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Annual cycle of plasma oestradiol-17β in the female trout *Salmo gairdneri*

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Summary. Levels of oestradiol in plasma of adult female rainbow trout *Salmo gairdneri* were determined during the annual reproductive cycle using a radioimmunoassay. From January to June, during previtellogenesis and the period of endogenous vitellogenesis plasma levels were low (1,3 ± 0,2 ng/ml). At the beginning of the period of exogenous vitellogenesis, the œstradiol level rose and a maximum (16,9 ± 0,2 ng/ml) was reached at the end of vitellogenesis in November, some weeks before spawning. A positive correlation (r = 0,58) was established between plasma œstradiol levels and the gonadosomatic index. From enzymatic cytochemical studies it was concluded that the granulosa cells, as well as the interstitial cells in the ovary, are involved in œstradiol production.

Introduction.

In oviparous vertebrates it is suggested that oestradiol-17β may trigger the liver to synthesize yolk proteins (for review : Clemens, 1974 ; Tata 1976). To test this hypothesis in *Salmo gairdneri*, we have studied the relation between oestradiol-17β levels in plasma during the annual cycle and the development of the oocytes ; we have also investigated the location of the 3β-hydroxysteroid dehydrogenase enzyme in the ovary.

Materials and methods.

Animals.

Adult female specimens of the rainbow trout were obtained from a hatchery in Vaassen (the Netherlands). After anaesthesia with MS 222, blood was taken from the *ductus Cuvieri* with a heparinized vacuum syringe, then centrifuged and the plasma stored at — 20 °C. The ovaries were removed and prepared for histological and enzyme-cytochemical examination as described by van den Hurk *et al.* (1978).

Assay method for plasma oestradiol-17β.

The organic solvents were redistilled once just before use. (2, 4, 6, 7(n)-^{3}H) Oestradiol-17β (Spec. act. 109 Ci/mmol) was obtained from the Radiochemical Centre (Amersham) and purified on TLC. The radioactivity was measured with a Nuclear Chicago Mark I scintillation counter with a scintillator of PPO (5.4 g) and POPOP (100 mg) in toluene-triton X 100 (2 : 1) mixture (1 L). Radioactive areas on the TLC plates were located by means of a Berthold thinlayer chromatogram scanner. Oestradiol-17β (E_2) was measured by radioimmunoassay. Antiserum (anti-E_2-6-CMO-BSA) was obtained from Dr. R. J. Scaramuzzi (MRC unit for Reproductive Biology, Edinburgh, Scotland, U. K.) through Dr. S. J. Dieleman (Clinic for Veterinary Obstetrics, Gynaecology and A. I., Utrecht, the Netherlands). The antiserum had a high specificity ; cross reactions with oestrone and oestradiol-17α were 3 p. 100 and 1.2 p. 100 respectively. For the assay of plasma E_2, 1 ml of plasma was added to an extraction tube together with 2 000 dpm of ^{3}H-E_2. After one hour at room temperature, extraction was performed with peroxide-free diethylether (2 × 5 ml). The combined ether fraction was evaporated under nitrogen at 40 °C and the residue dissolved in 2 ml of buffer containing Na_2HPO_4-$2H_2O$ (10.78 g), NaH_2PO_4-H_2O (5.4 g), NaN_3 (1 g), NaCl (9 g), gelatine (1 g) per litre aqua dest. From the sample 400 μl was counted for recovery determinations and 100 μl and 200 μl aliquots (both in duplo) were transferred to assay tubes containing 15 000 dpm ^{3}H-E_2. In all tubes the volume was raised, if necessary, to 200 μl with buffer. A standard curve was set up by adding 15 000 dpm ^{3}H-E_2 to a series of assay tubes containing 0, 25, 50, 100, 150, 200, 300 and 400 pg unlabeled E_2. After adding 200 μl of antiserum (1 : 60 000) and vortex mixing, the tubes were incubated overnight at 4 °C. To separate the free and bound E_2, all assay tubes were closed with plastic caps containing 200 μl of dextran-coated charcoal in buffer. Following incubation, the assay samples were simultaneously mixed with charcoal by shaking the tubes. After a contact time of 5 min. the charcoal was centrifuged and 100 μl of the supernatant, containing the bound E_2, was measured. The binding percentage was calculated and after adjustment for recovery (70-90 p. 100), values were expressed as ng/ml plasma. For determining the unspecific binding, a blank of 1 ml of buffer was run through the entire procedure. The value of the blanks was 0.017 ng/ml. Intra-assay precision was determined by a tenfold analysis in the optimal range from 25 to 200 pg. The coefficients of variation for analysis of 25, 50, 100, 150 and 200 pg were 10.7, 4.1, 2.1, 1.2 and 0.7 p. 100 respectively.

Results.

Histological studies showed annual changes in the growth and ripening of ovarian follicles. In the cycle, three periods could be distinguished. 1. The period of previtellogenesis, from January to June, characterized by the presence of young follicles (max. diameter 0.3 mm) and the differentiation of the follicular layer into granulosa and theca. 2. The period of endogenous vitellogenesis, which is restricted to May and June. In this period, the oocytes reach a diameter of 0.6 mm, as a result of endogenous yolk formation. Part of this process is the appearance of cytoplasmic vacuoles with chromophobic contents. 3. The period of exogenous vitellogenesis, during which

the oocytes take up yolk proteins and grow to 4.5 mm. This period lasts from July to December. In December or January, ovulation may take place, leading to the formation of postovulatory follicles. The enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) has been demonstrated in the granulosa cells of growing follicles during the first half of the period of exogenous vitellogenesis (fig. 1), in interstitial cells during the entire cycle (peak in January), and in the postovulatory follicles. In comparison with the strong activity of the surrounding interstitial cells, the 3β-HSD activity in the granulosa cells of the postovulatory follicles is rather weak (fig. 2). The plasma E_2 levels were determined at irregular intervals for 1 1/2 year. The individual E_2 levels are presented in table 1 and figure 3. Both in 1975 and 1976, they clearly followed a seasonal pattern.

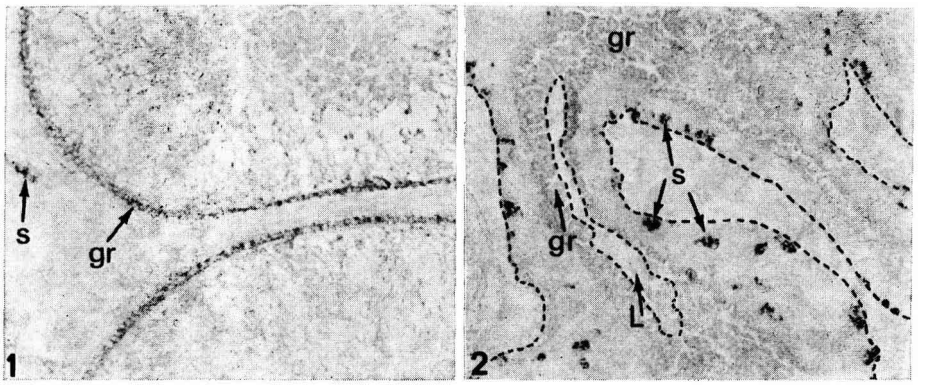

FIG. 1 and 2. — 3β-*HSD activity in the ovary of* Salmo gairdneri.

1. Detail of two growing follicles.
2. Detail of a corpus luteum :

gr : granulosa cells ; L : lumen of the corpus luteum ; s : stromal or interstitial cells.

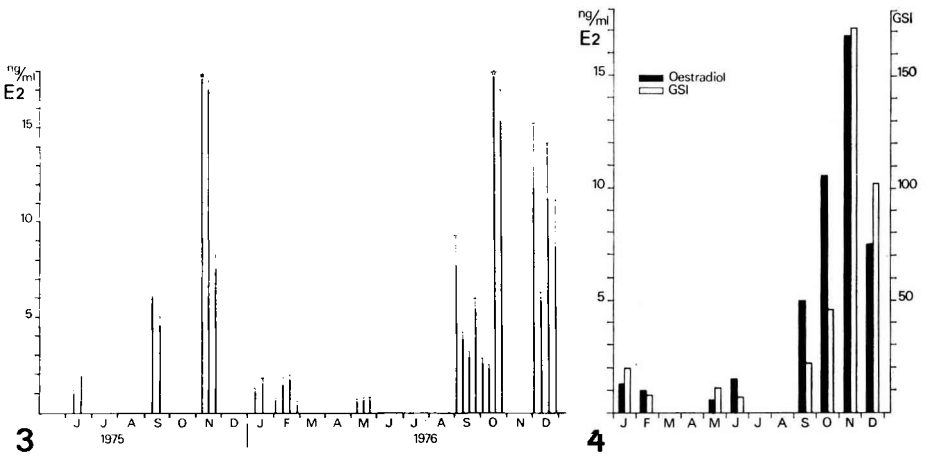

FIG. 3 and 4. — *Seasonal changes in the concentrations of plasma oestradiol-17β in female* Salmo gairdneri.

3. The individual E_2 levels (ng/ml) during 1975 and 1976.
4. The mean values of E_2 levels and gonado-somatic index (GSI p. 1 000) during an annual cycle.

The cyclical changes have been summarized in figure 4. A rise in E_2 level obviously starts before September, probably in July or August. After a maximum in November (16.9 $\pm$ 0.2 ng/ml), the values drop to 1.3 $\pm$ 0.2 ng/ml in January. Such low values last to June. Comparison of the plasma E_2 levels with the gonadosomatic index (GSI) of individual animals (table 1) indicate a positive correlation (correlation coefficient after Ferguson, 1966 : 0.58) (fig. 4).

TABLE 1

The oestradiol-17β (E2) levels in plasma of Salmo gairdneri. GSI : gonadosomatic index, (n) : number of assays

Date	GSI (p. 1 000)	E2 ng/ml (n)
3-6-1975	4.9	1.0 $\pm$ 0.1 (11)
	8.8	1.9 $\pm$ 0.2 (13)
24-9-1975	30.1	6.0 $\pm$ 1.1 (7)
	18.8	4.5 $\pm$ 0.5 (7)
19-11-1975	164.4	26.2 $\pm$ 0.6 (2)
	247.5	17.0 $\pm$ 0.9 (4)
	86.7	7.5 $\pm$ 1.3 (3)
7-1-1976	10.4	1.1 $\pm$ 0.2 (14)
	38.5	1.5 $\pm$ 0.3 (11)
25-2-1976	6.8	0.6 $\pm$ 0.1 (7)
	6.6	0.4 $\pm$ 0.1 (5)
	—	1.7 $\pm$ 0.2 (13)
5-5-1976	10.6	0.6 $\pm$ 0.1 (7)
	6.1	0.6 $\pm$ 0.1 (9)
	14.6	0.5 $\pm$ 0.1 (15)
23-9-1976	28.9	7.7 $\pm$ 1.6 (12)
	20.4	3.8 $\pm$ 0.4 (15)
	18.6	2.9 $\pm$ 0.4 (12)
	26.1	5.4 $\pm$ 1.9 (9)
21-10-1976	14.1	2.5 $\pm$ 0.4 (7)
	38.7	2.3 $\pm$ 0.2 (8)
	47.8	22.1 $\pm$ 3.6 (6)
	80.9	15.4 $\pm$ 1.6 (10)
2-12-1976	251.8	5.8 $\pm$ 0.4 (4)
	80.1	11.2 $\pm$ 3.1 (4)
	78.0	8.7 $\pm$ 2.5 (3)
	—	11.8 $\pm$ 3.4 (5)

Discussion.

Comparing radioimmunoassay and histological results, it appears that plasma E_2 levels are low during endogenous vitellogenesis and increase with the beginning of exogenous vitellogenesis. This increase continues throughout the period of exogenous yolk formation, and a maximum is reached some weeks before spawning. Schreck *et al.*, (1973) found 4.4 ng E_2 per ml plasma in adult female *Salmo gairdneri* in October. Judging from this value, the blood samples must have been collected before the E_2 levels reached maximum value. The correlation between the change in plasma E_2 level and exogenous vitellogenesis corresponds to the observations of Eleftheriou *et al.*, (1966), Schreck and Hopwood (1974) and Wingfield and Grimm (1977) in other

teleosts, and also with the hypothesis that E_2 triggers the synthesis of yolk proteins in the liver (Clemens, 1974 ; Tata, 1976). The continuous presence of E_2 in the blood corresponds to the uninterrupted activity of 3β-HSD in the interstitial cells of the ovary. The increase in plasma E_2 levels coincides with the development of 3β-HSD activity in the granulosa cells of the follicles. These correlations seem to indicate that E_2 is formed both in the interstitium and in the granulosa cells of growing follicles. During October and November, the plasma E_2 level continues to rise, but this is not reflected by an increased 3β-HSD activity in the ovary. On the contrary, during this period, 3β-HSD activity could not be demonstrated in the granulosa cells. This might be attributed to the fact that the oocytes become very large and cause stretching of the granulosa cells. A slight 3β-HSD activity is again noticeable in the granulosa cells after ovulation and collapse of the follicle. The presence of some 3β-HSD activity in the postovulatory follicles coincides with a maximum 3β-HSD activity in the interstitial tissue, which is not reflected by a rise in plasma E_2. This means that the 3β-HSD activity cannot be used as parameter for E_2 production, and that next to E_2 other steroids are being synthesized by the ovary during the annual cycle. On the other hand, the positive correlation in *Salmo gairdneri* between plasma E_2 level and GSI seems to indicate that the latter can be used for estimating E_2 production.

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Résumé. Les niveaux d'estradiol dans le plasma de femelles truites adultes ont été déterminés durant le cycle reproducteur annuel en utilisant un dosage radioimmunologique. De janvier à juin, pendant la prévitellogenèse et la période de vitellogenèse endogène, les niveaux plasmatiques sont faibles (1,3 $\pm$ 0,2 ng/ml). Au début de la période de vitellogenèse exogène les niveaux d'estradiol augmentent et un maximum (16,9 $\pm$ 0,2 ng/ml) est atteint à la fin de la vitellogenèse en novembre, quelques semaines avant la fraie. Une corrélation positive $r = 0{,}58$ a été calculée entre l'estradiol plasmatique et le rapport gonado-somatique. A partir d'études cytoenzymologiques on conclut que les cellules de la granulosa comme les cellules interstitielles de l'ovaire sont impliquées dans la production d'estradiol.

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