**, 220-231.
- Breton, B., Motin, A., Billard, R., Kah, O., Geoffre, S., and Precigoux, G. (1986). Immunoreactive gonadotropin-releasing hormone-like materiel in the brain and the pituitary gland during the preovulatory period in the brown trout (*Salmo trutta* L.): Relationships with the plasma and pituitary gonadotropin. *Gen. Comp. Endocrinol.* **61**, 109-119.
- Bromage, N. R., Whitehead, C., and Breton, B. (1982). Relationships between serum levels of gonadotropin, oestradiol-17 β , and vitellogenin in the control of ovarian development in the rainbow trout. II. The effects of alterations in environmental photoperiod. *Gen. Comp. Endocrinol.* **47**, 366-376.
- Counis, R., Dufour, S., Ribot, G., Querat, B., Fontaine, Y. A., and Jutisz, M. (1987). Estradiol has inverse effects on pituitary glycoprotein hormone α -subunit messenger ribonucleic acid in the immature European eel and the gonadectomized rat. *Endocrinology* **121**, 1178-1184.
- Crim, L. W., Wilson, C., St. Arnaud, R., Evans, D. M., and Harmin, S. A. (1987). Characteristics of fish pituitary LHRH receptors. In "Proceedings, Third Int. Symp. Fish Reprod. St John's, Newfoundland."
- De Leeuw, R., Beumer, P., Goos, H. J. Th., and Van Oordt, P. G. W. J. (1987). The effect of castration and steroid replacement on gonadotropin-releasing hormone (GnRH) receptor binding in the african catfish, *Clarias gariepinus*. In "Proceedings, Third Int. Sym. Fish Reprod. St John's, Newfoundland."
- Fahraeus-Van Ree, G. E., Guldenaar, S. E. F., and Gielen, J. T. (1982). Fine structure and function of isolated gonadotropic cells as revealed from pituitaries of immature rainbow trout (*Salmo gairdneri*), by means of a new enzymatic dispersion technique. *Cell Tissue Res.* **226**, 641-653.
- Fostier, A., and Jalabert, B. (1986). Steroidogenesis in rainbow trout (*Salmo gairdneri*) at various preovulatory stages: Changes in plasma hormone levels and *in vivo* and *in vitro* responses of the ovary to salmon gonadotropin. *Fish Physiol. Biochem.* **2**, 87-89.
- Fostier, A., Jalabert, B., and Terqui, M. (1973). Action prédominante d'un dérivé hydroxylé de la progestérone sur la maturation *in vitro* des ovocytes de la truite arc-en-ciel, *Salmo gairdneri*. *C.R. Acad. Sci. Paris* **277**, 421-423.
- Goos, H. J. Th. (1987). Steroid feedback on pituitary gonadotropin secretion. In "Proceedings, Third Int. Symp. Fish. Reprod. St John's, Newfoundland."
- Jalabert, B. (1976). *In vivo* oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*) and goldfish (*Carassius auratus*). *J. Fish. Res. Board Canad.* **33**, 974-988.
- Jalabert, B., Bry, C., Breton, B., and Campbell, C. (1976). Action de la 17 α hydroxy-20 β dihydroprogestérone et de la progestérone sur la maturation et l'ovulation *in vivo* et sur le niveau d'hormone gonadotrope plasmatique t-GtH. *C.R. Acad. Sci. Paris* **283**, 1205-1208.
- Jalabert, B., and Fostier, A. (1984). The follicular sensitivity *in vitro* to maturation-inducing hormones in rainbow trout, *Salmo gairdneri*: Role of oestradiol-17 β . *Aquaculture* **43**, 1-11.
- Lou, S. W., Aida, K., Hanyu, I., Sakai, K., Nomura, M., Tanaka, M., and Tazaki, S. (1984). Endocrine profiles in the females of a twice annually spawning strain of rainbow trout. *Aquaculture* **43**, 13-22.
- de Mones, A., Fostier, A., Cauty, C., and Jalabert, B. (1989). Ovarian early postovulatory development and oestrogen production in rainbow trout (*Salmo gairdneri*) from a spring-spawning strain. *Gen. Comp. Endocrinol.* **74**, 431-441.
- Ribeiro, L., Ahne, W., and Lichtenberg, V. (1983). Primary culture of normal pituitary cells of carp (*Cyprinus carpio*) for the study of gonadotropin release. *In Vitro* **19**, 41-45.
- Scott, A. P., and Sumpter, J. P. (1983). A comparison of the female reproductive cycles of autumn-spawning and winter-spawning strains trout. (*Salmo gairdneri* Richardson). *Gen. Comp. Endocrinol.* **52**, 79-85.
- Trinh, K. Y., Wang, N. C., Hew, C. L., and Crim, L. W. (1986). Molecular cloning and sequencing of salmon gonadotropin β subunit. *Eur. J. Biochem.* **159**, 619-624.
- Van Peutten, L. J. A., Peute, J., Van Oordt, P. G. W. J., Goos, H. J. Th., and Breton, B. (1981). Glycoprotein gonadotropin in the plasma and its cellular origins in the adenohipophysis of sham-operated and ovariectomized rainbow trout, *Salmo gairdneri*. *Cell Tissue Res.* **218**, 439-448.
- Weil, C. (1981). "La fonction gonadotrope de l'hypophyse au cours du cycle sexuel chez deux poissons téléostéens la Carpe commune (*Cyprinus carpio*) et la truite arc-en-ciel (*Salmo gairdneri*), son contrôle par l'hypothalamus, les gonades et les facteurs externes." Thèse de Doctorat d'Etat, Paris VI. *Microfiche* 870452. Institut d'Ethnologie, Paris 1987 (Archives et Documents-micro-édition)
- Weil, C., Billard, R., Breton, B., and Jalabert, B. (1978). Pituitary response to LH-RH at different

- stages of gametogenesis in the rainbow trout (*Salmo gairdneri*). *Ann. Biol. Anim. Biochem. Biophys.* 18, 863–869.
- Weil, C., Hansen, P., Hyam, D., Le Gac, F., Breton, B., and Crim, L. W. (1986). Use of pituitary cells in primary culture to study the regulation of gonadotropin hormone (GtH) secretion in rainbow trout: Setting up and validating the system as assessed by its responsiveness to mammalian and salmon gonadotropin releasing hormone. *Gen. Comp. Endocrinol.* 62, 202–209.
- Whitehead, C., Bromage, N. R., and Breton, B. (1983). Changes in serum levels of gonadotropin oestradiol-17 β and vitellogenin during the first and subsequent reproductive cycles of female rainbow trout. *Aquaculture* 34, 317–326.