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The seasonal variations in the *in vitro* metabolism of ^{14}C-pregnenolone in 2-year old rainbow trout (*Salmo gairdneri* R.) ovaries

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The seasonal changes of the steroid metabolism in developing 2-year old rainbow trout ovaries were examined *in vitro* with ^{14}C-pregnenolone as a precursor from females under natural rearing conditions.

There seems to be some seasonal variation in the intensity of ovarian steroid metabolism. The precursor was metabolized through DHA to androstenedione and 11β-hydroxytestosterone. Estrogens as metabolites could not be identified, though there was some activity in the phenolic fraction throughout the year.

The percentage yield of total androgens decreased from March till September to the minimum value, after which the yield started to increase again to its maximum in the following spring. The yield of total estrogens increased to the maximum in August and decreased to the minimum in April.

The metabolic activity of the ovarian tissue increased together with the increase in gonadosomatic index (GSI) value. Only the follicular tissue was metabolically active.

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1. Introduction

The cellular sites of steroidogenesis and *in vitro* steroid metabolism of salmonid fish gonads during the reproductive cycle have been reasonably well investigated (Hoar & Nagahama 1978, van den Hurk & Peute 1979, van Bohemen & Lambert 1981, and Nagahama et al. 1982), but the seasonal *in vitro* steroid metabolism of rainbow trout gonads is still an open question.

The aim of this study was to reveal using quite simple methods the seasonal, long lasting changes in the *in vitro* steroid metabolism of the 2-year old rainbow trout ovaries, and accordance with the ovarian growth. The farming of rainbow trout in extreme light (almost total darkness in winter time), and temperature (+22°–+0.2°) conditions provide some interesting background, which will be further investigated later.

2. Materials and methods

The experiment was run on a private fish farm belonging to Savon Taimen Co., at Rautalampi (Central Finland). The experimental fish, 2-year old females (*Salmo gairdneri* R., a spring spawning A 13 strain), were reared according to the normal routine at this farm, in outdoor ponds under natural annual environmental conditions. The first spawning time of these fish would have been in May 1980. The day lengths, water temperatures, and sampling dates are shown in Fig. 1.

For every sampling 10 fish were killed by a blow on the head. After this they were measured and weighed, the ovaries were dissected and weighed immediately, and approximately the same part of them (the middle) was used for the incubations.

The ovarian tissue was minced on ice with scissors. For every incubation 100 mg of this minced tissue was added to 2 ml of Cortland saline (Wolf 1963), with 2 mg of NADP (Boehringer Mannheim, Germany) as a cofactor. The precursor used was Δ^5-(4-^{14}C)-pregnenolone (Radiochemical Centre, Amersham), specific activity 53 mCi/mmol, 0.100 μCi (0.6 μg) per sample. Incubation commenced within 5 mins. of the beginning of the sampling, and it continued for 2 hours at +15° under conditions of continuous oxygen flow, and shaking. Incubation was stopped by adding

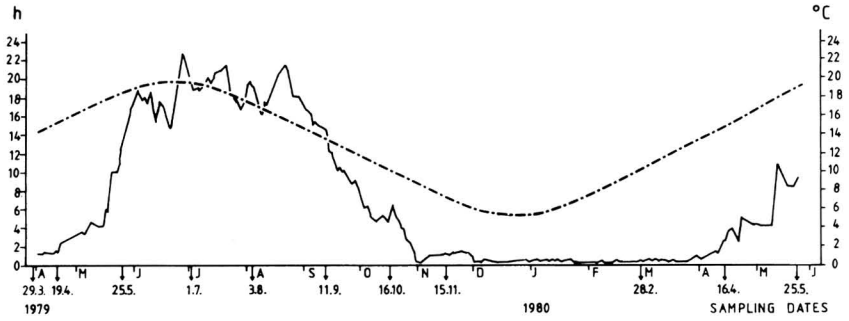

Fig. 1. The day lengths (broken line) and water temperatures at the fish farm belonging to Savon Taimen Co., in 1979–1980. The sampling dates are marked with arrows.

Table 1. Steroids and standards used.

Trivial name	IUPAC name
pregnenolone	3β-hydroxy-5-pregnene-20-one
17-α-hydroxypregnenolone	3β,17α-dihydroxy-5-pregnene-20-one
progesterone	4-pregnene-3,20-dione
17α-hydroxyprogesterone	17α-hydroxy-4-pregnene-3,20-dione
dehydroepiandrosterone (DHA)	3β-hydroxy-5-androsten-17-one
androstenedione	4-androstene-3,17-dione
testosterone	17β-hydroxy-4-androsten-3-one
11β-hydroxytestosterone	11β,17β-dihydroxy-4-androsten-3-one
11-ketotestosterone	17β-hydroxy-4-androsten-3,11-dione
estrone	estra-1,3,5(10)-triene-3-ol-17-one
estradiol-17β	estra-1,3,5(10)-triene-3,17-dione

1.5 ml of acetone. After this the samples were frozen (−20°) until further analysis was carried out.

For isolation, measurement and separation of the metabolites, the incubated samples were treated further as described by Teräväinen & Saure (1976). The recovery of the radioactivity extracted from the samples was measured from aliquotes by liquid scintillation counting (Wallac LKB Beta, in Leiras Ltd, Turku), before TLC separation. The solvent system for the separation in TLC plates (Merck Keselgel 60) was acetone:chloroform (15:85) for neutral fractions, and diethylether:chloroform (1:3) for phenolic fractions.

The radioactivity on the TLC-plates was quantified by a TLC-scanner (LB 2721, Berthold, Co., Wildbad, G.F.R., in the Institute of Medical Biology, Helsinki University). Reference steroids were visualized after spraying with p-toluene sulphonic acid (20% in ethanol). The trivial and IUPAC names of the steroids and standards used are listed in Table 1.

The identification of the metabolites in this study was quite simple, being based on the Rf-values of the standard steroids added on every TLC plate, and on the Rf-values of the reliable radioactive metabolite peaks. The results are expressed as a group mean ± *SD* (*n*).

3. Results

Regardless of the sampling time, more than 90% of the radioactivity added to the incubation was recovered (Fig. 2). Most of the radioactivity was recovered as neutral fractions (androgens), but small amounts of phenolic fractions (estrogens) were also found. The relative recoveries of these two fractions depended on the season. In the neutral fraction (Fig. 2) the minimum was reached in September, after which the percentage yield increased sharply till its maximum in October, and stayed at this high level till April. In the phenolic fraction (Fig. 2) the percentages were quite the opposite. The maximum was reached in August and thereafter the percentage yield decreased to the minimum, to remain at this low level until the middle of April.

Table 2. The percentage yields (mean ± *SD* and *n*) of the identified metabolites on the TLC runs, with 14-C-pregnenolone as a precursor.

Date	Precursor: pregnenolene	Metabolites: 17α-OH-pregnenolene	DHA	androstenedione	11β-OH-testosterone
29.3.1979		19.2 (1)	48.6 (1)	13.6 (1)	14.5 (1)
			46.7± 7.6 (8)	23.3±10.4 (8)	22.1± 8.2 (8)
19.4.	18.9±8.9 (5)		31.0± 9.2 (5)	18.6± 7.3 (5)	19.4±11.2 (5)
		48.6±11.6 (3)	8.0± 2.5 (3)		33.9±12.6 (3)
25.5.		22.3± 2.9 (2)	48.9± 6.5 (2)	11.3± 2.5 (2)	11.4± 3.5 (2)
			35.8± 5.4 (6)	32.8± 5.4 (6)	21.1± 1.4 (6)
1.7.			54.9±11.9 (7)	19.3± 5.1 (7)	17.2± 3.9 (7)
3.8.		55.0± 7.8 (2)	12.1± 1.6 (2)		18.6± 6.9 (2)
			66.7±16.8 (2)	8.6± 4.4 (2)	17.5± 9.5 (2)
12.9.		6.8± 1.9 (6)	60.9±14.1 (6)		16.9± 6.5 (6)
16.10.		11.2 (1)	73.2 (1)		8.7 (1)
			60.3± 9.6 (7)	12.6± 8.3 (7)	17.3± 7.7 (7)
15.11.			61.4±11.6 (7)	11.6± 7.1 (7)	18.1± 7.8 (7)
28.2.1980			64.9± 9.6 (9)	8.2± 2.8 (9)	18.4± 7.0 (9)
16.4.			69.5±11.5 (8)	10.5± 8.3 (8)	11.9± 4.8 (8)
25.5.			67.3±11.8 (5)	9.3± 4.4 (5)	7.4± 4.8 (5)

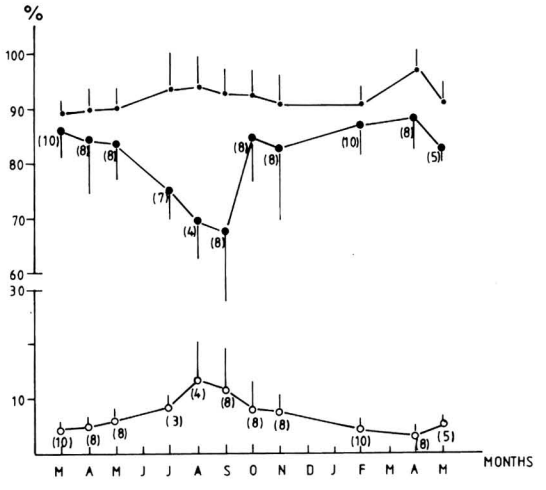

Fig. 2. The percentage yields of the neutral (androgens) ●, and phenolic (estrogens) ○ fractions, and the recovery percentages of the total steroid fractions ★.

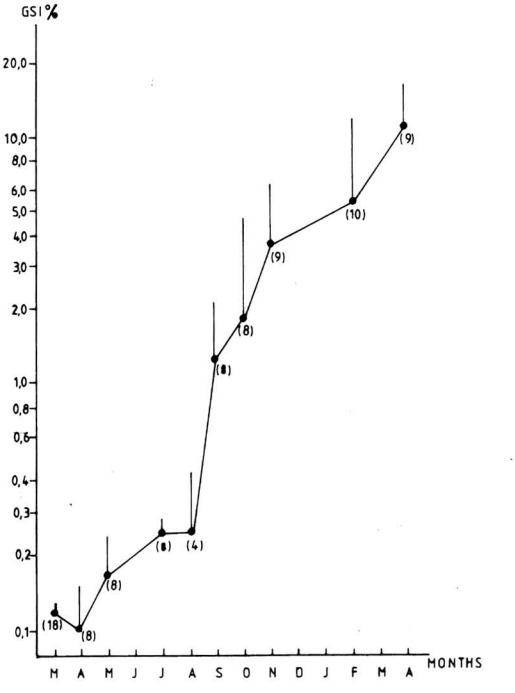

Fig. 3. The ovarian weights, expressed as gonadosomatic indices (GSI), of the rainbow trout.

Although estrogens were detected in the liquid scintillation counting none of them could be identified in thin layer chromatography.

Pregnenolone was metabolized through dehydroepiandrosterone (DHA) to androstenedione and 11β-hydroxytestosterone (Table 2).

All of the metabolic activity took place in the follicle cells. Ripe eggs, incubated at the time they could be completely separated from the follicle cells (May 1980), had no metabolic activity, while the follicular tissue metabolized the precursor completely. Apparently this activity increases in parallel with the GSI value, as in April we incubated ovarian tissue with quite large inseparable eggs, and the metabolic activity of these tissue samples was exactly the same as that of the pure follicle tissue in May. The GSI values are given in Fig. 3 (GSI = gonadal weight as percentage of body weight).

4. Discussion

Ovarian follicle layers are believed to be the major cellular sites of ovarian steroidogenesis (Hoar 1965, Guraya 1976, Hoar & Nagahama 1978, and Nagahama et al. 1982). Our results agree well with these investigations and reveal that eggs when ovulated are metabolically totally inactive. It has also been stated that of the follicle layers the thecal cell layer is responsible for androgen production, and the granulosa layer is the site of aromatization of androgens (Kagawa et al. 1982).

The size of ovarian sample chosen for incubation in this investigation was perhaps too large to reveal the development of total metabolic activity of the ovarian tissue, because each time, except in the second sampling, the precursor was completely metabolized, in spite of the fact that the ratio of metabolically active tissue grew smaller when the size of the eggs increased (during the 2nd experimental year, the fish being 3 years old). On the other hand, the large variation in the GSI values also indicates great differences in the phase of ovarian growth, and the sharp changes in the percentage yields of the metabolites may partly be due to the changes in the degree of ovarian maturity.

In this work pregnenolone throughout the year was completely metabolized through DHA to androstenedione and 11β-hydroxy-testosterone. Lambert (1978) and Tesone & Charreau (1980) have obtained the same results with *Brachydanio rerio* H.-B., and *Jenynsia lineata*, Jenyns. Many authors have shown that pregnenolone is metabolized through progesterone (Eckstein 1970, and Eckstein & Katz 1971, with *Tilapia aurea*, Eckstein & Eylath 1970, with *Mucil cephalus*, and Hirose et al. 1975, with *Eptatretus burgeri*). Colombo & Colombo Belvedere (1976, with *Anguilla anguilla*), and Lambert & van Bohemen (1979, with *Salmo gairdneri*), have stated that both ways are possible. The reasons for this discrepancy remain open.

Ovaries of these rainbow trouts metabolized pregnenolone also to some extent to phenolic steroids, but no estrogens as metabolites could be detected. Low yields could easily have been lost in the background activity.

When we compare our results to the literature, the extreme lighting (continuous light in the summer and darkness in the winter) and temperature (+22°−+0.2°) conditions in our hatcheries, which obviously have effects on the endocrine system of these fish, are important considerations.

On the basis of this preliminary work it is difficult to determine any details of the *in vitro* steroid metabolism of rainbow trout ovaries during the natural reproductive cycle in such extreme environmental conditions as those on our fish farms, but the results reveal that only the follicular tissue metabolizes the precursor, pure eggs being completely inactive. Also there seems to be some kind of circannual or perhaps size related variation in the intensity of ovarian steroid metabolism. These results are important, because with these very simple methods we can clarify the possible differences in the *in vitro* gonadal steroid metabolism of rainbow trout exposed to different annual photoperiod cycles.

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