Short-term profiles of plasma gonadotropin and estradiol-17β levels in the female rainbow trout, from early ovarian recrudescence and throughout vitellogenesis

Y. Zohar, Bernard Breton, Alexis Fostier

To cite this version:

Y. Zohar, Bernard Breton, Alexis Fostier. Short-term profiles of plasma gonadotropin and estradiol-17β levels in the female rainbow trout, from early ovarian recrudescence and throughout vitellogenesis. General and Comparative Endocrinology, Elsevier, 1986, 64 (2), pp.172-188. 10.1016/0016-6480(86)90002-X. hal-01600969

HAL Id: hal-01600969
https://hal.archives-ouvertes.fr/hal-01600969

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

Y. ZOHAR, B. BRETON, AND A. FOSTIER

Laboratoire de Physiologie de Poissons, INRA, Campus de Beaulieu, 35042 Rennes, France

Accepted May 12, 1986

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-17β (E217β), and estrone levels were measured in the plasma samples. At early ovarian recrudescence (March), short-term (1–2 hr), high-amplitude (ΔGtH = 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. E217β 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 E217β (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 (ΔGtH = 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 E217β up to levels ranging from 6 to 30 ng/ml. In contrast to the GtH profiles, the E217β profiles showed continuous and progressive variations, superimposed on the abrupt GtH pulses, and a high degree of synchronization. E217β levels increased during the photophase and reached maxima toward and during early scotophase.

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-17β (E217β) 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
levels of E\textsubscript{2}17β and testosterone rise considerably during vitellogenesis and decline progressively from its final stages (brown trout: Billard \textit{et al.}, 1978; Breton \textit{et al.}, 1983; rainbow trout: Fostier \textit{et al.}, 1978; Scott and Sumpter, 1983; Scott \textit{et al.}, 1980; Bromage \textit{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, \textit{Carassius auratus} (Breton \textit{et al.}, 1972; Hontela and Peter, 1978, 1980a; Vodicnik \textit{et al.}, 1978; Gillet and Billard, 1981; Gillet \textit{et al.}, 1981) and in the male and female of the common carp, \textit{Cyprinus carpio}; the silver carp, \textit{Hypophthalmichthys molitrix}; and the grass carp, \textit{Ctenopharyngodon idellus} (Pan \textit{et al.}, 1980). Circulating levels of testosterone, E\textsubscript{2}17β, and estrone have been shown to fluctuate during the day in the catfish, \textit{Heteropneustes fossilis} (Lamba \textit{et al.}, 1983). In salmonids, data on circadian changes in the levels of reproductive hormones are rare. Our previous reports (Zohar, 1980; Zohar \textit{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 ultradian) profiles of plasma GtH and main sex-steroid levels. Since our previous data (Zohar, 1980; Zohar \textit{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 E\textsubscript{2}17β from early ovarian recrudescence and throughout vitellogenesis, whereas our following one (Zohar \textit{et al.}, 1986) describes short-term profiles of GtH and 17α-hydroxy, 20β-dihydroprogesterone at the periovulatory period.

**MATERIALS AND METHODS**

\textbf{(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±2°C between December and May and at 15±2°C 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.

\textbf{(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).

\textbf{(c) Blood Sampling}

Before catheterization, 0.3–0.5 ml of blood was removed from the caudal vasculature of every fish. Hor-
Gonadotropin was measured by radioimmunoassay (RIA) according to Breton and Billard (1977). The antibody used was anti-trout GtH at a final dilution of 1:1,300,000 to 1:200,000. A highly purified salmon GtH (Breton et al., 1978) was used as 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-17β 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-17β 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-ke- toestradiol-17β, 8% for 16-epiestriol, and less than 0.5% for estriol, estradiol-17α, testosterone, 11- ketotestosterone, and 17α-hydroxy,20β-dihydroprogesterone. The estrone antibody was used at a final dilution of 1:4,800, and its degree of cross-reactivity was less than 0.8% for estradiol-17β, estradiol, testosterone,
TABLE 1
THE INTRAASSAY VARIABILITY OF THE RADIOIMMUNOASSAYS

| Hormone  | Level (ng/mL) | Mean Range of | Number of | Number of |
|----------|---------------|---------------|-----------|-----------|
| GtH      |               | CV (%)        | CV (%)    | samples/assay | assays |
| 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 17β | 6.3–12.4 | 6            | 3          |
| 1–2      | 9.6           | 2.6–8.7       | 3         | 3          |
| 3–4      | 5.3           | 8.8–9.4       | 5         | 2          |
| 20–22    | 9.1           | 4.3–6.7       | 6–10      | 2          |
| 33–35    | 5.5           | 9.6–11.4      | 5         | 2          |

11-ketotestosterone, and 17α-hydroxy,20β-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 a χ² 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 ≤ 2 × 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.

(I) Irregular profiles. The hormonal concentrations composing each of the profiles were divided into two subgroups. The first one included the low concentrations, 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$_2$17β (broken lines) in female trout at early ovarian recrudescence (March). Females were bled every 4 hr over a period of 24 hr. Individual GtH profiles with significant fluctuations in hormone levels, and the mean of profiles (n = 4 fish) with stable GtH levels, are presented. The number of each female is indicated. The statistical significance of the hormonal fluctuations is indicated by the following symbols: for GtH, ▲ = nonsignificant and ▼ = significant; for E$_2$17β, ▼ = nonsignificant and ▼ = 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-17β 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 constant. 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 hr 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
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 E$_2$17β 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
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
GONADOTROPIN AND ESTRADIOL-17β PROFILES IN TROUT

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-17β 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 E<sub>2</sub>17β profiles was different from that established for the GtH. E<sub>2</sub>17β levels did not change abruptly, but revealed continuous, relatively synchronized daily
Fig. 5. Individual profiles of plasma levels of GtH (continuous lines), E_{17\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 minimal 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 (x ± tSE) of plasma levels of GtH (lower curves—24 hr) and of E$_{17\beta}$ (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 $5/2$ 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 $E_2$ levels in the female trout during spring, other studies have shown a slow increase in plasma $E_2$ 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 $E_2$ 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 $E_2$ 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 E$_2$17$\beta$ output considerably (Zohar et al., 1982b; Zohar, Fostier, and Breton, unpublished results). *In vivo*, the GtH pulses might maintain constant E$_2$17$\beta$ 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 E$_2$17$\beta$ (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 E$_2$17$\beta$, up to levels ranging from 6 to 30 ng/ml. At advanced exogenous vitellogenesis, individual E$_2$17$\beta$ 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 E$_2$17$\beta$ profiles. This was reflected by an average E$_2$17$\beta$ profile showing regular significant daily fluctuations (Fig. 6). E$_2$17$\beta$ 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 E$_2$17$\beta$ 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 E$_2$17$\beta$ 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 exogen-
ous vitellogenesis, rather than a change in the hormone absolute levels, is respon-
sible for the marked E$_2$17B 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 be-
tween the individual GtH profiles of dif-
ferent females. This fact results in the de-
tection of a significant daily cycle in GtH levels when different groups of fish are sam-
ped every 4 hr. Our present data dem-
strate that in the female rainbow trout, the individual GtH profiles are not
synchronized since the short-term in-
creases in GtH levels occur at different
times in different females. Thus, the GtH
fluctuations can be detected only if indi-
vidual fish are sampled repeatedly. Aver-
ging the individual GtH profiles masked
the existence of GtH pulsatility, and re-
sulted 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 indi-
vidual 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 vitel-
logenesis, 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 ex-
ample might reflect the initiation of an in-
crease 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. Consi-
dered together, all of the GtH profiles char-
acterizing exogenous vitellogenesis indi-
cate a pulse duration of 1 to 3 hr. Bleeding
fish at even higher frequencies than that re-
ported here is necessary for a precise anal-
ysis of the dynamics of the GtH pulses.
Our present data do not demonstrate a
clear circadian rhythmicity in GtH levels at
advanced exogenous vitellogenesis. How-
ever, 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$_2$17B secretion patterns in the female trout
at advanced exogenous vitellogenesis
cannot be precisely determined on the
basis of the present in vivo study. They are
considered in more detail elsewhere
(Zohar, Fostier, and Breton, in prepara-
tion), taking into account the results of
both in vivo and in vitro studies. However,
the relationship between the GtH and
E$_2$17B secretion patterns differs from that
described in most mammalian species, in
which pulses are considered as basic
signals at both the pituitary and the gonadal
level. A pulsation of LH is immediately fol-
lowed by a pulsation of a corresponding go-
nadal steroid in the mouse (Desjardins,
1981), the rabbit (Moor and Younghai,
1975), the goat (Muduuli et al., 1979), the
dog (De Palatis et al., 1978), the ewe
(Baird, 1978), the ram (Lincoln, 1976), the
rhesus monkey (Steiner et al., 1980), and
man (Backstrom *et al*., 1982). In the female rainbow trout, however, the short-term GtH pulses are accompanied by regular E$_2$17β variations of longer duration. The same phenomenon was also observed in *vitro* (Zohar *et al*., 1982b and unpublished data). The greater abundance of GtH pulses during the day may indicate the importance of their frequency in the regulation of the progressive increase in E$_2$17β 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.

**ACKNOWLEDGMENTS**

We thank Aline Solari for her valuable help in the statistical analysis of our data, and Elisabeth Sambrony and Odile Marcuzzi for their technical assistance. This work was supported by a grant from the “Yad Hanadiv” Foundation to Y.Z.

**REFERENCES**

Backstrom, C. T., McNeilly, A. S., Leask, R. M., and Baird, D. T. (1982). Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the human menstrual cycle. *Clin. Endocrinol.* 17, 29–42.

Baird, D. T. (1978). Pulsatile secretion of LH and ovarian estriadiol during the follicular phase of the sheep estrous cycle. *Biol. Reprod.* 18, 359–364.

Barr, G. D., and Barraclough, C. A. (1978). Temporal changes in basal hypothalamic LH-RH correlated with plasma LH during the rat estrous cycle and following electrochemical stimulation of the medial preoptic area in pentobarbital-treated proestrous rats. *Brain Res.* 148, 413–423.

Barr, W. A. (1963). The endocrine control of the sexual cycle in the plaice, *Pleuronectes platessa*. II. The endocrine control of oogenesis. *Gen. Comp. Endocrinol.* 3, 205–215.

Billard, R., and Breton, B. (1978). Rhythms of reproduction in teleost fish. In “Rhythmic Activity of Fishes” (J. E. Thorpe, ed.), pp. 31–53. Academic Press, London/New York.

Billard, R., Breton, B., Fostier, A., and Welt, 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/North Holland, Amsterdam.

Bliss, C. I. (1967). “Statistics in Biology.” McGraw–Hill, New York.

Breton, B., and Billard, R. (1977). Effects of photoperiod and temperature on plasma gonadotropin and spermatogenesis in the rainbow trout (*Salmo gairdneri* R.). *Ann. Biol. Anim. Biochim. Biophys.* 17, 331–340.

Breton, B., and Derrien-Guimard, M. C. (1983). Actions de stimulations gonadotropes pulsatiles sur l’incorporation de vitellogenine *in vitro* par des follicules de Truites incubens dans un system de perfusion ouvert. *C. R. Acad. Sci. (Paris) Ser. III* 296, 857–860.

Breton, B., Billard, R., Jalabert, B., and Kann, G. (1972). Dosage radioimmunologique des gonadotropines plasmatiques chez *Carassius auratus* au cours du nycthemere et pendant l’ovulation. *Gen. Comp. Endocrinol.* 18, 463–468.

Breton, B., Fostier, A., Zohar, Y., Le Bail, P. Y., and Billard, R. (1983). Gonadotropine glycoproteique maturante et oestradiol-17β 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. Biochim. Biophys.* 18(4), 759–765.

Breton, B., Zohar, Y., and Billard, R. (1985). Photoperiod and gonadotropin control of the reproductive cycle in the female rainbow trout. In “Current Trends in Comparative Endocrinology” (B. Lofts and W. N. Holmes, eds.), pp. 1243–1246. Hong Kong Univ. Press, Hong Kong.

Brinkley, H. J. (1981). Endocrine signaling and female reproduction. *Biol. Reprod.* 24, 22–43.

Bromage, N. R., Whitehead, C., and Breton, B. (1982a). Relationships between serum levels of gonadotropin, oestradiol-17β and vitellogenin in the control of ovarian development in the rainbow trout. II. The effects of alterations in environ-
mental photoperiod. *Gen. Comp. Endocrinol.* 47, 366–376.

Bromage, N. R., Whitehead, C., Elliot, J., Breton, B., and Matty, A. J. (1982b). Investigations into the importance of day length on the photoperiodic control of reproduction in the female rainbow trout. In “Reproductive Physiology of Fish” (C. J. J. Richter and H. J. Th. Goos, eds.), pp. 233–236. Pudoc, Wageningen.

Bry, C., and Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*). II. Glucocorticoid levels, hematological data and resumption of feeding for five days after surgery. *Reprod. Nutr. Dev.* 20, 1825–1834.

Burzawa-Gerard, E. (1982). Existe-t-il plusieurs gonadotropines (GtH) chez les poissons? Données biochimiques et vitellogenèse exogène. In “Reproductive Physiology of Fish” (C. J. J. Richter and H. J. Th. Goos, eds.), pp. 19–22. Pudoc, Wageningen.

Crim, L. W., and Idler, D. R. (1978). Plasma gonadotropin, estradiol, and vitellogenin and gonad phosvitin levels in relation to the seasonal reproductive cycles of female brown trout. *Ann. Biol. Anim. Biochim. Biophys.* 18, 1001–1005.

De Palatis, L., Moore, J., and Falvo, R. E. (1978). Plasma concentrations of testosterone and LH in the male dog. *J. Reprod. Fertil.* 52, 201–207.

Desjardins, C. (1981). Endocrine signaling and male reproduction. *Biol. Reprod.* 24, 1–21.

Dixon, W. J. (1953). Processing data for outliers. *Biometrics* 9, 74–89.

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.

Gillet, C., and Billard, R. (1981). Effets de la température, de la photoperiode et des niveaux alimentaires sur la gonadotropine plasmatique et hypophysaire et la gametogenèse du poisson rouge. *Cah. Lab. Montereau.* 11, 41–48.

Gillet, C., Billard, R., and Breton, B. (1981). La reproduction du poisson rouge (*Carassius auratus*) eleve a 30°C: Effet de la photoperiode, de l'alimentation et de l'oxygénation. *Cah. Lab. Montereau.* 11, 49–56.

Grubbs, F. E. (1969). Procedures for detecting out-lying observations in samples. *Technometrics* 11, 1–21.

Hanyu, I., and Tamura, T. (1978). Electroretinograms and spectral sensitivity in the ayu, a salmonid fish. *Bull. Japan. Soc. Sci. Fish.* 44, 401–406.

Hoar, W. S., Wiebe, J., and Hui Wai, E. (1967). Inhibition of the pituitary gonadotropic activity of fishes by a dithiocarbamoylhydrazine derivative. *Gen. Comp. Endocrinol.* 8, 101–109.

Hontela, A., and Peter, R. E. (1978). Daily cycles in serum gonadotropin levels in the goldfish: Effects of photoperiod, temperature, and sexual condition. *Canad. J. Zool.* 56, 2430–2442.

Hontela, A., and Peter, R. E. (1980a). Effects of pinealectomy, blinding, and sexual condition on serum gonadotropin levels in the goldfish. *Gen. Comp. Endocrinol.* 40, 168–179.

Hontela, A., and Peter, R. E. (1980b). Synchronization of daily gonadotropin cycles with temperature, feeding, and light in the goldfish. *Amer. Zool.* 20, 728.

Hyder, M. (1972). Endocrine regulation of reproduction in *Tilapia*. *Gen. Comp. Endocrinol.* 3, (suppl.), 729–740.

Idler, D. R. (1982). Some perspectives on fish gonadotropins. In “Reproductive Physiology of Fish” (C. J. J. Richter and H. J. Th. Goos, eds.), pp. 4–13. Pudoc, Wageningen.

Idler, D. R., and Ng, T. B. (1983). Teleost gonadotropins: isolation, biochemistry and function. In “Fish Physiology” (W. S. Hoar, D. J. Randall, and E. M. Donaldson, eds.), pp. 187–221. Academic Press, New York.

Khoo, K. H. (1979). The histochemistry and endocrine control of vitellogenesis in goldfish ovaries. *Canad. J. Zool.* 57, 617–626.

Kobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Rec. Prog. Horm. Res.* 36, 53–88.

Lamba, V. J., Goswami, S. V., and Sundararaj, B. I. (1983). Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17β, estrone, and testosterone) correlated with the an-
nual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol. 50, 205–225.

Lambert, J. G. D., Bosman, G. I. C. G. M., Van den Hurk, R., and Van Qordt, P. G. W. J. (1978). Annual cycle of plasma oestradiol-17β in the female trout (*Salmo gairdneri*). Ann. Biol. Anim. Biochim. Biophys. 18, 923–927.

Lincoln, G. A. (1976). Seasonal variation in the episodic secretion of luteinizing hormone and testosterone in the ram. J. Endocrinol. 69, 213–226.

Lincoln, G. A., and Short, R. V. (1980). Seasonal breeding: Nature’s contraceptive. Recent Prog. Horm. Res. 36, 1–52.

Mackay, N. J. (1973). The effects of gonadotropin preparations and steroid hormones on the ovaries of intact and gonadotropin deprived Gudgeons (*Hypseleotris galii*). Gen. Comp. Endocrinol. 21, 278–286.

Moor, B. C., and Younglai, E. V. (1975). Variations in peripheral levels of LH and testosterone in adult male rabbits. J. Reprod. Fertil. 42, 259–266.

Muduuli, D. S., Sanford, L. M., Palmer, W. M., and Howland, B. E. (1979). Secretory patterns and circadian and seasonal changes in luteinizing hormone, follicle stimulating hormone, prolactin and testosterone in the male pygmy goat. J. Anim. Sci. 49, 543–553.

Pui, J., Waug, R., Slen, R., Xu, G., Pau, X., and Xu, W. (1980). On the mode of pituitary gonadotropin releasing in carps. J. Fish China 4, 121–127.

Peter, R. E. (1981). Gonadotropin secretion during reproductive cycles in teleosts: Influences of environmental factors. Gen. Comp. Endocrinol. 45, 294–305.

Scott, A. P., and Sumpter, J. P. (1983). A comparison of the female reproductive cycles of autumn-spawning and winter spawning strains of rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol. 52, 79–85.

Scott, A. P., Bye, F. J., and Baynes, S. M. (1980). Seasonal variations in sex steroids of female rainbow trout (*Salmo gairdneri*). J. Fish. Biol. 17, 587–592.

Steiner, R. A., Peterson, A. P., Yu, J. Y. L., Conner, H., Gilbert, M., Terpenning, B., and Bremmer, W. (1980). Ultradian luteinizing hormone and testosterone rhythms in the adult male monkey, (*Macaca fascicularis*). Endocrinology 107, 1489–1493.

Sumpter, J. P., Scott, A. P., Baynes, S. M., and Witthames, P. R. (1984). Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 43, 235–242.

Sundararaj, B. I., Anand, T. C., and Donaldson, F. M. (1972). Effects of partially purified salmon pituitary gonadotropin on ovarian maintenance, ovulation, and vitellogenesis in the hypophysectomized catfish (*Heteropneustes fossilis*) (Bloch). Gen. Comp. Endocrinol. 18, 102–114.

Upadhyay, S. N., Breton, B., and Billard, R. (1978). Ultrastructural studies on experimentally induced vitellogenesis in juvenile rainbow trout (*Salmo gairdneri* R.). Ann. Biol. Anim. Biochim. Biophys. 18, 1019–1025.

Van Bohemen, Ch.G., and Lambert, J. G. D. (1981). Estrogen synthesis in relation to estrone, estradiol, and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 45, 105–114.

Van den Hurk, R., and Peute, J. (1979). Cyclic changes in the ovary of the rainbow trout, (*Salmo gairdneri*), with special reference to sites of steroidogenesis. Cell. Tissue Res. 199, 289–306.

Vivien, J. H. (1939). Role de l'hypophyse dans le determinisme du cycle genital femelle d'un teleosceen, (*Gobius paganellus*). C. R. Hebd. Seances Acad. Sci. 207, 948–949.

Vodicnik, M. J., Kral, R. E., and De Vlaming, V. L. (1978). The effects of pinealectomy on pituitary and plasma gonadotropin levels in *Carassius auratus* exposed to various photoperiod-temperature regimes. J. Fish. Biol. 12, 187–196.

Whitehead, C., Bromage, N. R., and Breton, B. (1983). Changes in serum levels of gonadotropin, oestradiol 17β and vitellogenin during the first and subsequent reproductive cycle of female rainbow trout. *Aquaculture* 4, 317–326.

Whitehead, C., Bromage, N. R., and Forster, J. R. M. (1978a). Seasonal changes in reproductive function of the rainbow trout (*Salmo gairdneri*). J. Fish. Biol. 12, 601–608.

Whitehead, C., Bromage, N. R., Forster, J. R. M., and Matty, A. J. (1978b). The effects of alterations in photoperiod on ovarian development and spawning time in the rainbow trout (*Salmo gairdneri*). Ann. Biol. Anim. Biochim. Biophys. 18, 1035–1043.

Yamazaki, F. (1965). Endocrinological studies on the reproduction of the female goldfish, *Carassius auratus* L., with special reference to the function of the pituitary gland. Mem. Fac. Fish. Hokkaido Univ. 13, 1–64.

Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*). I. Its validity in the study of blood gonadotropin patterns. *Reprod. Nutr. Dev.* 20, 1811–1823.
Zohar, Y., Breton, B., and Billard, R. (1982a). Short-term profiles of plasma gonadotropin levels in the female rainbow trout throughout the reproductive cycle. *Gen. Comp. Endocrinol.* 46, 369.

Zohar, Y., Breton, B., and Fostier, A. (1982b). 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.

Zohar, Y., Breton, B., and Fostier, A. (1986). Short-term profiles of plasma gonadotropin and 17α-hydroxy-20β-dihydroprogesterone levels in the female rainbow trout at the periovulatory period. *Gen. Comp. Endocrinol.* 64, 189–198.