Gonadotropin hormone (GtH) receptors in the ovary of the brown trout *Salmo trutta* L. in vitro studies

Bernard Breton, Florence Le Gac, Elisabeth Sambroni

► To cite this version:

Bernard Breton, Florence Le Gac, Elisabeth Sambroni. Gonadotropin hormone (GtH) receptors in the ovary of the brown trout *Salmo trutta* L. in vitro studies. *General and Comparative Endocrinology*, 1986, 64 (2), pp.163-171. 10.1016/0016-6480(86)90001-8 . hal-01600840

HAL Id: hal-01600840

<https://hal.science/hal-01600840>

Submitted on 2 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - ShareAlike| 4.0 International License

Gonadotropin Hormone (GtH) Receptors in the Ovary of the Brown Trout *Salmo trutta* L. *in Vitro* Studies

BERNARD BRETON, FLORENCE LE GAC, AND ELISABETH SAMBRONI

Laboratoire de Physiologie des Poissons, INRA, Campus de Beaulieu, F 35042 Rennes Cédex, France

Accepted April 22, 1986

A method has been developed suitable for the study of the binding of salmon (*Oncorhynchus tshawytscha*) gonadotropic hormone (GtH) to brown trout (*Salmo trutta*) ovarian particulate fractions. Several proteolytic enzyme inhibitors were tested to preserve the binding capacity of particulate fractions (1000, 20,000, and 110,000g) from yolky oocytes. The best preservation was obtained with trypsin inhibitor. The effects on binding of the pH, time, and temperature of the incubation were investigated. Binding increased linearly with amount of particulate fraction, and is saturable. In both the 20,000 and 110,000g fractions, there are two classes of receptors: one with a high-affinity constant (3.14 and $0.95 \cdot 10^{10} M^{-1}$ for the 20,000 and 110,000g fractions, respectively) and a low capacity (1.76 and 0.63 fmol/g of ovary), the other with a higher-affinity constant (3.44 and $1.78 \cdot 10^9 M^{-1}$) but a higher capacity (7.37 and 7.42 fmol/g of ovary). GtH binding was not affected by ovine luteinizing hormone (oHL), ovine follicle-stimulating hormone (oFSH), and human chorionic gonadotropin (hCG), but was partially inhibited by $1 \mu g$ of bovine thyroid-stimulating hormone (bTSH) and salmon prolactin (sPRL). The results are discussed in relation to the biological specificity of fish GtH and to the unsolved problem concerning the number of gonadotropins in fish. © 1986 Academic Press, Inc.

Numerous reports exist on the circulating levels of the maturational gonadotropin (GtH) during ovarian development in salmonids (Billard *et al.*, 1978; Fostier and Jalabert, 1982; Breton *et al.*, 1983) which have given a good picture of the long-term gonadotropic control of the female reproductive cycle. Furthermore, short-term studies of the GtH secretion (Hontela and Peter, 1978; Gilet *et al.*, 1978; Zohar, 1982; Zohar *et al.*, 1982) reveal the existence of daily and pulsatile rhythms of secretion in teleost fish. In both cases a better understanding of the physiological roles of GtH has been obtained after studying the *in vivo* and *in vitro* effects of the hormone on ovarian steroidogenesis (for review, see Fostier *et al.*, 1983). These effects are, in fact, the result of a complex chain of events in which GtH acts primarily after binding to ovarian receptors, and they differ according to the mode of GtH secretion (circadian or pulsatile) as demon-

strated by Zohar (1982). For example, pulsatile GtH secretion induces a circadian rhythm of estradiol secretion during vitellogenesis in trout. Such results emphasize the necessity to study the physiological significance of these rhythms by investigating the local action of GtH and its regulation at the receptor level. Human chorionic gonadotropin (hCG) binding has recently been demonstrated in a particulate membrane preparation from preovulatory rainbow trout (Schulz *et al.*, 1985), but the GtH receptors are not so well characterized. GtH binding to the goldfish ovary was first demonstrated using autoradiography (Cook and Peter, 1980). Later GtH binding was demonstrated *in vitro* in the ovary of immature chum salmon (*Oncorhynchus keta*) (Van der Kraak, 1983) and in a membrane preparation from the amago salmon (*Oncorhynchus rhodurus*) ovary (Salmon *et al.*, 1984). However, these limited results precluded characterization of GtH re-

ceptors. In this present study, we have tried to develop a suitable method to prepare and characterize the *in vitro* specific binding of purified maturational salmon GtH to vitellogenic brown trout ovarian particulate fractions.

MATERIALS AND METHODS

Experimental animals. Three-year-old female brown trout *Salmo trutta* L. originated from the INRA Gournay fish farm and were kept at 12° in a recirculating water system. They were fully vitellogenic females with a gonadosomatic index greater than 10%.

Hormones. Salmon gonadotropin (sGtH) was purified from *Oncorhynchus tshawytscha* pituitary glands as previously described (Breton *et al.*, 1978). Salmon prolactin (sPRL) was prepared according to Prunet and Houdebine (1984). Human chorionic gonadotropin was purchased from Boehringer; the immunological potency was 9000 UI/mg in terms of the second international standard. Bovine thyroid-stimulating hormone (bTSH), ovine luteinizing hormone (oLH), and ovine follicle-stimulating hormone (oFSH-S15) were obtained from the National Institute of Health (NIH), Bethesda, Maryland.

Receptor preparation. The entire procedure was carried out on ice using chilled buffers. Immediately following decapitation of the fish, the ovaries were removed and either deep-frozen by plunging into liquid nitrogen or placed in a medium adjusted to the osmotic pressure and ionic composition of the trout sera (Jalabert *et al.*, 1974). The medium was buffered by hepes at pH 7.8 and contained one or a combination of the following proteolytic enzyme inhibitors: Soya bean trypsin inhibitor, Sigma type 1, paminobenzamidine, *N*-tosyl-L-lysine chloromethyl ketone (TLCK), *D*-tosyl-L-arginine methyl ester (TAME), *L*-tosyl-2-phenylethylchloromethyl ketone (TPCK). The oocytes were crushed in this medium. The resultant mixture was filtered through a nylon cloth (0.5 mm mesh). Ovarian tissue collected from the filter was washed twice with the same medium in order to remove the remaining yolk. It was resuspended in a 0.015 *M* Tris–10 *mM* MgCl₂ buffer at pH 7.6 containing 0.15 *M* sucrose and the proteolytic enzyme inhibitor. Tissues were homogenized in a polytron tissue grinder with two successive bursts at high speed for 10 sec per burst (1 vol of tissue for 10 vol of buffer). The homogenate was passed through a nylon filter (150 µm per mesh) to remove unground pieces of tissue and was further homogenized with five strokes in a glass Teflon homogenizer having a 0.1 mm space between the piston and the cylinder. The final homogenate was centrifuged at 1000*g* for 15 min at 4°. The pellet was kept and the supernatant centrifuged again at 20,000*g* for 30 min at 4°. After the pellet was saved, the re-

maining supernatant was centrifuged at 110,000*g* for 90 min at 4°. The final pellet was kept and an aliquot of the last supernatant was kept to test its binding capacity. The protein contents of all these fractions were determined according to Lowry (1951).

Preparation of ¹²⁵I-sGtH. Pure sGtH (5 µg) was iodinated by the lactoperoxidase method (Thorell and Johansson, 1971) as modified by Hirano *et al.* (1985). The chemicals and the methodology were the same as those described for the study of trout testis receptors (Le Gac *et al.*, 1986). The method for the determination of the specific activity of the labeled hormone was equally described by the same authors (Le Gac *et al.*, 1986) and was generally between 20 and 40 µCi/µg.

Binding assay procedure. The assay conditions were determined after studying the effects of proteolytic enzyme inhibitors, pH, temperature, and time of incubation. Assays were performed in 5-ml plastic tubes containing 1 or 2 mg of pellet protein with 100 µl of Tris–MgCl₂ buffer containing 1% bovine serum albumin. The required number of counts per minute (cpm), and if necessary the unlabeled hormone, were added under 50 µl and the incubation volume was made up to 500 µl with the Tris buffer according to the type of assay, direct or competition binding. After being vortexed, the mixture was allowed to incubate and the incubation was stopped by adding 1 ml of chilled incubation buffer. The tubes were then centrifuged at 2000*g* for 30 min at 4°. The supernatants were discarded and the resultant pellets counted in a gamma counter (75% efficiency).

The nonspecific binding (NSB) was determined in the presence of 1 µg of unlabeled GtH. The specifically bound (SB) was calculated by subtracting the nonspecifically bound from the total bound (TB):

$$SB = TB - NSB$$

$$SB \% = \frac{TB - NSB \times 100}{TC}$$

(TC = total count).

Both competition and saturation studies were performed and the data were used for Scatchard plot analysis for the determination of the binding capacity (*R*₀) and the binding affinity (*K*_a).

RESULTS

Receptor Preparation

When particulate fractions were prepared without removal of the vitellus, or in buffer which did not contain proteolytic enzyme inhibitor, we did not get any specific binding (less than 1%) with any kind of

pellet. On the contrary, when the yolk material was removed in a medium containing proteolytic enzyme inhibitors, sGtH bound to every kind of pellet and there was no binding in the 110,000g supernatant (Table 1). The specific binding per milligram of protein was generally the highest in 1000g pellets except in the presence of the trypsin inhibitor, in which the amount of specifically bound hormones was the same in 1000 and 20,000g pellets. SB in 110,000g pellets was lower than in 20,000g pellets. The non-specific binding was especially elevated in the 1000g pellets in which it represented 40 to 60% of the total binding according to the proteolytic enzyme inhibitor used. In contrast, this value was low in the two other fractions, ranging from 6 to 28% of the TB except when *para*-aminobenzamidine was used, in which case NSB reached 40% of the TB. Considering the total amount of binding the three fractions, and the values of the NSB, it is suggested that trypsin in-

hibitor is the most suitable for preserving the binding capacity of the ovarian particulate preparations.

Expecting the same situation as in mammals where the 110,000g pellets corresponding to the microsomes contain the most pure binding fraction, we also use these kind of pellets in most of the remainder of the study in order to have theoretically the best conditions for the characterization of the ovarian GtH receptors, although 110,000g pellets bind less than the 20,000g pellets. Both the 20,000 and 110,000g pellets have been used in competition and saturation studies.

Parameters Influencing GtH Binding

The effect of pH was tested between 6.5 and 8.5. The best binding was obtained at 7.6 (Fig. 1). Figure 2 presents the kinetics of the binding at 4, 12.5, 20, and 37°. Whatever the temperature, the same apparent equilibrium was reached and the binding

TABLE 1
BINDING OF ^{125}I -SGtH (27.5 Ci/ μg) TO TROUT OVARIAN PARTICULATE FRACTIONS

Proteolytic inhibitor	Pellet								
	1000g			20,000g			110,000g		
	NSB	SB cpm	SB %TC	NSB	SB cpm	SB %TC	NSB	SB cpm	SB %TC
None	3504 13.9	108	0.43	1636 84.2	206	0.81	5534	0	0
<i>para</i> -aminobenzamidine (1 mM)	5244 41.2	7446	29.55	2311 39.5	3534	14.02	372 17.0	1813	7.19
trypsin inhibitor (0.25 mg/ml)	7198 51.5	6779	25.19	387 5.9	6166	24.47	360 14.1	2185	8.67
TLCK (1 mM)	7571 61.4	4751	18.95	475 11.4	3710	14.70	314 11.5	2323	9.22
TAME (1 mM)	7103 55.1	5772	22.90	666 11.7	5024	20.09	420 12.0	3085	12.24
TPCK (1 mM)	2817 38.3	4540	18.0	1365 23.7	4386	17.10	548 28.5	1371	5.44

Note. Results are expressed in cpm bound per mg of particulate protein and in percentage of the total binding for the NSB.

Total count in tubes: 2.5×10^4 cpm.

NSB: nonspecifically bound.

SB: specifically bound.

The values are the means of triplicates. Tubes were incubated for 20 hr at 16°.

FIG. 1. ^{125}I -sGtH specific binding to 110,000g ovarian pellets (1 mg/tube) according to the pH of incubation. Incubation was performed at 20° for 20 hr. TC = 75,000 cpm. Results are expressed in cpm specifically bound per mg of particulate protein.

remained stable for 12 hr once it had reached its maximum value. At 37° there was no equilibrium state; a transient increase occurred between 1 and 2 hr after the beginning of the incubation but it decreased rapidly, becoming very low at 4 hr. The specific binding was also a function of the pellet concentration in the assay tubes. It increased to between 0.5 and 4 mg of 110,000g pellet protein per tube (Fig. 3).

For the next part of the study the following parameters were chosen: pH 7.6; incubation temperature 16°; incubation

FIG. 2. Kinetics of ^{125}I -sGtH specific binding to 110,000g ovarian pellets (1 mg/tube) according to the temperature. TC = 75,000 cpm. Results are expressed in cpm specifically bound per mg of particulate protein: 4° (○), 12.5° (△), 20° (□), 37° (●).

FIG. 3. ^{125}I -sGtH binding to 110,000g ovarian pellets according to the quantity of ovarian particulate fractions expressed in mg of protein per assay tube. Total count = 75,000 cpm. Results are expressed in cpm specifically bound per mg of particulate protein: (□) SB, (■) NSB.

time 16 hr; quantity of particulate protein per assay tube, 1 or 2 mg. With these conditions 110,000g pellets prepared from liver and muscle were tested for their ability to bind ^{125}I -sGtH. There was no specific binding in a range of concentration of 1 to 4 mg of membrane protein per tube.

Saturation and Competition Studies

When a constant amount of 110,000g particulate fraction was incubated with increasing doses of ^{125}I -sGtH, the nonspecific binding increased linearly while the specific binding tended to become saturated (Fig. 4). When a constant amount of 110,000g particulate fraction was incubated with a constant amount of ^{125}I -sGtH (50,000 cpm) and increasing doses of unlabeled sGtH, the binding was competed by the nonlabeled gonadotropin (Fig. 5). Inhibition was significant with as little as 0.5 ng, 50% with around 2 ng, and complete in the presence of 40 ng per assay tube. The mammalian glycoprotein hormone hCG, oLH, oFSH, and well as bGH at a dose of 1 μg per tube, did not inhibit the binding. In contrast, the same amount of sPRL and bTSH significantly competed with the sGtH binding to ovarian particulate fractions; 1 mg of sPRL or bTSH inhibited 55 or 43% of the binding, respectively. The same percentages of inhibition with sGtH were obtained with 500 to 1000 times less

FIG. 4. Binding of ^{125}I -sGtH as a function of ^{125}I -sGtH concentration 1 mg of 110,000g protein fraction was incubated with increasing amounts of ^{125}I -sGtH: (■) SB, (○) TB, (□) NSB. $\text{SB} = \text{TB} - \text{NSB}$.

sGtH. In another series of experiments, ^{125}I -hCG labeled using the same method as for sGtH iodination did not specifically bind to 110,000g ovarian pellets.

Equilibrium Affinity Constants and Binding-Site Concentrations

Receptor binding data were analyzed using the Scatchard method, where the ratio of bound-free hormone is plotted

against the specifically bound hormone concentration. Transformation of the saturation curve gives a linear line and suggests a receptor population with a high affinity ($K_a = 3.125 \cdot 10^{10} \text{ M}^{-1}$) and a low capacity ($R_0 = 2.9 \text{ fmol/g}$ of ovary, including yolk material; Fig. 6). In another particulate

FIG. 5. Competitive inhibition of ^{125}I -sGtH specific binding (○) to trout ovarian receptor by increasing concentration of native sGtH; (■) bTSH; (□) sRPL.

FIG. 6. Scatchard plot from saturation data. The study was done with 110,000g pellets.

preparation from the same stage of ovarian development, competition studies have been done with both 20,000 and 110,000g pellets and analyzed using the same mathematical transformation. The resulting curve for 20,000g pellets is shown in Fig. 7, and Table 2 gives R_0 and K_a values for the two preparations. The Scatchard plot was non-linear and could be dissociated into two parts as shown in Fig. 7 for the 20,000g ovarian pellets. The first part, up to 20 to 30 ng of sGtH per tube, was similar to the curve obtained from the saturation studies, giving R_0 and K_a values in the same order of magnitude (Table 2). Higher concentrations of unlabeled sGtH gave another part of the curve which suggested a second type of binding site in 20,000 and 110,000g pellets having a lower affinity than in the first, in the range of $10^9 M^{-1}$, but a higher capacity (Table 2). This study was not conducted with 1000g pellets.

DISCUSSION

In this work we have demonstrated the existence of specific and saturable sites for the binding of sGtH to vitellogenic ovaries in the brown trout. The high affinity value found is compatible with the basal and peak circulating levels of GtH during vitello-

genesis in trout ($2 \cdot 10^{-10}$ mol/liter $<$ GtH $<$ $10 \cdot 10^{-10}$ mol/liter) (Breton *et al.*, 1983; Zohar, 1982). As for the testes (Le Gac *et al.*, in press), proteolytic enzyme inhibitors were necessary to recover the binding capacity of the different ovarian fractions. But in addition, removal of the vitellus before crushing the ovarian tissue was an essential condition to preserve the binding properties, indicating that vitellus may contain a high proteolytic activity.

The binding was found in 1000, 20,000, and 110,000g pellets, but in 1000g pellets the nonspecific binding was rather high, making it difficult to use such a preparation to well characterize the receptors. However, this fraction must be taken into account to study the total number of receptors in the ovary. The total ovarian particulate protein content of 1000g pellets has been determined. During vitellogenesis it is 61.2 ± 24.7 mg ($n = 7$) versus 12.1 ± 2.78 mg ($n = 7$) for both the 20,000 and 110,000g pellets. Thus the 1000g preparation represents a great part of the ovarian binding capacity.

The characteristics of NSB and K_a of the 20,000 and 110,000g pellets are quite similar. For further preparations the 1000g supernatant is directly centrifuged at 110,000g thus avoiding one run and increasing the yield of pellets having a low NSB. The specificity of the binding for the recognition of GtH is important in the female. The binding of ^{125}I -sGtH was not competed for by hCG, oLH, and oFSH while in the male high doses (1 μ g) of these hormones did compete with sGtH (Le Gac *et al.*, in press). These results confirm the *in vivo* species specificity of the fish GtH (Breton *et al.*, 1972) and the absence of biological effects of mammalian gonadotropin on the induction of vitellogenesis in salmonids. More surprising is the binding inhibition induced by sPRL and bTSH. The GtH contamination of sPRL has been measured by RIA according to Breton *et al.*

FIG. 7. Scatchard plot from competition data. The study was done with 20,000g pellets.

TABLE 2
VALUES OF AFFINITY CONSTANTS AND BINDING CAPACITY OF 20,000 AND 110,000g PELLETS OBTAINED FROM VITELLOGENIC FEMALE BROWN TROUT AFTER SCATCHARD TRANSFORMATION OF COMPETITION CURVES

	R_0	K_a	R_0	K_a
20,000g pellets	1.76 fmol/g ovary	$3.14 \cdot 10^{10} M^{-1}$	7.37 fmol/g ovary	$3.44 \cdot 10^9 M^{-1}$
110,000g pellets	0.63 fmol/g ovary	$0.95 \cdot 10^{10} M^{-1}$	7.42 fmol/g ovary	$1.78 \cdot 10^9 M^{-1}$

Note. The unity of ovary is related with fresh ovary included yolk material.

(1978) and biological assay on the *in vitro* trout oocyte maturation assay (Jalabert *et al.*, 1974). Neither technique reveals any contamination, ruling out the possible competition induced by GtH contamination. Only one dose of each hormone sPRL and bTSH was used which precludes the determination of the specificity of the competition. Further work using dilutions of each of them is necessary before the possible functional significance of such competition can be elucidated. On the contrary of Schultz *et al.* (1985) in rainbow trout ovary, and of Schlaghecke (1983) and Schulz and Schlaghecke (1984) in rainbow trout testis, we did not find any specific binding of hCG to our preparation. This could be related to the purity of hCG used for labeling, our preparation being less biologically potent. Another explanation can be the disappearance of the hCG biological activity after labeling. In another study (Le Gac *et al.*, in press) it was demonstrated that only 30 to 40% of the labeled GtH had the capacity to bind to trout testis receptor, suggesting a partial degradation of the hormone after labeling which would be more important for hCG in our conditions. The affinity constant found for the type I receptors is one order of magnitude higher than those described for sGtH in immature chum salmon ovary (2.5 to $3.1 \cdot 10^9 M^{-1}$) (Van der Kraak, 1983) and amago salmon granulosa cell (Salmon *et al.*, 1984), and also for hCG in preovulatory rainbow trout ovary (Schulz *et al.*, 1985) values which correspond to the affinity constant of our second binding sites. The competition experiments with

unlabeled GtH give two different slopes in the Scatchard analysis, and similar results were obtained in the male (Le Gac *et al.*, in press). This could indicate the presence of two types of receptors with different affinity constants as proposed by Fontaine (1980). An alternative situation exists when there is only one gonadotropin in fish. Salmon *et al.* (1984), studying GtH binding to amago Salmon ovary, described the existence of binding sites on both thecal and granulosa cells. Unfortunately, there were no data on the characteristics of these binding sites, except a range of $10^9 M^{-1}$ for the affinity constant of the granulosa cell binding sites. Our two kinds of receptors could correspond to GtH binding on granulosa and thecal cells or may be present only during specific stages of gametogenesis and especially vitellogenesis, for which a requirement for two different gonadotropins has been hypothesized (Idler, 1982). A single gonadotropin acting on different types of receptors according to the stage of ovarian development (Fontaine, 1980) might also be possible. Although the two classes of receptors have been found separately in male and female, this conclusion must be regarded as provisory. A nonlinear Scatchard plot may be due to other causes, for example, a negative cooperativity or contamination of the tracer by damaged hormone (Rodbard, 1973).

ACKNOWLEDGMENTS

We are very grateful to the NIH for the gift of pituitary hormones bGH, oLH, oFSH, and bTSH. sPRL was kindly supplied by P. Prunet (INRA Rennes,

France). This work was supported by the French National Institute for Agronomic Research (INRA).

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 model. In "Comparative Endocrinology" (P. J. Gaillard and H. H. Boer, eds.), pp. 37-48. Elsevier, North-Holland, Amsterdam.
- Breton, B., Billard, R., and Jalabert, B. (1972). Spécificité d'action et relations immunologiques des hormones gonadotropes de quelques poissons téléostéens. *Ann. Biol. Anim. Biochem. Biophys.* **13**, 347-362.
- Breton, B., Fostier, A., Zohar, Y., Le Bail, P. Y., and Billard, R. (1983). Gonadotropine glycoprotéique maturante et estradiol 17 β pendant le cycle reproducteur chez la truite fario (*Salmo trutta*) femelle. *Gen. Comp. Endocrinol.* **49**, 220-231.
- Breton, B., Prunet, P., and Reinaud, P. (1978). Sexual differences in Salmon gonadotropin. *Ann. Biol. Anim. Biochem. Biophys.* **18**, 754-765.
- Cook, A. F., and Peter, R. E. (1980). The metabolism of gonadotropin in goldfish, *Carassius auratus*: Tissue uptake and distribution during the reproductive cycle. *Gen. Comp. Endocrinol.* **42**, 91-100.
- Fontaine, Y. A. (1980). Les hormones gonadotropes de l'hypophyse: Biochimie et biologie comparée, spécificité et évolution. *Reprod. Nutr. Dev.* **20**, 381-418.
- Fostier, A., and Jalabert, B. (1982). Physiological basis of practical means to induce ovulation in fish. In "Reproductive Physiology of Fish" (C. J. J. Richter and H. J. Th. Goos, eds.), pp. 164-173. Pudoc, Wageningen.
- Fostier, A., Jalabert, B., Billard, R., Breton, B., and Zohar, Y. (1983). The gonadal steroids. In "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldson, eds.), pp. 277-372. Academic Press, New York/Orlando.
- Gilet, C., Breton, B., and Billard, R. (1978). Seasonal effects of exposure to temperature and photoperiod regimes on gonad growth and plasma gonadotropin in goldfish (*Carassius auratus*). *Ann. Biol. Anim. Biochem. Biophys.* **18**, 1045-1050.
- Hirano, T., Prunet, P., Kawachi, H., Takahashi, A., Ogasawara, T., Kubota, J., Nishioka, R. S., Bern H. A., Takada, K., and Ishii, S. (1985). Development and validation of a salmon prolactin radioimmunoassay. *Gen. Comp. Endocrinol.*, **59**, 266-276.
- Hontela, A., and Peter, R. E. (1978). Daylycycles in serum gonadotropin levels in the goldfish effect of photoperiod, temperature and sexual condition. *Canad. J. Zool.* **56**, 2430-2442.
- Idler, D. R. (1982). Some perspectives on fish gonadotropins. In "Reproductive Physiology" (C. J. J. Richter and H. J. Th. Goos, eds.), p. 413. Pudoc, Wageningen.
- Jalabert, B., Breton, B., and Billard, R. (1974). Dosage biologique des hormones gonadotropes de poissons par le test de maturation in vitro des ovocytes de truite. *Ann. Biol. Anim. Biochem. Biophys.* **4**, 217-228.
- Le Gac, F., Breton, B., and Bougoussa, M. (1986). Gonadotropin hormone (GtH) receptors in the testis of the trout *Salmo gairdneri*: In vitro studies. *J. Fish. Physiol. Biochem.*, in press.
- Lowry, O. H. (1951). Protein measure with the Folin phenol reagent. *J. Biol. Chem.* **195**, 265-275.
- Prunet, P., and Houdebine, L. M. (1984). Purification and biological characterization of chinook salmon prolactin. *Gen. Comp. Endocrinol.* **53**, 49-57.
- Rodbard, (1973). "Mathematics of Hormone Receptor Interaction I: Basic Principles in Receptors for Reproductive Hormones" (B. W. O'Malley and A. R. Means, eds.), pp. 289-327. Plenum, New York.
- Salmon, C., Kagawa, H., Adachi, S., Nagahama, Y., and Fontaine, Y. A. (1984). Mise en évidence de sites de liaison spécifique de la gonadotropine de saumon chum (*Oncorhynchus keta*) dans des préparations membranaires de granulosa d'ovaire de saumon amago (*Oncorhynchus rhodurus*). *C. R. Acad. Sci. Paris Ser III* **298**, 337-340.
- Schlaghecke, R. (1983). Binding of 125I-hCG to rainbow trout (*Salmo gairdneri*) testis in vitro. *Gen. Comp. Endocrinol.* **49**, 261-269.
- Schulz, R., and Schlaghecke R. (1984). A receptor preparation from testes of *Salmo gairdneri* (Richardson): In vitro binding studies with 125I-labeled hCG and trout pituitary proteins. *Gen. Comp. Endocrinol.* **54**, 132-138.
- Schulz, R., Schlaghecke, R., and Blum, V. (1985). A particulate membrane preparation from ovaries of preovulatory rainbow trout (*Salmo gairdneri*): Binding studies with 125I-human chorionic gonadotropin. *Comp. Biochem. Physiol. A* **82**, 429-433.
- Thorell, J. I., and Johansson, B. G. (1971). Enzymatic iodination of polypeptides with I¹²⁵ to high specific activity. *Biochem. Biophys. Acta* **251**, 363-369.
- Van der Kraak, G. (1983). An introduction to gonadotropin receptor studies in fish. In "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldson, eds.), Reproduction Vol. IX A, pp. 405-441. Academic Press, New York/Orlando.

- Zohar, Y. (1982). "L'évolution de la Pulsatilité et des Cycles Nycthéméraux de la Sécrétion Gonadotrope Chez la Truite arc-en-ciel Femelle, en Relation Avec le Cycle Sexuel Annuel et Par Rapport à l'Activité Stéroïdogène de L'ovaire." These doctorat d'état, Paris VI.
- Zohar, Y., and Billard, R. (1984). Annual and daily changes in plasma gonadotropin and sex steroids in relation to teleost gonad cycles. *Trans. Amer. Fish. Soc.* 113, 444-451.
- Zohar, Y., Breton, B., and Fostier, A. (1982). Gonadotropic function during the reproductive cycle of the female rainbow trout *Salmo gairdneri* in relation to ovarian steroid secretion: In vivo and in vitro studies. In "Reproductive Physiology of Fish" (C. J. J. Richter and H. J. Th. Goos, eds.), pp. 14-18. Pudoc, Wageningen.