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Plasma 11-Oxotestosterone and Gonadotrophin in Relation to the Arrest of Spermiation in Rainbow Trout (Salmo gairdneri)

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Plasma gonadotrophin (GTH) and 11-oxotestosterone levels have been studied in relation to sperm production during the last weeks of the spermiation period. The level of 11-oxotestosterone decreased in parallel to sperm production (coefficient of correlation: $\rho = 0.59; P < 0.01$) and reached its lowest values ($X \pm SE = 0.33 \pm 0.19 \text{ ng/ml}; n = 11$) when spermiation was over. No significant variations were found in GTH levels; however, there was a slight positive correlation between sperm production and GtH levels ($\rho = 0.39; P < 0.05$).

The endocrine control of spermiation in salmonids is still not clearly understood (Billard et al., 1982). High levels of glycoprotein gonadotrophin have been reported to occur during the spawning season (Crim et al., 1973, 1975; Crim and Evans, 1978; Billard et al., 1978; Whitehead et al., 1979; Stuart-Kregor et al., 1981). However, a slow decrease in plasma gonadotrophin was recorded in rainbow trout during the first 6 weeks of sperm release (Billard and Breton, 1977; Sanchez-Rodriguez et al., 1978; Fostier et al., 1982), after which gonadotrophin rose (Billard and Breton, 1977; Sanchez-Rodriguez et al., 1978). No precise data are available concerning the end of spermiation. The analysis of monthly or bimonthly samples has shown a peak of plasma testosterone occurring before full spermiation, when 11-ketotestosterone and 11$\beta$-hydroxytestosterone reach their maxima in brook trout (Sangalang and Freeman, 1974), rainbow trout (Scott et al., 1980), and brown trout (Kime and Manning, 1982). Idler et al. (1971) reported stable levels of testosterone and increasing levels of 11$\beta$-hydroxytestosterone and 11-oxotestosterone during spermiation in Atlantic salmon. However, in other studies (and thus probably in other strains), concomitant variations were reported between testosterone and 11-oxotestosterone in rainbow trout (Scott and Baynes, 1982) and Atlantic salmon (Hunt et al., 1982).

To appreciate better the relationships between the hormonal environment and the spermiation process, we thought it would be useful to closely follow endocrine changes when the physiological phenomenon, i.e., spermiation, appeared and disappeared. In a previous study (Fostier et al., 1982) we analyzed weekly relationships between plasma gonadotrophin and 11-oxotestosterone and sperm production at the onset of spermiation. This paper reports a comparable study performed during the last 6 weeks of sperm production.

**MATERIALS AND METHODS**

*Animals.* Twenty male rainbow trout (mean body weight 630 g), handled for the first time for sperm collection (stripping), were individually tagged and kept in recycled fresh water under natural photoperiod and temperature ($6 \text{ to } 13^\circ$) between January and March. During the experiment, seven fish died.

*Sampling.* Blood was sampled once a week and sperm every 2 weeks, as described previously by Fostier et al. (1982). Changes in the spermatozoa concentration were estimated by measuring the optical den-
sity, at a wavelength of 410 nm, of sperm diluted in a solution of NaCl (8%), knowing that the optical densities are linearly related to the spermatozoa concentration (Billard et al. 1971). Two males were still producing sperm at the end of the experimental period; thus only the other 11 fish were used for data analysis.

**Hormone measurement.** Plasma gonadotrophin (t-GTH) and 11-oxotestosterone (11-oxoT) were measured using the radioimmunoassay already described by Fostier et al. (1982). Intrassay variability was CV = 28% (X = 2.7 ng/ml; n = 7) for GTH and CV = 9.3% (X = 26.7 ng/ml; n = 14) for 11-oxoT.

**Statistical analysis.** Groups of samples taken on different dates were compared using variance analysis. The coefficients of correlation or Spearman rank correlations were calculated (Snedecor and Cochran, 1971).

**RESULTS AND DISCUSSION**

The 11 trout studied stopped spermiating between 8/2 and 22/2. The volume of collected sperm decreased continuously from 4 weeks before the end of spermiation (Fig. 1h). The gonadosomatic index measured at the end of the experiment (X ± SD = 1.0 ± 0.5%) was similar to that of spent males (Billard et al., 1971). No difference in spermatozoa concentrations, estimated by the optical density measurements, was detected between fish or between dates of sampling (variance analysis). The general mean estimated at 4.1 ± 1.3 x 10^9 spermatozoa/ml was low, as already observed for sperm collected at the end of the spawning season (Billard, 1974). Because of the homogeneity between samples of the spermatozoa concentrations, the volume of sperm collected has been taken as an indicator of the spermatozoa production.

When the whole experimental population was taken into account, no significant variations were found between plasma GTH levels at the different sampling dates. However, the individual profiles of five fish fluctuated before the end of spermiation (three have been plotted in Figs. 1a–c); the other six fish showed more regular GTH levels (four have been plotted in Figs. 1d–g). These differences between the two groups as to plasma GTH pattern could not be related to any particular characteristic of sperm production or to 11-oxoT levels.

Plasma 11-oxoT levels decreased significantly in parallel to the volume of collected sperm (Fig. 1h). When no more sperm could be stripped from the males, mean 11-oxoT concentration reached 0.33 ng/ml (individual values between 0 and 2.04 ng/ml) and has to be compared to the 61 ng/ml recorded 6 weeks earlier (individual values between 30.8 and 91.8 ng/ml).

Sperm volume was positively correlated with the 11-oxoT levels found at the date of sampling (coefficient of correlation = +0.59; P < 0.01) and slightly correlated with the GTH levels (CR = +0.39; P < 0.05). No significant correlation was found between the 11-oxoT and GTH levels.

As when spermiation begins (Fostier et al., 1982), the plasma 11-oxoT levels were correlated with the quantities of sperm collected during the last weeks of sperm production. Furthermore, there was a clear temporal relationship between the disappearance of 11-oxoT from the plasma and the arrest of spermiation. Unfortunately, there are few experimental studies on salmonid spermiation using 11-oxoT. Weekly injections of that androgen (5 mg/fish) in sockeye salmon induced spermiation after a treatment of 2–3 weeks (Idler et al., 1961), but Silastic implants of it (capsule length = 4 cm; Φ = 2 mm) in 150-day old rainbow trout did not significantly stimulate sperm production (Billard et al., 1982). In the latter case, steroid release was low compared to the high levels of intratesticular 11-oxoT; this would be expected, considering the plasma levels. Besides, these experiments were carried out on nonhypophysectomized animals, although negative 11-oxoT feedback on GTH secretion was shown at the spermiation stage (Billard, 1978).

The high levels of 11-oxoT during spermiation could maintain GTH secretion at a low level. However, at the onset of spermiation the higher GTH levels might stim-
ulate 11-oxoT secretion by the testis (Ng and Idler, 1980; Magri et al., 1982; Lebail et al., 1983); this production would then be amplified by the gonadal–adrenal–hepatic positive feedback loop (Kime, 1978). The decrease of 11-oxoT secretion at the end of spermiation would not be related primarily to a drop in GTH levels but to a break in the positive feedback loop as, for instance, a qualitative and/or quantitative modification of the steroidogenic potentialities of the testis or of the other components of the loop. Nevertheless, we must be careful not to misinterpret the role of the pituitary since patterns of short-term blood GTH might show transitory peaks, as demonstrated in female (Zohar et al., 1981).

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