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Christian Bry

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Plasma Cortisol Levels of Female Rainbow Trout (*Salmo gairdneri*) at the End of the Reproductive Cycle: Relationship with Oocyte Stages

C. BRY

Laboratoire de Physiologie des Poissons, INRA 78350-Jouy-en-Josas, France

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The concentrations of plasma cortisol were determined in two populations of female rainbow trout at the end of their reproductive cycles. In females ending vitellogenesis or having oocytes with germinal vesicles in peripheral positions, mean cortisol levels were low (about 8 to 10 ng/ml). Cortisol concentrations remained low during oocyte maturation (about 8 and 11 ng/ml). In contrast, a 3-fold cortisol increase was observed in females sampled in the postovulation period (about 26 and 31 ng/ml). These results are discussed in relation to other studies on endocrinological changes during the spawning season with emphasis on the possible role of cortisol in reproduction in female teleosts. © 1985 Academic Press, Inc.

Seasonal elevations of plasma cortisol or corticosteroid levels during the spawning period have been reported in a number of female teleost fishes: migratory Salmonidae (e.g., Hane and Robertson, 1959; Idler *et al.*, 1959; Schmidt and Idler, 1962; Fagerlund and Donaldson, 1970; Heyl and Carpenter, 1972); a landlocked salmonid fish, *Coregonus lavaretus* (Fuller *et al.*, 1976); nonmigratory rainbow trout, *Salmo gairdneri* (Robertson *et al.*, 1961) and hatchery-reared brown trout, *Salmo trutta* (Pickering and Christie, 1981); the plaice, *Pleuronectes platessa* (Wingfield and Grimm, 1977); and the goldfish, *Carassius auratus* (Cook *et al.*, 1980). Despite such apparently converging evidence, no clear pattern has yet emerged with regard to the stages of the maturation-ovulation-oviposition sequence effectively associated with an increase in the corticosteroid hormone. In the goldfish, for example, a surge in serum cortisol was found a few hours prior to ovulation and cortisol levels declined at the time of ovulation and thereafter (Cook *et al.*, 1980). In contrast, brown trout sampled in the postovulation period had elevated cortisol concentrations (Pickering and

Christie, 1981) and in *C. lavaretus* the highest cortisol levels during the spawning season were recorded in ovulated females caught on the spawning grounds (Fuller *et al.*, 1976). On the other hand, *in vitro* experiments with rainbow trout follicles suggest that some corticosteroids might play an indirect role in oocyte maturation by lowering the threshold of oocyte sensitivity to the maturation-inducing factor (Jalabert, 1975, 1976).

The present investigation was accordingly undertaken in an attempt to establish with precision the temporal links between circulating cortisol levels and the final stages of ovarian evolution in the rainbow trout. Based on macroscopic modifications of the oocytes, several preovulation stages have been defined in the trout (Jalabert *et al.*, 1976) and related with the levels of reproductive hormones (e.g., Fostier *et al.*, 1978). Another advantage of the trout model when compared with fish reproducing in warmer waters is the relatively long duration of oocyte maturation (Jalabert, 1978; Bry, 1981), a feature which may allow a more accurate description of temporal relationships.

In addition, the choice of a hatchery-reared species offers the possibility of reducing the time interval between capture and sampling to a minimum compatible with the measurement of normal (i.e., not influenced by handling) cortisol levels.

MATERIALS AND METHODS

Experimental conditions. Two similar experiments were carried out in October–November 1979 and November–December 1982. Forty-eight and sixty female rainbow trout (*S. gairdneri* Richardson; weight range 900–1700 g) were used, respectively. These females were in their third year and at the end of their second reproductive cycle. The 1979 trout originated from a commercial trout hatchery, whereas the 1982 fish belonged to a population raised by our geneticist colleagues and characterized by a high level of overall genetic variability. For 5–7 weeks before the start of the sampling period the fish were acclimated to outdoor circular tanks (600 liters) spaced away from each other, supplied with running river water (flow rate 40 liters/min, trout stocking density 30 kg/m³, or 0.5 kg/liter per min) and fed once a day between 0900 and 0930 hr with dry pellets (1% body wt/day). As no fungal infection was observed in the experimental fish, no sanitary treatment was performed. Water temperature range was 8.5–10.5° during the 1979 and 1982 sampling periods.

Sampling protocol. On sampling days the fish were fed as usual and care was taken to avoid activity in the vicinity of the experimental tanks. Between 1230 and 1300 hr one fish was quickly removed from each tank and immobilized. A blood sample was withdrawn with a heparinized syringe from the dorsal aorta within 30–50 sec of introducing the hand net into the tank. This time interval is well within the few minutes lag that takes place before cortisol levels begin to rise in rainbow trout captured and left struggling out of water (Bry, unpublished data). On some occasions, two fish were taken together from each tank, but the aforementioned timing was respected. There was an interval of 1–2 days in 1979 and 3–10 days in 1982 between two successive sampling dates. Once sampled for blood, each female was anesthetized in a 2-phenoxyethanol solution (0.3 ml/liter) and several oocytes were collected by abdominal stripping. The stage of oocyte development was determined under a binocular microscope according to the following classification (adapted from Jalabert *et al.*, 1976):

- 1: Immature oocyte with migrating germinal vesicle (G.V.). Oocytes having a G.V. in subperipheral position were included in this category.
- 2: Immature oocyte with peripheral G.V.
- 3: Maturing oocyte. In most cases these oocytes

were in a relatively advanced state of maturation, i.e., at or after G.V. breakdown time.

- 4: Matured and ovulated oocyte.

Following blood and oocyte sampling, the fish were not returned to the experimental tanks. In 1982 the nonovulated females were put aside after sampling and checked twice a week in order to estimate the time lag between sampling and ovulation.

Ovulated females were first detected on October 30, 1979 (second sampling session) and on December 6, 1982 (third sampling session). In 1982, 2 weeks elapsed between the first detection of ovulated females and the date of the last sampling. Half of the population was estimated as ovulated by November 7, 1979, and by December 14, 1982.

Cortisol assay. Each plasma sample (0.2 ml) was assayed in triplicate for cortisol, using a competitive protein binding assay systematically preceded by a chromatographic step (Sephadex LH-20) as previously described (Bry and Zohar, 1980). This procedure removed potentially interfering steroids such as progesterone, 17 α -hydroxyprogesterone, 11-deoxycortisol, and cortisone. The assay showed a negligible cross-reactivity with 17 α -hydroxy-20 β -dihydroprogesterone, a progestagen which is elevated at the time of oocyte maturation and ovulation in the rainbow trout (Fostier *et al.*, 1981; Scott *et al.*, 1983). In our working conditions, the detection limit was close to 1 ng/ml. The coefficients of variation were below 6% (within assay) and below 13% (between assays) for plasma cortisol concentrations between 5 and 25 ng/ml.

Statistical analyses. The cortisol data were logarithmically transformed in order to meet variance homogeneity and subjected to a two-way analysis of variance with oocyte stage and year of experiment as factors. When the *F* value for a given parameter was significant, the data were further analysed using Kramer's (1956) modification of Newman–Keuls multiple range test.

RESULTS

Cortisol levels exhibited significant variations with respect to ovarian stages at the time of sampling (Fig. 1, $P < 0.005$) and the patterns of cortisol changes observed in the 1979 and 1982 rainbow trout populations were not significantly different ($P > 0.05$). In females having oocytes with a migrating or peripheral G.V. (stages 1 and 2) as well as in maturing females (stage 3), the mean concentrations of circulating cortisol were low and ranged from 7.7 to 10.5 ng/ml, with no significant difference according to oocyte stage ($P > 0.05$). In contrast, a

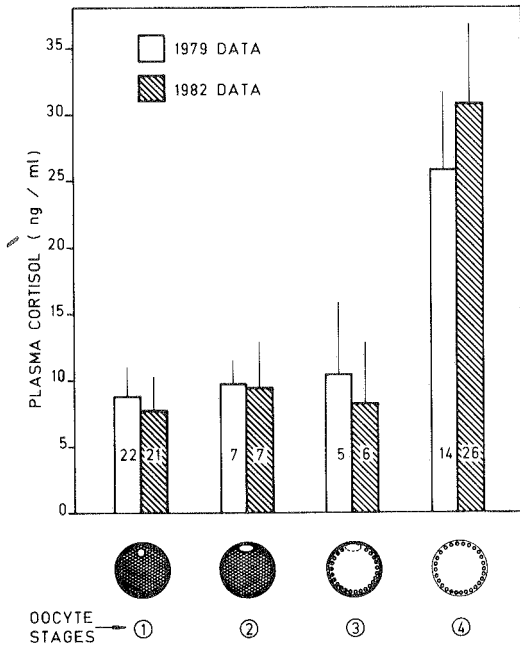


FIG. 1. Plasma cortisol concentrations (arithmetic mean \pm SEM) of female rainbow trout at the end of the reproductive cycle, in relation to oocyte stages. The experiment was conducted in October–November 1979 and replicated in November–December 1982. The numbers of fish in each category are indicated inside the vertical bars. The stages of oocyte maturation are classified as follows: (1) immature oocyte with migrating germinal vesicle (G.V.); (2) immature oocyte with peripheral G.V.; (3) maturing oocyte (see details under Materials and Methods); (4) matured and ovulated oocyte. The levels of plasma cortisol at stage 4 (postovulation) are significantly different from the values associated with stages 1,2,3 ($P < 0.01$, derived from analysis of variance on log-transformed data).

three-fold elevation of cortisol levels was observed in females sampled postovulation (stage 4; mean values: 25.8 and 30.9 ng/ml). In the 1982 trout population, no significant difference in cortisol titers was found between ovulated females sampled at the beginning of the population ovulation period and ovulated females sampled 10–15 days later (31.8 and 26.8 ng/ml, respectively; $P > 0.05$). The average time interval between the sampling date of a female at a defined ovarian state and the date of ovulation was

35 days for stage 1, 8 days for stage 2, and 3 days for stage 3.

DISCUSSION

The results of our investigation indicate a marked elevation of plasma cortisol levels in the female rainbow trout during the post-ovulation period, in comparison with the low cortisol concentrations observed during several preovulatory stages. The timing of this cortisol increase is not known, due to the strict requirements of our sampling protocol which precluded handling of the fish at any time before the sampling date and therefore did not permit us to record the ovulation dates for the females sampled postovulation. It appears probable, however, that cortisol elevation lasts some time, since cortisol levels were similarly elevated in ovulated females sampled at the beginning of the population ovulation period and in ovulated females sampled 10–15 days later (in the latter lot, the average time interval between ovulation and sampling being presumably longer). Our results are consistent with the findings of Pickering and Christie (1981) who reported a 4-fold difference in cortisol levels between nonovulated and ovulated female brown trout (13.5 ng/ml and 53.1 ng/ml, respectively). In contrast, serum cortisol levels of ovulatory and nonovulatory female goldfish did not differ at the time of ovulation or after ovulation, although a peak in serum cortisol was found in the ovulatory fish group 4–8 hr prior to ovulation (Cook *et al.*, 1980). At present, we can only speculate on the biological significance of a cortisol increase in ovulated trout. Cortisol might be involved in the control of ovulation *sensu stricto* (i.e., the expulsion of the mature oocyte from the follicle). In this respect, Hirose and Ishida (1974) have shown that high dosages of cortisol administered to ayu (*Plecoglossus altivelis*) could induce hydration of the oocytes and ovulation. On the other hand, cortisol might play a role in spawning be-

havior. In connection with the latter hypothesis, it may be noted that a rise in corticosterone, peaking at the time of oviposition, was demonstrated in laying hens (Beuving and Vonder, 1981). Interestingly, the plasma concentrations of several other hormones are elevated for prolonged periods of time after spontaneous ovulation in some salmonids; glycoprotein gonadotrophin (Jalabert and Breton, 1980; Scott *et al.*, 1983) and 17α -hydroxy-20 β -dihydroprogesterone (Fostier *et al.*, 1981; Scott *et al.*, 1983) in the rainbow trout; prostaglandins in the brook trout (Goetz and Cetta, 1983). It should be emphasized, however, that the situation of ovulated trout in most endocrinological studies (including the present study) is necessarily somewhat artificial, since spawning behavior does not usually take place in captive female trout under conditions of intensive rearing in tanks. A better understanding of the possible involvement of cortisol (and other hormones) in the oviposition of female trout will obviously require work on fish undergoing the complete spawning sequence in a seminatural environment.

Another possible interpretation of our results is that the extended presence of ova and associated fluid within the body cavity might induce a state of moderate ill-being, resulting in an increased cortisol secretion. Histological studies on female rainbow trout in possession of overripened ova (several weeks after ovulation) showed degenerative changes in hepatic and urinary tissues and a hyperfunctional state in the interrenal gland (Takashima *et al.*, 1975). Additional studies on the kinetics of cortisol elevation in ovulated trout and on the general behavior of these fish would help to clarify this issue.

On the basis of the results of the present study, it appears that there is no elevation of plasma cortisol in female rainbow trout during oocyte maturation at or after G.V. breakdown time. This result does not rule

out the possibility of a short-lived cortisol increase at the time of natural, maturation-inducing gonadotrophin release (oocyte with peripheral G.V., shortly before the initiation of macroscopic modifications) and/or at the very beginning of oocyte maturation (maturing oocyte before G.V. breakdown). This hypothesis would be difficult to test, however, due to the transient nature of such stages and to the impossibility of handling the fish and sampling oocytes repeatedly, if a valid measurement of cortisol levels at rest is to be made.

The cortisol concentrations observed in preovulatory rainbow trout (this study: stages 1, 2, 3; about 8 to 11 ng/ml) are comparable to the daily means found in June–July in catheterized female rainbow trout (about 5 to 10 ng/ml; Bry, 1982) and are among the lowest values reported in non-ovulated salmonid fish during the reproductive period. The notable differences in cortisol levels between various studies of salmonid fish during reproduction may be attributed to differences in species or in situations (wild migrating fish vs hatchery-reared fish). These discrepancies may also result from a lack of methodological standardization concerning: (1) in the case of a domesticated species, the conditions of acclimatization to the experimental environment (for an illustration of the importance of stocking density, see Pickering and Pottinger, 1983); (2) the time interval between capture and blood sampling; and (3) the specificity of cortisol assay.

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