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Short-Term Profiles of Plasma Gonadotropin and 17α -Hydroxy, 20β -dihydroprogesterone Levels in the Female Rainbow Trout at the Periovulatory Period

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Individual free-swimming female rainbow trout in which oocytes underwent final stages of germinal vesicle migration, maturation, or ovulation were bled via a dorsal-aortic catheter at frequencies of once every 1, 3, or 4 hr over periods of 9 to 36 hr. Gonadotropin (GtH) and 17α -hydroxy, 20β -dihydroprogesterone ($17\alpha, 20\beta$ -OHP) levels were measured in the plasma samples. GtH levels were elevated and showed wide and progressive daily variations. A high degree of synchronization appeared among the GtH profiles of individual fish. Two distinct daily GtH surges were observed, one at early photophase and the other during the mid-scotophase. The onset of the GtH increases was closely related to the beginning of the photophase and the scotophase, respectively. In females undergoing oocyte maturation or in ovulated females, $17\alpha, 20\beta$ -OHP levels were increasing or high, showing progressive daily fluctuations that were either synchronized with the GtH changes or somewhat phase-shifted in relation to them. These data are discussed in relation to the seasonal changes in the short-term profiles of reproductive hormones in the trout. © 1986 Academic Press, Inc.

In teleosts, oocyte maturation and ovulation are accompanied by a significant increase in plasma gonadotropin (GtH) levels (salmonids: Crim *et al.*, 1975; Fostier *et al.*, 1978, 1981a; Fostier and Jalabert, 1986; Breton *et al.*, 1983; Scott *et al.*, 1983; Scott and Sumpter, 1983; Bromage *et al.*, 1982; Whitehead *et al.*, 1983; Young *et al.*, 1983; cyprinids: Breton *et al.*, 1972; Stacey *et al.*, 1979a; Breton *et al.*, 1980; cypriniformes: Stacey *et al.*, 1984). In various salmonid species, ovulation is preceded by a continuous decrease, first in estradiol- 17β ($E_2 17\beta$) and then in testosterone levels, and by a drastic increase in 17α -hydroxy, 20β -dihydroprogesterone ($17\alpha, 20\beta$ -OHP), the most probable maturation-inducing steroid in salmonidae (rainbow trout: Fostier *et al.*, 1978, 1981a; Fostier and Jalabert, 1982,

1986; Scott and Baynes, 1982; Scott *et al.*, 1982, 1983; amago salmon: Young *et al.*, 1983; Atlantic salmon and coho salmon: Wright and Hunt, 1982; masu salmon: Yamauchi *et al.*, 1984). The same general pattern also occurs in the white sucker (cypriniformes: Scott *et al.*, 1984). In the goldfish (Cyprinidae), $E_2 17\beta$ levels stay high during maturation and ovulation while there might be a short and relatively shallow peak of $17\alpha, 20\beta$ -OHP preceding oocyte maturation (Stacey *et al.*, 1983; Kagawa *et al.*, 1983; Peter *et al.*, 1984).

Although a great deal of attention has been drawn to hormonal changes at the periovulatory period in teleosts, only a few studies have explored short-term (circadian and ultradian) fluctuations of the hormones. Pan *et al.* (1980) recorded daily variations in plasma GtH levels in three carp species at their spawning time. In the catfish, Lamba *et al.* (1983) demonstrated daily changes in plasma levels of cortisol, testosterone, $E_2 17\beta$, and estrone during the

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"spawning period." In the goldfish, Hon-tela and Peter (1978) found more pronounced daily variations in plasma GtH levels in "mature" fish, as compared to fish at earlier stages of ovarian development. In the same species, Stacey *et al.* (1979a) demonstrated a preovulatory surge of GtH, the duration of which was approximately 24 hr. Our previous paper (Zohar *et al.*, 1986) demonstrated, in the female rainbow trout, that important changes occur in the short-term profiles of blood GtH and E_2 17 β levels from early ovarian recrudescence and throughout vitellogenesis. The present paper describes the short-term GtH and 17 α ,20 β -OHP profiles in individual rainbow trout females at the periovulatory period.

MATERIALS AND METHODS

(a) Experimental Conditions

The experiments were carried out from December through January each year over a 3-year period. Female trout aged 3 to 4 years and weighing 1.5 to 3 kg were used. All of them had reached sexual maturity at least once before. Animal stocking conditions and dorsal aorta catheterization procedures have been described in the previous paper (Zohar *et al.*, 1986). After catheterization, fish were allowed to recover for at least 3 days before they were bled. Only fish which had 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 every 1, 3, or 4 hr over periods of 9 to 36 hr. Blood sampling was done according to Zohar *et al.* (1986). Blood samples were centrifuged (15 min at 5000 rpm and at 4°), and the plasma was stored at -30° until further analysis.

(b) Determination of Ovarian Developmental Stages

At the beginning and the end of each experiment, a few oocytes were extracted from the ovaries by abdominal pressure (stripping). The stage of oocyte development was determined under a binocular microscope according to Jalabert *et al.* (1976).

Maturation. The meiotic maturation is preceded by the migration of the germinal vesicle (GV) toward the animal pole of the oocyte (GVM). The GV becomes more visible as it approaches the oocyte periphery. The visible phenomena of the maturation start with the progressive fusion of the vitellin spheres and the

peripheral migration of the lipid globules (cytoplasmatic maturation), and terminate with germinal vesicle breakdown (GVBD).

Ovulation. The oocytes, expelled from their follicular envelopes, are found free in the central cavity.

(c) Hormonal Measurements

The procedures of the radioimmunoassay (RIA) for the GtH as well as the RIA precision have been given in detail by Zohar *et al.* (1986).

The RIA of 17 α ,20 β -OHP was carried out according to Fostier *et al.* (1981b), using the same antibody. The intraassay variability was 5.9% for hormone levels of 9–10 ng/ml and 7.4% for hormone levels between 65 and 80 ng/ml.

(d) Analytical Methods

The mathematical and statistical analysis of our data has been described in detail in the previous paper (Zohar *et al.*, 1986). In the present study, we dealt only with regular fluctuating hormonal profiles.

RESULTS

In most females sampled, oocytes either were in the final stages of germinal vesicle migration or were undergoing maturation. In some cases, ovulation occurred within the 72-hr recovery period between catheterization and successive bleeding (OV in figures).

The overall mean GtH level in the periovulatory period (12.9 ± 2.8 ng/ml for all sampling times of all females) was significantly higher (Student *t* test, $P < 0.01$) than the mean basal GtH level recorded at early ovarian recrudescence (2.3 ± 0.6 ng/ml), or at early (1.8 ± 0.4 ng/ml) or advanced (2.4 ± 0.6 ng/ml) exogenous vitellogenesis (Zohar *et al.*, 1986). In 21 of 25 fish sampled during the periovulatory period, GtH profiles fluctuated significantly. The nature of the changes in the GtH levels during this period was different from that found in earlier ovarian developmental stages (Zohar *et al.*, 1986). Episodic changes in GtH levels no longer occurred, and those levels instead showed wide, progressive, and continuous daily fluctuations. A consistent daily cycle was observed in all sampled fish, independent of the sampling frequency (Figs.

1–5). There was a synchronization among individual profiles.

Figure 1 shows GtH profiles in 10 female trout sampled every hour over a period of 9 hr. Significant fluctuations in GtH levels were observed in eight of them. In all sampled females, GtH levels decreased progressively from early photophase (lights were on at 0720 hr) to minimal values found at the middle and the second part of the photophase, i.e., between 1100 and 1600 hr, according to the females. Gonadotropin levels increased again just before and at the beginning of the scotophase (see also Fig.

2). Bleeding fish every hour over a period of 12 hr (Fig. 2) showed that the above-described decrease in GtH levels during the photophase is, in fact, the descending part of a large GtH surge associated with the onset of the photophase (highly significant in four of the females and at the limit of significance in the fifth). The increase in GtH levels in all fish started shortly before or at the onset of light, the maximal levels being achieved within 2 to 3 hr. As in Fig. 1, after the GtH decline, the start of a subsequent upsurge was observed (Fig. 2).

The high degree of synchronization

FIG. 1. Individual profiles of plasma GtH levels in female trout at the periovulatory period (December–January). Females were bled every hour over a period of 9 hr. The number of each female is indicated. The symbol OV refers to females in which ovulation occurred within the 72-hr recovery period between catheterization and successive bleeding. The symbol ▲ means that the GtH profile fluctuated significantly, whereas the symbol △ means that GtH fluctuations were not significant.

FIG. 2. Individual profiles of plasma GtH levels in female trout at the periovulatory period (December). Females were bled every hour over a period of 12 hr. For additional details, see the legend of Fig. 1.

among the individual GtH profiles of the fish sampled hourly (Figs. 1 and 2) can be seen in their averages as presented in Fig. 3. These average profiles show, in a significant manner ($P < 0.01$), the GtH surge associated with "dawn" (Fig. 3b), the gradual GtH decrease starting from early morning and continuing through the major

part of the photophase, and the increase which follows it toward and during early scotophase (Figs. 3a, b).

Figure 4 shows the average profile of GtH changes in five female trout sampled every 4 hr over a period of 24 hr. This profile confirms the above results and shows a significant circadian cycle in plasma GtH

FIG. 3. Mean profiles ($\bar{x} \pm tSE$) of plasma GtH levels in female trout at the periovulatory period (December–January). The mean profiles were established from the individual GtH profiles shown in Fig. 1 (for (a)) and Fig. 2 (for (b)). Fish were bled every hour over a period of 9 (a) or 12 (b) hr. **Significant difference at $p < 0.01$.

FIG. 4. Mean profile of plasma GtH levels in female trout at the periovulatory period (December). This profile was established from individual GtH profiles (not shown) of females which were bled every 4 hr over a period of 24 hr. **Significant difference at $P < 0.01$.

levels. Gonadotropin levels increased significantly from early scotophase and remained elevated during the dark period. The decrease in GtH levels in the second part of the scotophase, which precedes the "dawn" GtH surge, was not significant in this case, probably due to the relatively low frequency of bleeding. Fish sampled every 3 hr over 36 hr also showed the same general daily pattern of GtH levels (two profiles of such fish, Nos. 206 and 207, are shown in Fig. 5). In this case, the GtH decrease at late scotophase was more evident.

Figure 5 shows the individual profiles of plasma GtH and $17\alpha,20\beta$ -OHP levels in seven females. Some of them were bled every hour over a period of 9 or 12 hr, and others every 3 hr over 36 hr. Female No. 199 was bled every 4 hr over a period of 24 hr. Three of the females underwent oocyte maturation (Mat) at the end of the sampling period, whereas in the others ovulation (OV) was completed. All females showed daily fluctuations in GtH levels, similar to those described above. Plasma levels of $17\alpha,20\beta$ -OHP also fluctuated significantly in all the fish (Fig. 5). These fluctuations were continuous and progressive. In one female (No. 206) showing early signs of oocyte maturation (cytoplasmic maturation,

the GV being still intact), $17\alpha,20\beta$ -OHP levels increased sharply during the sampling period, from 20 to 220 ng/ml. In two other females (Nos. 194 and 207) showing more advanced stages of oocyte maturation (GVBD), circulating $17\alpha,20\beta$ -OHP levels were high (250–500 ng/ml) and had a pattern closely parallel to that of GtH. In the four females which eventually ovulated, the levels of $17\alpha,20\beta$ -OHP were either as high as or lower than those recorded in the fish which underwent oocyte maturation. In female No. 184, the plasma levels of $17\alpha,20\beta$ -OHP closely paralleled those of GtH, whereas in the other fish the $17\alpha,20\beta$ -OHP profiles were phase-shifted in relation to GtH.

DISCUSSION

The present study confirms the well-documented increase in plasma GtH levels which occurs in the female of different salmonid species at the periovulatory period. It shows, in addition, that from final stages of germinal vesicle migration there is a drastic change in the short-term profiles of plasma GtH levels. The intermittent, episodic pattern of GtH release, characterizing the advanced stages of vitellogenesis (Zohar *et al.*, 1986), was no longer observed, and GtH levels instead showed continuous and gradual daily changes. Also, a high degree of synchronization was apparent among the individual GtH profiles. Plasma GtH levels showed two maxima, one in the early photophase and the other at the middle of the scotophase, the initiation of the increase in GtH levels coinciding closely with "dawn" and "dusk," respectively. This fact suggests a regulatory role of the photoperiod in the daily GtH fluctuations. In the goldfish, Stacey *et al.* (1979a, b) have demonstrated a preovulatory GtH surge which is synchronized with the photoperiod. Maximal GtH levels were reached during the

FIG. 5. Individual profiles of plasma levels of GtH (continuous lines) and $17\alpha,20\beta$ -OHP (broken lines) in female trout at the periovulatory period (December–January). Females were bled either every hour over a period of 9 or 12 hr, every 3 hr over 36 hr, or every 4 hr over 24 hr. For additional details, see the legend of Fig. 1.

night, and ovulation took place toward the end of the scotophase.

A circadian rhythmicity of ovulation or spawning time has been observed in several species of teleosts: *Oryzias latipes* (Robinson and Rugh, 1943; Egami, 1954), *Rivulus marmoratus* (Harrington, 1963), *Trichopsis vittatus* and *Trichopsis pumilus* (Marshall, 1967), *Pagrus ehrenbergii* (Stepkina, 1973), *Pomatomus saltatrix* (Norcross *et al.*, 1974), *Menidia audens* (Hubbs, 1976), and *Sparus aurata* (Zohar and Gordin, 1979). In most of these cases, as well as in the goldfish (Stacey *et al.*, 1979b), the time of ovulation or spawning seems to be dependent on photoperiod.

Thus, a daily rhythmicity of ovulation and spawning, entrained by the photoperiod, seems to be a fairly common phenomenon in fish. This rhythmicity might reflect circadian changes in the gonadotropin function, as was suggested for *Oryzias latipes* (Iwamatsu, 1978), and observed in the goldfish (Stacey *et al.*, 1979a) and in the rainbow trout (the present study). In the rainbow trout, we still have to establish the temporal relationships between the daily variations in circulating GtH and the processes of oocyte maturation and ovulation, as well as the dependence of both phenomena on photoperiod. Yet, when analyzing the two different models of the gold-

fish and the trout, a certain analogy might be established between their GtH profiles throughout oocyte maturation and ovulation. In goldfish, which spawn in warm water, maturation and ovulation are rapid (24 hr or less; Jalabert *et al.*, 1973; Sokolowska *et al.*, 1984) and are preceded by one daily GtH surge (Stacey *et al.*, 1979a). In trout, which spawn in cold water, maturation and ovulation take much longer (3 to 6 days; Jalabert, 1978; Bry, 1981). In the latter case, several successive daily GtH surges might constitute the gradual GtH increase accompanying oocyte maturation and ovulation. In both species, however, the gonadotropic signal related to oocyte maturation and ovulation might be composed of daily elevations of GtH, probably synchronized with the photoperiod.

As long as the first signs of oocyte maturation (cytoplasmatic maturation) were not visible, no 17 α ,20 β -OHP was detected in the circulation, although GtH levels were already elevated prior to this stage (at germinal vesicle migration). The levels of this steroid increased rapidly at the stage of cytoplasmatic maturation and before germinal vesicle breakdown. These observations are in agreement with data showing that in the female rainbow trout, the GtH stimulates, *in vivo* (Scott *et al.*, 1982; Wright and Hunt, 1982; Fostier and Jalabert, 1986) and *in vitro* (Fostier *et al.*, 1981b; Fostier and Jalabert, 1986; Zohar *et al.*, 1982), a drastic increase in the secretion of 17 α ,20 β -OHP, which is followed by oocyte maturation. Once the levels of 17 α ,20 β -OHP reached high values, when oocytes were more advanced in the process of maturation or were ovulated, they presented regular circadian fluctuations. These fluctuations either were closely synchronized with the GtH changes or were phase-shifted in relation to them. Such temporal relationships suggest a role of the circadian GtH variations, which are established first, in the regulation of the daily fluctuations of 17 α ,20 β -OHP. The shift between the GtH

and the 17 α ,20 β -OHP profiles which appears after ovulation might reflect the appearance of a negative feedback exerted by 17 α ,20 β -OHP on GtH secretion (Jalabert *et al.*, 1980).

In the rainbow trout, the increase in GtH levels prior to oocyte maturation is preceded by a progressive decline in E₂17 β (see introduction). Such a change might indicate that at the periovulatory period, the continuous rather than episodic elevated levels of GtH are the reflection, at the pituitary level, of the removal of a negative E₂17 β feedback on GtH secretion (Bommelaer *et al.*, 1981). On the other hand, the drop in E₂17 β and the following elevation in 17 α ,20 β -OHP levels might be the reflection of a change in the ovarian steroidogenic responsiveness to the GtH signals, which occurs at this time. In fact, in a separate study, we have shown that both short GtH pulses and constant elevated GtH levels stimulated E₂17 β secretion from vitellogenic follicles incubated *in vitro*. However, continuous elevated levels of GtH reduced E₂17 β secretion from follicles undergoing maturation, yet at the same time were necessary to induce 17 α ,20 β -OHP release (Zohar, 1982; Zohar *et al.*, 1982). The circadian rhythm in GtH levels at the periovulatory period might be related to a parallel rhythm in the sensitivity of the ovary to GtH. A daily cycle in the responsiveness of the ovary (indicated by its growth rate) to gonadotropin treatment has been observed in *Notemigunus crysoleucas* (de Vlaming and Vodcnik, 1977) and in the goldfish (Peter *et al.*, 1982). The last study showed that under some environmental conditions, there was a synchronization between the temporal changes in ovarian sensitivity to GtH and the daily cycle of GtH levels in the plasma. Moreover, it has been shown that the gonadal response to different preparations of gonadotropin, as estimated by measuring the production of steroids in *Fundulus grandis* (MacGregor *et al.*, 1985) and the production of c-AMP in

the *Mugil cephalus* (Kuo and Watanabe, 1978), fluctuated during the day.

In conclusion, this and our previous study (Zohar *et al.*, 1986) have demonstrated that important modifications occur in the short-term profiles of plasma GtH and gonadal steroid levels in the female rainbow trout throughout ovarian development. At the stage of early ovarian recrudescence, short-term, high-amplitude GtH pulses occur in association with low and stable $E_217\beta$ levels. At the stage of early exogenous vitellogenesis, GtH and $E_217\beta$ levels are both low and constant. Throughout vitellogenesis, GtH pulsatility again appears accompanied by a marked increase in $E_217\beta$ levels. Thus, at advanced stages of exogenous vitellogenesis, short-term episodic GtH pulses of moderate amplitude are accompanied by wide and progressive daily variations of $E_217\beta$ levels (Zohar *et al.*, 1986). At the periovulatory period, GtH levels become continuously elevated and fluctuate in a regular circadian pattern, probably entrained by the photoperiod. Plasma levels of $17\alpha,20\beta$ -OHP rise rapidly just before the onset of oocyte maturation and then show wide daily fluctuations related to those of the GtH.

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