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Short-Term Profiles of Plasma Gonadotropin and Estradiol-17 β Levels in the Female Rainbow Trout, from Early Ovarian Recrudescence and throughout Vitellogenesis

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At various stages throughout the annual reproductive cycle, female rainbow trout (*Salmo gairdneri*) were fitted with a catheter in the dorsal aorta. They were bled via the catheter at frequencies of once every 30, 60, or 240 min over periods of 5 to 24 hr. Gonadotropin (GtH), estradiol-17 β (E₂17 β), and estrone levels were measured in the plasma samples. At early ovarian recrudescence (March), short-term (1–2 hr), high-amplitude (Δ GtH = up to 100 ng/ml), episodic pulses of GtH were recorded in the plasma of 12 of the 26 studied fish. In the others, GtH levels remained low and constant. No synchronization was found among the individual GtH profiles. E₂17 β levels in the same fish were low and constant while estrone was not detectable. At early stages of exogenous vitellogenesis (June), plasma GtH (1–3 ng/ml) and E₂17 β (0.5–1.5 ng/ml) levels were low and constant. At advanced stages of exogenous vitellogenesis (September–October), fluctuating GtH levels were found again in most of the females; basal GtH concentrations were only slightly higher than those recorded in June. The fluctuations consisted of short-term (1–3 hr) random GtH pulses of moderate amplitude (Δ GtH = up to 5 ng/ml), occurring at a relatively high frequency (up to 5 per 12 hr). Although no regular synchronous daily pattern of GtH was noted, most of the GtH pulses were observed during the photophase and early scotophase. The appearance of GtH pulsatility during exogenous vitellogenesis was accompanied by a large increase in plasma E₂17 β up to levels ranging from 6 to 30 ng/ml. In contrast to the GtH profiles, the E₂17 β profiles showed continuous and progressive variations, superimposed on the abrupt GtH pulses, and a high degree of synchronization. E₂17 β levels increased during the photophase and reached maxima toward and during early scotophase. © 1986 Academic Press, Inc.

The seasonal variations in plasma gonadotropin (GtH) and sex-steroid levels were thoroughly studied in the female trout (*Salmo gairdneri*). A careful examination of the various studies reveals some discrepancies amongst them. A transient increase in GtH levels has been observed by some authors at early stages of ovarian development (spring) in the rainbow (Breton *et al.*, 1985) and brown (Billard *et al.*, 1978; Breton *et al.*, 1983) trout, whereas others have reported constant or only slightly varying levels of the hormone (rainbow

trout: Scott and Sumpter, 1983; Sumpter *et al.*, 1984). At the same time, estradiol-17 β (E₂17 β) levels have been reported as low and constant (rainbow trout: Lambert *et al.*, 1978; Whitehead *et al.*, 1978a, b; Scott *et al.*, 1980; Van Bohemen and Lambert, 1981) or increasing steadily (brown trout: Billard *et al.*, 1978; Breton *et al.*, 1983; rainbow trout: Sumpter *et al.*, 1984). During vitellogenesis plasma GtH levels have been reported as either (a) increasing moderately (brown trout: Breton *et al.*, 1983; Billard *et al.*, 1978; Crim and Idler, 1978), (b) remaining constant (rainbow trout: Scott and Sumpter, 1983), or (c) initially increasing and then declining continuously (rainbow trout: Bromage *et al.*, 1982a, b; Whitehead *et al.*, 1983). Plasma

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levels of E₂17 β and testosterone rise considerably during vitellogenesis and decline progressively from its final stages (brown trout: Billard *et al.*, 1978; Breton *et al.*, 1983; rainbow trout: Fostier *et al.*, 1978; Scott and Sumpter, 1983; Scott *et al.*, 1980; Bromage *et al.*, 1982a, b).

One possible explanation for the above-mentioned discrepancies might be the fact that the long term (seasonal) profiles only partially reflect the hormonal function. In fact, in the majority of the mammalian species studied in this respect, the fundamental signals composing the hormonal message are generally of very short duration, i.e., from a few minutes up to a few hours (see review in Knobil, 1980; Lincoln and Short, 1980; Brinkley, 1981; Desjardins, 1981). Similarly, daily variations in the circulating levels of the reproductive hormones have been reported in different fish species. Daily cycles in plasma GtH levels have been demonstrated in the female goldfish, *Carassius auratus* (Breton *et al.*, 1972; Hontela and Peter, 1978, 1980a; Vodicnik *et al.*, 1978; Gillet and Billard, 1981; Gillet *et al.*, 1981) and in the male and female of the common carp, *Cyprinus carpio*; the silver carp, *Hypophthalmichthys molitrix*; and the grass carp, *Ctenopharyngodon idellus* (Pan *et al.*, 1980). Circulating levels of testosterone, E₂17 β , and estrone have been shown to fluctuate during the day in the catfish, *Heteropneustes fossilis* (Lamba *et al.*, 1983). In salmonids, data on circadian changes in the levels of reproductive hormones are rare. Our previous reports (Zohar, 1980; Zohar *et al.*, 1982a) have presented some data showing that in the female rainbow trout, daily (short-term) fluctuations in plasma GtH levels do occur and are superimposed on the seasonal variations of the hormone.

The aim of the present work was to study, at some characteristic stages of the reproductive cycle of the female rainbow trout, the short-term (circadian and ultra-

dian) profiles of plasma GtH and main sex-steroid levels. Since our previous data (Zohar, 1980; Zohar *et al.*, 1982a) had indicated a lack of synchronization between fish in the GtH secretion pattern, we studied hormonal profiles of individual free-swimming trout, fitted with a catheter in the dorsal aorta. The present paper describes short-term profiles of GtH and E₂17 β from early ovarian recrudescence and throughout vitellogenesis, whereas our following one (Zohar *et al.*, 1986) describes short-term profiles of GtH and 17 α -hydroxy, 20 β -dihydroprogesterone at the periovulatory period.

MATERIALS AND METHODS

(a) Animals and Stocking Conditions

Female trout, aged 3 to 4 years and weighing 1.5 to 3 kg, were used in the present study. All of them had reached sexual maturity at least once before. About 2 to 3 months before the beginning of the acclimation to experimental conditions, fish were transferred from farm to indoor tanks supplied with recycled water. Then, 3 to 4 weeks before the initiation of the experiments the fish were transferred into tanks (70–150 liters) in which they were kept individually. Fish were exposed to a natural photoperiod. Water temperature was maintained at $10 \pm 2^\circ$ between December and May and at $15 \pm 2^\circ$ between June and November. Fish were fed with artificial food granules at a daily ratio of 1% of their body weight. Feeding time was between 1000 and 1200 hr.

(b) Dorsal Aorta Catheterization

At various times of each year over a 3-year period, catheters were implanted in the dorsal aortas of 10–20 female trout. Only fish which had showed active feeding behavior for at least 1 week were catheterized. The procedure for implanting a catheter in the dorsal aorta has been described in detail by Zohar (1980). The same study (Zohar, 1980) and Bry and Zohar (1980) showed that the resumption of normal feeding behavior after catheterization reflected the recovery of fish from the stress situation related to the surgery. In such fish, the surgery and the presence of the catheter in the dorsal aorta did not affect gonadotropin levels in the blood or ovarian function (Zohar, 1980).

(c) Blood Sampling

Before catheterization, 0.3–0.5 ml of blood was removed from the caudal vasculature of every fish. Hor-

mone levels in these samples were compared to those recorded in blood sampled successively via the dorsal aortic catheter during experimentation, as described by Zohar (1980). After catheterization, fish were allowed to recover for at least 3 days before further bleeding.

In agreement with our previous findings (Zohar, 1980; Bry and Zohar, 1980), only fish which resumed normal feeding behavior after surgery were selected for experimentation. Individual free-swimming catheterized trout were bled repeatedly via the catheter at frequencies of once ever $\frac{1}{2}$, 1, or 4 hr over periods of 5 to 24 hr. In order to bleed the fish, the cannula, its stopper (a scaled syringe needle), and its cork floater were gently removed from the water. The cannula was unplugged and fitted with a 1-ml syringe. A volume of 250 μ l, corresponding to 50 μ l more than the dead volume of the catheter, was removed and discarded. The cannula tip was then fitted with a clean 1-ml syringe previously rinsed with lithium heparinate solution (100–150 iu of heparine in 0.7% of saline). A volume of 300 to 400 μ l of blood was withdrawn and transferred to ice-cold 3-ml tubes. At the end of each sampling, the blood filling the catheter was pushed back by 200 μ l of lithium-heparinate saline solution (as above) and the cannula was plugged. Bleeding during the dark period was made using a dim red light to which wavelength salmonids adapted to dark are not sensitive (Hanyu and Tamura, 1978). Blood samples were centrifuged (15 min at 5000 rpm and at 4°), and the plasma was stored at -30° until further analysis.

(d) Determination of Ovarian Developmental Stages

At the end of each experiment, the females were sacrificed and their ovaries submitted to histological analysis. We distinguished three major ovarian developmental stages (based on Van den Hurk and Peute, 1979):

1. *Early ovarian recrudescence (March)*. Ovaries contained isolated or grouped oogonia, oogonia undergoing mitotic divisions, oocytes at different stages of the prophase of the first meiotic division, and different proportions of oocytes undergoing previtellogenic growth, endogenous vitellogenesis, and the very beginnings of exogenous vitellogenesis. Previtellogenic oocytes (50 and 500 μ m in diameter) were characterized by the presence of Balbiany bodies. Their nuclei were relatively big and many nucleoli were scattered at its periphery. Their follicular layers were not completely organized. Oocytes which undergo endogenous vitellogenesis (400–900 μ m in diameter) were characterized by the successive appearance of two structures: the vitellin vesicles (the future cortical alveoli) which appeared first in the ooplasm periphery and spread later toward its center, and the lipid globules which appeared in the perinuclear area, fused,

and gained size. The zona radiata and the follicular layers were already organized in such oocytes. Oocytes undergoing early exogenous vitellogenesis are described below.

2. *Early exogenous vitellogenesis (June)*. Ovaries contained mainly oocytes undergoing initial stages of exogenous vitellogenesis. Such oocytes (which measured 1 to 2 mm in diameter) were characterized by the progressive appearance, in the periphery of the ooplasm, of vitellin granules.

3. *Advanced exogenous vitellogenesis (September–October)*. Ovaries contained mainly vitellogenic oocytes with diameters of up to 4–5 mm. They were characterized by the massive appearance of vitellin granules all over the ooplasm. These granules coalesce to form spheric structures (vitellin spheres) which occupy most of the oocyte volume.

(e) Hormone Measurements

Gonadotropin was measured by radioimmunoassay (RIA) according to Breton and Billard (1977). The antibody used was anti-trout GtH at a final dilution of 1:130,000 to 1:200,000. A highly purified salmon GtH (Breton *et al.*, 1978) was used for the standard curve and for the radioactive labeling. Each unknown sample was measured in triplicate. All samples from a single experiment were measured in the same RIA run. GtH concentration in most of the samples was measured twice, in two different RIA runs, in order to confirm the existence of fluctuations in the hormonal concentration and to eliminate any error due to the measuring technique (see also Zohar, 1980).

The sensitivity of the RIA varied between 15 and 30 pg GtH per tube which corresponds to 0.3 and 0.6 ng GtH/ml of plasma. The intraassay variability (Table 1) was estimated by repetitive measurements, in each of the RIA runs, of the GtH concentrations in different pools of plasma. These pools were sampled from fish at different physiological stages and contained various GtH levels, covering the entire range of the standard curve.

The radioimmunoassays of estradiol-17 β and estrone were carried out according to Fostier *et al.* (1978), except that bound steroid was precipitated with polyethylene glycol (Fostier *et al.*, 1982). Both steroids were measured after being extracted from the plasma and separated from each other on an LH-20 column. Unknown samples were measured in triplicate. The estradiol-17 β antibody was used at a final dilution of 1:50,000, and its degree of cross-reactivity with other steroids was 11% for estrone, 9% for 16-ketoestradiol-17 β , 8% for 16-epiestriol, and less than 0.5% for estriol, estradiol-17 α , testosterone, 11-ketotestosterone, and 17 α -hydroxy,20 β -dihydroprogesterone. The estrone antibody was used at a final dilution of 1:4800, and its degree of cross-reactivity was less than 0.8% for estradiol-17 β , estriol, testosterone,

TABLE 1
THE INTRAASSAY VARIABILITY OF THE RADIOIMMUNOASSAYS

Hormone	Level (ng/mL)	Mean CV (%)	Range of CV (%)	Number of samples/assay	Number of assays
GtH	1-3	9.3	6.0-10.9	5-7	7
	4-6	7.7	4.1-12.3	5-7	7
	7-12	9.9	7.3-11.9	5-7	9
	14-20	6.6	4.0-9.9	5-7	8
	40-50	6.8	2.3-8.8	5-6	5
	105-125	4.9	3.6-6.9	5-7	5
Estradiol 17 β	1-2	9.6	6.3-12.4	6	3
	3-4	5.3	2.6-8.7	5	3
	20-22	9.1	8.8-9.4	5	2
	33-35	5.5	4.3-6.7	6-10	2
Estrone	1.5-2.0	10.5	9.6-11.4	5	2

11-ketotestosterone, and 17 α -hydroxy,20 β -dihydroprogesterone. The sensitivity of both radioimmunoassays was approximately 10 pg/tube, and the intraassay variability is given in Table 1.

(f) Analytical Methods

The mean, coefficient of variation (CV) and variance were calculated for all the values composing each of the individual hormonal profiles. A second (hypothetical) variance was calculated for each profile, based on the highest CV obtained for a plasma pool (Table 1) having a hormone concentration close to the mean of the values composing the tested profile. This hypothetical variance (and CV) is thus the expected one, supposing that the observed hormonal fluctuations reflected only the variations of the RIA. The real and hypothetical variances were compared by χ^2 test. On the basis of this comparison, all profiles having a real CV at least twice as large as the hypothetical one were considered as fluctuating significantly, due to physiological reasons. These fluctuating profiles showed a highly significant ($P < 0.005$) larger real CV than a hypothetical one. For the nonfluctuating profiles (real CV $\leq 2 \times$ hypothetical CV), the mean of all values composing each of them was considered as the "basal level."

We distinguished two types of fluctuating profiles: (1) Irregular profiles, showing random, episodic fluctuations of hormonal levels. In this group there was no synchronization between individual profiles of animals representing the same physiological stage; (2) Regular profiles, showing gradual and continuous changes in the hormonal levels. In this group there was a certain synchronization between profiles of individual animals. The two types of profiles were further analyzed by different methods.

(1) *Irregular profiles.* The hormonal concentrations composing each of the profiles were divided into two subgroups. The first one included the low concentra-

tions, representing the "baseline" levels or "inliers." The second subgroup included the high hormonal concentrations, representing "pulsation" levels or "outliers." The statistical detection of outliers was affected according to tests adapted for one (Dixon, 1953) or many (Grubbs, 1969) outliers. High hormonal levels which were characterized as "outliers" were then eliminated one by one, and the fluctuating nature of the resting values of the profile was tested each time as described above. This analysis was stopped when the CV of the resting values ("inliers") became equal to or smaller than twice the hypothetical CV.

(2) *Regular profiles.* A global analysis of the profiles of all the animals representing a given ovarian developmental stage was realized by a two-factorial analysis of variance (the two factors analyzed were number of animals and sampling time). When significant differences were found, the analysis was continued by the Duncan test (Bliss, 1967). This enabled the identification of significant differences, during the experimental period, between groups of mean hormonal levels.

RESULTS

(a) Early Ovarian Recrudescence: March (Figs. 1 and 2)

In trout which were sampled every 4 hr for 24 hr, we observed in five of the nine fish studied significant short-term increases in plasma GtH levels (Fig. 1). In two of the females (Nos. 113 and 115), the amplitude of the increases reached around 100 and 50 ng/ml, respectively, whereas in the others the amplitude varied between 4 and 8 ng/ml. No synchronization was observed among the individual profiles. In

FIG. 1. Profiles of plasma levels of GtH (continuous lines) and $E_217\beta$ (broken lines) in female trout at early ovarian recrudescence (March). Females were bled every 4 hr over a period of 24 hr. Individual GtH profiles with significant fluctuations in hormone levels, and the mean of profiles ($n = 4$ fish) with stable GtH levels, are presented. The number of each female is indicated. The statistical significance of the hormonal fluctuations is indicated by the following symbols: for GtH, Δ = nonsignificant and $\blacktriangle$ = significant; for $E_217\beta$, $\circ$ = nonsignificant and $\bullet$ = significant. The symbol * situated above a peak indicates that it is significantly higher than the basal level.

four females, GtH levels remained constant. Four of the GtH peaks were observed in the middle or toward the end of the photophase, whereas another peak occurred during the scotophase. In the fish in which they were measured, estradiol-17 β levels were found to be low and constant, whereas estrone levels were below the detection limits of the RIA (0.15 ng/ml).

Bleeding fish every hour for 12 hr, during the day (for one group) or throughout the night (for another), showed short-term increases (pulses) in plasma GtH levels in seven of the 17 sampled females (Fig. 2). In the other fish, GtH levels remained con-

stant. The amplitude of the GtH increases was relatively large in some of the cases, and ranged from 5 to about 100 ng/ml. In most of the females, a GtH pulse included only one point (sample) after which GtH returned to its basal level within 1 hr. In one female (No. 122), in which the GtH peak value was the highest (118 ng/ml), GtH returned to its basal level more gradually, through an intermediate value observed 1 h after the peak.

Although no synchronization was evident among individual profiles, six of eight significant GtH pulses occurred during the night, whereas the others occurred in the

FIG. 2. Profiles of plasma levels of GtH (continuous lines) and E₂17 β (broken lines) in female trout at early ovarian recrudescence (March). Females were bled every hour over a period of 12 hr, one group from 0900 to 2100 hr and a second group from 2100 to 0900 hr. For other details, see the legend of Fig. 1.

late photophase (Fig. 2). In all the fish exhibiting GtH pulses, only one pulse was detected throughout the 12-hr sampling period, with the exception of one female, for which we observed two significant GtH elevations. In three females showing GtH pulses, we measured low and constant E₂17 β levels, whereas estrone was not detectable (Fig. 2, female Nos. 122–124).

(b) *Early Exogenous Vitellogenesis: June (Fig. 3)*

At this stage of ovarian development, GtH levels were low (1–3 ng/ml) and relatively stable. When females were sampled once every 4 hr for 24 hr (Fig. 3a), we observed slight fluctuations in GtH levels in two of six fish, whereas in the others these

FIG. 3. Individual profiles of plasma levels of GtH (continuous lines) and E₂17β (broken lines) in female trout at early exogenous vitellogenesis (June). Females were bled either every 4 hr over a period of 24 hr (a), or every hour over 9 hr (b). For other details, see the legend of Fig. 1.

levels remained constant. In all females sampled every 1 hr for 10 hr (Fig. 3b), GtH levels were low and stable. When measured, E₂17β levels were found to be low and constant (Fig. 3b).

(c) *Advanced Exogenous Vitellogenesis: September–October (Figs. 4–7)*

Due to space limitations, we include here only a representative number of individual hormonal profiles out of nearly 50 made on fish at this stage of ovarian development.

Of the 47 analyzed individual GtH profiles of females undergoing advanced exogenous vitellogenesis, 29 exhibited significant daily fluctuations.

In Fig. 4 are shown individual GtH profiles of nine fish which were bled hourly for 9 hr. For two females (Nos. 145 and 147), two GtH profiles are shown which were determined in two separate RIA runs. Seven of the nine GtH profiles fluctuated significantly. In all these cases, one to two significant GtH pulses were found during the 9-hr sampling period.

In Fig. 5 are shown individual profiles of GtH, E₂17β, and estrone in eight females which were bled once every hour for 12 hr. Six of the GtH profiles fluctuated significantly, whereas in another one (No. 155) fluctuations were very close to being significant. In four of the females, significant

FIG. 4. Individual profiles of plasma GtH levels in female trout at advanced exogenous vitellogenesis (September). Females were bled every hour over a period of 9 hr. The two curves in continuous and broken lines correspond, for the same female, to results obtained in two different runs of RIA. For other details, see the legend of Fig. 1.

GtH pulses were recorded, whereas in another (No. 154) a long GtH elevation was observed. In both groups of profiles (Figs. 4 and 5), most of the GtH pulses included only one high GtH value (except in female No. 159).

No synchronization existed among the individual GtH profiles of fish bled hourly. However, we noted 13 significant GtH pulses in the eight females which were sampled between 0800 and 2000 hr (Fig. 5), and only six significant pulses in seven females sampled between 2000 and 0800 hr (individual curves not shown). The lack of synchronization between the individual GtH profiles is reflected by their mean profile (Fig. 6, low curve), which masks the existence of the GtH pulsatility and shows very constant GtH levels over the 24-hr sampling period. Figure 7 shows GtH profiles in eight females sampled once every 30 min for 5½ hours. In this case, significant fluctuations in GtH levels occurred in five of the females. Due to the more frequent

samplings, most of the elevations in GtH levels included at least two high GtH values.

The analysis of all GtH profiles which we recorded showed that when oocytes undergo advanced exogenous vitellogenesis, the dynamics of GtH secretion differs from that found at earlier stages of gonadal development. One to five GtH pulses occur over a period of 12 hr; their duration ranges from 1 to 3 hr and their amplitude reaches around 5 ng/ml.

As far as estradiol-17 β is concerned, its mean level was much more elevated than at earlier stages of ovarian development (Fig. 6, Student *t* test, $P < 0.01$). The levels of this steroid fluctuated significantly during the day in eight of 11 studied females (Fig. 5 for the profiles of seven females sampled between 0800 and 2000 hr). The pattern of the individual E₂17 β profiles was different from that established for the GtH. E₂17 β levels did not change abruptly, but revealed continuous, relatively synchronized daily

FIG. 5. Individual profiles of plasma levels of GtH (continuous lines), $E_217\beta$ (broken lines), and estrone (dotted lines) in female trout at advanced exogenous vitellogenesis (October). Females were bled every hour over a period of 12 hr. For other details, see the legend of Fig. 1.

variations overriding the GtH pulses (Fig. 5). In the six fish showing fluctuating profiles out of the seven sampled from 0800 to 2000 hr, $E_217\beta$ levels were lower in the first part of the photophase, increased progressively later on, and reached maxima during the later part of the photophase or at the beginning of the scotophase. In some of these females, $E_217\beta$ tended to decrease after reaching maxima. This tendency continued in fish sampled hourly between 2000 and 0800 hr (curves not shown). The relative synchronization between the individual $E_217\beta$ profiles is reflected in their mean profile (Fig. 6, upper curve), which shows a progressive, highly significant increase of $E_217\beta$ during the day, from min-

imal levels at 0800 to 1000 hr up to maxima reached between 1500 and 1700 hr, followed by an initiation of a decline. Plasma $E_217\beta$ profiles were recorded only in four females sampled between 2000 and 0800 hr. These profiles (not shown) exhibited relatively high individual variation, and the calculation of their average was thus meaningless. More fish should be studied in order to confirm the $E_217\beta$ pattern during the nocturnal part of the 24 hr.

We measured estrone levels in five females (Fig. 5). Those levels were very low, close to the detection limit of the RIA. In some of the females (Nos. 156 and 160), the estrone profiles were parallel to those of $E_217\beta$.

FIG. 6. Mean profiles ($\bar{x} \pm \text{tSE}$) of plasma levels of GtH (lower curves—24 hr) and of E₂17 β (upper curve—12 hr) in female trout at advanced exogenous vitellogenesis (October). The mean profiles were established from individual profiles of fish sampled every hour over a period of 12 hr (Fig. 5 for the group sampled from 0900 to 2100 hr). **Significantly different at $P < 0.01$.

DISCUSSION

Annual changes in GtH levels in the female rainbow trout have been described previously (see introduction). The present study together with our following one (Zohar *et al.*, 1986) reveal that in addition to the seasonal variations in the hormone concentration, important modifications occur in the pattern of the short-term profiles of GtH levels throughout oocyte development.

A transient GtH elevation has previously been observed in the circulation of the female rainbow and brown trout undergoing ovarian recrudescence. This elevation occurred either in spring (March) in fish exposed to natural photoperiodic regime (Billard *et al.*, 1978; Breton *et al.*, 1983), or earlier (between January and March) when the photoperiodic regime was altered (Breton *et al.*, 1985). Our present data suggest that this increase in GtH is the reflection of short-term, high-amplitude, episodic

elevations in the hormone level. The GtH pulses observed in March were not synchronized among the individual fish and high GtH levels were recorded during no more than 2 hr. Thus, a point sampling of a group of fish undergoing early ovarian recrudescence (March), for the study of seasonal GtH variations, might show a mean elevated GtH level accompanied by high individual variation, as was actually the case (Breton *et al.*, 1983, 1985).

High-amplitude GtH pulses were observed in only 50% of the fish studied in March. This might be due to the short duration of the pulses and to their low frequency (in most of the cases only one GtH pulse was observed during the 12-hr sampling period). However, the absence of GtH pulses in half of the females should also be considered together with the fact that in June no more such GtH pulses were observed, and with the findings of Breton *et al.* (1983, 1985) concerning the transient nature of the spring GtH surge. Such a con-

FIG. 7. Individual profiles of plasma GtH levels in female trout at advanced exogenous vitellogenesis (October). Females were bled every 30 min over a period of 5½ hr, between 1430 and 2000 hr. For other details, see the legend of Fig. 1.

sideration might indicate that the period during which the high-amplitude GtH pulses occur in each female is short, and that they characterize a precise given physiological state. A low degree of physiological synchronization among the studied fish might explain the differences found in their individual GtH profiles.

According to Van den Hurk and Peute (1979) and Breton *et al.* (1983, 1985), exogenous vitellogenesis in the rainbow trout (as observed histologically) first occurs in May, 1 to 2 months following the spring GtH rise (March). However, a recent study of the same species (Sumpter *et al.*, 1984) has indicated that exogenous vitellogenesis can begin as early as March. Although Lambert *et al.* (1978), Whitehead *et al.* (1978a, b), Scott *et al.* (1980), and Van Bohemen and Lambert (1981) found very low and constant $E_217\beta$ and vitellogenin levels in the female trout during spring, other studies have shown a slow increase in plasma $E_217\beta$ levels starting in March (Billard *et al.*, 1978; Breton *et al.*, 1983) or

even earlier (Fostier and Le Bail, unpublished data; Sumpter *et al.*, 1984). Sumpter *et al.* (1984) reported an increase in circulating vitellogenin levels in the rainbow trout as early as January, and the beginning of its incorporation in late March. In the present study, the first signs of vitellogenin uptake were also histologically visible in the fish bled in March, in which the high-amplitude GtH pulses were found. These considerations indicate that the GtH pulses might play a role in the induction of vitellogenin uptake by the follicles. In fact, Breton and Derrien-Guimard (1983) have demonstrated that physiological GtH pulses stimulate vitellogenin incorporation into *in vitro* perfused oocytes of rainbow trout. However, our data, showing that $E_217\beta$ levels in fish exhibiting high-amplitude GtH pulses were constant, do not necessarily mean that GtH pulses do not play a role in controlling the synthesis and/or secretion of $E_217\beta$ from follicles at this time of the year. In a separate study, we have demonstrated that the administration of

physiological GtH pulses to *in vitro* perfused ovarian fragments undergoing early recrudescence stimulated their E₂17 β output considerably (Zohar *et al.*, 1982b; Zohar, Fostier, and Breton, unpublished results). *In vivo*, the GtH pulses might maintain constant E₂17 β levels over short periods.

The exact role of the pituitary in the control of the different phases of oogenesis has not yet been satisfactorily defined. Whereas the previtellogenic growth of oocytes seems to be independent of the pituitary (Vivien, 1939; Barr, 1963; Yamazaki, 1965), it has been shown in various teleost species that oogonial proliferation (Barr, 1963; Yamazaki, 1965) and vitellogenesis are pituitary-dependent processes (Barr, 1963; Yamazaki, 1965; Sundararaj *et al.*, 1972; Khoo, 1979), the last one definitely being controlled by gonadotropin (Hoar *et al.*, 1967; Hyder, 1972; Mackay, 1973; Upadhyay *et al.*, 1978). On the basis of these observations, we cannot exclude the possibility that the high-amplitude GtH pulses found in March might also be related to the numerous oogonial mitoses and oocyte meioses, or to endogenous vitellogenesis, which were observed in the ovaries of the studied females.

In June, when most of the ovarian follicles were in the early stages of exogenous vitellogenesis, GtH (1–3 ng/ml) and E₂17 β (0.5–1.5 ng/ml) levels were low and constant. In September–October, when exogenous vitellogenesis was in its advanced stages, plasma GtH levels fluctuated again. In most of the studied females, episodic, short-term GtH pulses of moderate amplitude were recorded, while basal GtH levels (2.4 ± 0.6 ng/ml) were only slightly higher than those recorded in June (1.8 ± 0.4 ng/ml). The appearance of GtH pulsatility during exogenous vitellogenesis was accompanied by a large increase of plasma E₂17 β , up to levels ranging from 6 to 30 ng/ml. At advanced exogenous vitello-

genesis, individual E₂17 β profiles showed continuous and progressive variations superimposed on the abrupt GtH fluctuations. In contrast with the GtH profiles, a relative synchronization existed among the individual E₂17 β profiles. This was reflected by an average E₂17 β profile showing regular significant daily fluctuations (Fig. 6). E₂17 β levels increased during the photophase and reached maxima toward and during early scotophase; a decrease later on might occur, which should be confirmed.

In the goldfish, Hontela and Peter (1978) found constant plasma GtH levels in "regressed" fish, in which oocytes undergoing endogenous vitellogenesis dominated the ovary. Daily fluctuations in GtH levels appeared later on, in "maturing" and in "mature" females. In the ovaries of these females, the proportion of oocytes undergoing advanced stages of vitellogenesis was high. In all groups, basal GtH levels were the same. Based on these results, Hontela and Peter (1978) and Peter (1981) suggested that the daily fluctuations in GtH levels might be more important for the stimulation and the maintenance of the ovarian activity than the progressive long-term changes in the concentrations of the hormone. On the basis of our present results, a similar hypothesis might be proposed for the female rainbow trout. Whereas previous studies suggested that the important increase in circulating E₂17 β during exogenous vitellogenesis is accompanied either by a moderate increase or continuous decrease in the mean plasma GtH levels or by constant concentrations of the hormone (see introduction), our study suggests an important change in the short-term GtH profiles during the same processes. The constant levels of GtH characterizing the beginning of exogenous vitellogenesis become pulsatile in its advanced stages, while E₂17 β increases drastically. The basal GtH levels remain low in both cases. This sug-

gests that in the female rainbow trout the appearance of GtH pulsatility during exogenous vitellogenesis, rather than a change in the hormone absolute levels, is responsible for the marked $E_217\beta$ increase. However, the rainbow trout model might be more complicated than the goldfish one. The results obtained by Hontela and Peter (1978), and confirmed by Hontela and Peter (1980a, b) and Gillet *et al.* (1981), indicate that there is a relative synchronization between the individual GtH profiles of different females. This fact results in the detection of a significant daily cycle in GtH levels when different groups of fish are sampled every 4 hr. Our present data demonstrate that in the female rainbow trout, the individual GtH profiles are not synchronized since the short-term increases in GtH levels occur at different times in different females. Thus, the GtH fluctuations can be detected only if individual fish are sampled repeatedly. Averaging the individual GtH profiles masked the existence of GtH pulsatility, and resulted in stable mean GtH levels over the 24-hr sampling period (Fig. 6). A similar situation has been described by Pan *et al.* (1980) in three carp species; when individual GtH profiles were recorded during the spawning season, short-term GtH pulses were observed. As in the case of the rainbow trout at advanced exogenous vitellogenesis, those profiles were relatively nonsynchronized among individual fish.

Although it is difficult to determine the frequency of GtH pulses during advanced exogenous vitellogenesis, we observed up to 5 GtH pulses within a 12-hr period when the bleeding interval was 1 hr. However, the number of pulses varied between the females, and in some of them GtH levels were quite constant. This might reflect a lack of total physiological synchronization among the studied females or might be the result of not bleeding frequently enough in relation to the duration of the pulses. In

many of the profiles for which bleeding was done at 1-hr intervals, a pulse included only one value of high GtH level. The pulses were longer in a few cases, and in one female (Fig. 5, No. 154) the increase in GtH levels lasted a few hours. This last example might reflect the initiation of an increase in the basal GtH levels, related to a possible initiation of the germinal vesicle migration (see Zohar *et al.*, 1986). When the sampling interval was reduced to 30 min, most of the GtH pulses included more than one value of high GtH level. Considered together, all of the GtH profiles characterizing exogenous vitellogenesis indicate a pulse duration of 1 to 3 hr. Bleeding fish at even higher frequencies than that reported here is necessary for a precise analysis of the dynamics of the GtH pulses. Our present data do not demonstrate a clear circadian rhythmicity in GtH levels at advanced exogenous vitellogenesis. However, they indicate a possible decrease in the frequency of the GtH pulses during the night, which should be further confirmed.

The relationship between the GtH and $E_217\beta$ secretion patterns in the female trout at advanced exogenous vitellogenesis cannot be precisely determined on the basis of the present *in vivo* study. They are considered in more detail elsewhere (Zohar, Fostier, and Breton, in preparation), taking into account the results of both *in vivo* and *in vitro* studies. However, the relationship between the GtH and $E_217\beta$ secretion patterns differs from that described in most mammalian species, in which pulses are considered as basic signals at both the pituitary and the gonadal level. A pulsation of LH is immediately followed by a pulsation of a corresponding gonadal steroid in the mouse (Desjardins, 1981), the rabbit (Moor and Younglai, 1975), the goat (Muduuli *et al.*, 1979), the dog (De Palatis *et al.*, 1978), the ewe (Baird, 1978), the ram (Lincoln, 1976), the rhesus monkey (Steiner *et al.*, 1980), and

man (Backstrom *et al.*, 1982). In the female rainbow trout, however, the short-term GtH pulses are accompanied by regular E₂17 β variations of longer duration. The same phenomenon was also observed *in vitro* (Zohar *et al.*, 1982b and unpublished data). The greater abundance of GtH pulses during the day may indicate the importance of their frequency in the regulation of the progressive increase in E₂17 β levels during the photophase, as well as in the possible decrease of these levels later on.

Recently, the physiological function of fish gonadotropin(s) in relation to vitellogenesis has been a matter of discussion (e.g., Idler, 1982; Idler and Ng, 1983; Burzawa-Gerard, 1982). In this discussion, references have been made to results which are related to the circulating levels of the glycoproteic GtH. Our present study is the first to demonstrate that in the female rainbow trout, changes in the short-term secretion pattern of GtH occur from early ovarian recrudescence and throughout vitellogenesis, in addition to the seasonal evolution of the hormone level. Such short-term secretion patterns should be considered when the glycoproteic GtH role in vitellogenesis is discussed.

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