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Ovarian Early Postovulatory Development and Oestrogen Production in Rainbow Trout (*Salmo gairdneri* R.) from a Spring-Spawning Strain

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Ovarian early postovulatory development and oestrogen production were studied in rainbow trout from a spring-spawning strain, 5 to 50 days postovulation (PO). Early postovulatory development was assessed by the ovarian development index, corresponding to the relative volume occupied in the ovary by oocytes in an advanced stage of differentiation (containing cortical alveoli). This index was shown to be related to the maximal oocyte diameter and to the time since ovulation, as well as to metabolic criteria. In contrast, gonadosomatic index was not correlated with any of these, confirming its unreliability for assessing the gonadal development, at least at this stage. Size of follicles and presence of vitellus (lipoprotein) in the oocytes of several animals indicate that ovarian development was more advanced in our females than in other studies. It is suggested that young follicles start developing before ovulation of the preceding batch of oocytes. Oestradiol (E_2) plasma levels were low but detectable, and showed, in some females, peak values (max 5.5 ng/ml) within the first month PO. Besides, *in vitro* metabolism was studied using two isotopes of androstenedione as precursors. No sharp discrepancies were observed between the two types of incubation. Conjugation of the precursor or its metabolites was high but decreased with time postovulation. This suggests that it proceeds from postovulatory follicles. E_2 production potential was demonstrated from the shortest time postovulation. Moreover, it increased with ovarian development, suggesting that E_2 is synthesized in developing follicles. © 1989

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Oestradiol- 17β plays an important role in female reproduction. In fish, its most well-known action is the stimulation of vitellogenin synthesis by the liver. However, other activities have been suspected, like a mitogenic effect on germinal cells (for review see Fostier *et al.*, 1983). In salmonids, whose sexual cycle is annual, oestradiol (E_2) plasma concentrations are highest during active vitellogenesis, a few weeks before spawning, and then drop to reach basal levels before ovulation (Fostier *et al.*, 1978). E_2 levels are not thought to rise again before a couple of months. However, Jalabert and Fostier (unpublished) detected transient high E_2 plasma concentrations shortly after ovulation in rainbow trout.

Moreover, even though Lambert and van Bohemen (1979) did not detect aromatase (oestrogen synthetase) activity about 1 month postovulation in ovarian homogenates from the same species, Sire and Dépêche (1981) report a 3% aromatization of androstenedione by ovarian fragments 0 to 15 days after ovulation. Unfortunately, it was not possible to precisely compare the stage of ovarian development in these two studies.

In order to clarify our knowledge of the endocrinological events happening during the first few weeks postovulation, we undertook to follow E_2 production during this period, in relationship with gonadal development.

Assessment of the latter cannot always be achieved accurately by gonadosomatic index (GSI) measurement as has been shown in several species (Delahunty and de Vlaming, 1980; de Vlaming *et al.*, 1982). The ovary is made up of various tissues (after spawning: somatic tissues, oogonia, developing follicles, postovulatory, and atretic follicles) occupying highly variable relative volumes and having different activities. Hence, general weight data such as GSI are insufficient for assessing ovarian development and activity. We chose to evaluate gonadal development using histological determination of the relative volume of the gonad occupied by oocytes in an advanced stage of differentiation, defined as oocytes containing cortical alveoli.

E₂ production was followed at two levels: E₂ plasma concentrations were regularly measured by radioimmunoassay (RIA) and ovarian aromatase activity was evaluated by *in vitro* incubation of ovarian tissue. Androstenedione (A₄) was chosen as the precursor since it has been shown to be metabolized more efficiently than testosterone by trout ovarian aromatase (Sire and Dépêche, 1981; de Monès, 1987). In addition, since several authors studying aromatization obtained divergent results from radioactive and unlabeled precursor metabolism analysis, two isotopes of A₄ were used for our ovarian incubations: inert A₄ leading to E₂ RIA measurement and ¹⁴C-labeled A₄, leading to purification and counting of ¹⁴C-labeled E₂.

MATERIALS AND METHODS

Animals. Sixteen 3-year-old female rainbow trout (weight, 1–2 kg) belonging to a spring strain (spawning, March) maintained by INRA (Gournay-sur-Aronde, Oise) were transferred into the laboratory at least 1 month before the beginning of the experiments. They were kept in a recirculating water system at 10–12° and submitted to a natural photoperiod. During the expected spawning period, fish were checked for ovulation once or twice a week and ovulated animals were stripped of eggs. Ovulation occurred between February 25 and March 18, 1983, that is, 5 to 50 days before

the *in vitro* experiments. Blood was sampled about twice a week as described by Fostier and coauthors (1978).

Fish were killed at various times after ovulation. Their ovaries were quickly removed and weighed before and after removal of atretic and unovulated residual large follicles (total ovary weight and “developing ovary weight”). The ovarian lamellae were cut apart in petri dishes maintained on ice and were used for histological analysis and *in vitro* metabolism studies. For each female, unincubated lamellae were kept to measure initial tissue E₂ concentrations.

Analysis of ovarian development. The relative volume of the gonad occupied by oocytes in an advanced stage of differentiation was used as an indicator of gonadal development. One ovarian lamella per female was fixed in a Bouin–Hollande solution. Three to ten consecutive 10-μm-thick sections every 60 were kept on a slide. They were stained by Heidenhain’s azan (azocarmine G and Heidenhain’s blue) (Gabe, 1968).

The following germ cell types were identified: (1) oogonia and young primary oocytes; (2) oocytes at the “perinucleolar stage” (Wallace and Selman, 1981), some of them displaying basophilic Balbiani bodies; (3) oocytes in advanced stage of differentiation. Three structures appear as oocytes develop: cortical alveoli (stained blue with azan), lipid globules (appearing empty after histological processing), and, later, yolk globules (stained red with azan). Oocytes in advanced stage of differentiation are defined as those in which cortical alveoli are present. Cortical alveoli contain protein and carbohydrate components endogenously synthesized by the oocyte. They have also been called vitellin or yolk vesicles. However, such a designation should be avoided since their content is not used as nutrient reserve for the embryo but is released at the ovule periphery at fertilization (cortical reaction). Yolk globules or bodies (stained red with azan) are membranous vesicles containing a lipoprotein complex derived from vitellogenin internalization in the oocytes. We consider that these granules characterize true vitellogenic oocytes. (4) Postovulatory and atretic follicles.

In order to work on a sample representative of the whole tissue, one section on each slide was analyzed with Haug’s lattice points system (Solari, 1973) (16 to 48 microscopic fields/female, depending on the quantity of tissue fixed; magnification of 63). The percentage of total points superposed over oocytes containing cortical alveoli or their zona radiata, as related to total points superposed over any kind of ovarian tissue, gave an estimation of the total ovarian ratio, and was called ovarian development index (ODI).

In addition, for each female, mean diameter of the largest observed oocyte was measured (referred to as maximal oocyte diameter).

Steroids. Unlabeled steroids used as precursor (an-

drostenedione, abbreviated A₄), as carriers (oestrone, E₁; oestradiol, E₂; testosterone, T; androstenedione), or as reference for radioimmunoassay (RIA) (E₂, A₄) were purchased from Steraloids, Sigma or Merck (recrystallized E₁). Labeled steroids were obtained from Amersham. [4-¹⁴C]Androstenedione (2.2 GBq/mmol) was purified by thin-layer chromatography (dichloromethane:methanol 4:1) before use. [1,2,6,7-³H]A₄, [2,4,6,7-³H]E₂, and [1,2,6,7-³H]T were used as tracers to estimate recovery during incubation analysis.

In vitro incubation of ovarian lamellae. Incubations were carried out in siliconized glass petri dishes containing 5 ml trout balanced salt solution (TBSS; Jalabert and Fostier, 1984). For each female, lamellae from both ovaries were distributed at the rate of one-tenth of "developing ovary weight" (weight of ovaries freed from large unovulated and atretic follicles) per dish (1.4 ± 0.1 g). Androstenedione was added in 30 µl (inert A₄) or 20 µl ([¹⁴C]A₄) ethanol at the beginning of incubation to reach a final concentration of 2 × 10⁻⁶ M (580 ng/ml, about 4 KBq/ml for [¹⁴C]A₄). After 18 hr with constant agitation and under renewed atmosphere of 50% N₂-50% O₂, incubations were stopped by adding 10 ml distilled ethanol. Samples were kept frozen (-20°) until analysis.

Measurement of E₂ and A₄ concentrations in plasmas. Before extraction, tritiated tracers were added to estimate recovery after analysis (2000 dpm). Plasma (0.3 ml) was extracted twice with 2 ml cyclohexane:ethylacetate 1:1. Dry residues were dissolved in 0.3 ml dichloromethane:methanol 95:5 and were chromatographed on Sephadex LH 20 (Pharmacia) columns (φ: 0.5 cm, h: 8 cm) using dichloromethane:methanol 95:5 as eluant. Androstenedione was collected in the first fraction (1.4 ml) along with testosterone, the second fraction was discarded (1.4 ml), and oestradiol was collected in the last 1.6 ml. After evaporation under air, dry residues of the A₄ and E₂ fractions were dissolved in phosphate buffer for radioimmunoassays, which were done according to Fostier and colleagues (1982). Oestradiol and androstenedione antibodies were provided by Dr. Terqui (INRA). Characteristics of the oestradiol assay have been given elsewhere (Fostier *et al.*, 1978; Fostier and Jalabert, 1986). Cross-reactions for androstenedione antibody were: adrenosterone, 50%; 11β-hydroxyandrostenedione, 13%; testosterone, 1%; 11-ketotestosterone, 0.3%; and dihydroepiandrosterone, 0.2%.

Evaluation of aromatase activity from unlabeled A₄ incubations. After addition of 6000 dpm [³H]E₂, incubated lamellae (1.4 ± 0.1 g) were homogenized in their medium (incubation medium - 5 ml - plus ethanol - 10 ml), and unincubated tissues (1.2 ± 0.0 g) in NaCl (7 g/liter; 5 ml) plus ethanol (10 ml). After freezing, homogenates were centrifuged and pellets were reextracted with 80% ethanol. Aqueous ethanolic fractions were pooled, partially evaporated, and then extracted

with dichloromethane (2 × 10 ml). Dry residues were dissolved in 0.5 ml dichloromethane:methanol 95:5 and chromatographed on Sephadex LH 20 (φ: 1 cm × hr: 12 cm). The following fractions were obtained: 3.5 ml, discarded; 2 ml, A₄; 2.5 ml, T; 3.5 ml, E₁; 3.5 ml, discarded; 7 ml, E₂. E₂ and A₄ concentrations were measured by RIA as described above. The difference between E₂ levels in lamellae incubated with A₄ and in unincubated tissues gave an estimate of net E₂ production from exogenous androstenedione during the incubation time; this production being the result of both E₂ synthesis and E₂ further metabolism.

Measurement of aromatase activity from ¹⁴C-labeled A₄ incubations. Steroids were extracted as described above after addition of carriers (A₄ and T, 1 µg; E₁ and E₂, 5 µg) and tracers ([³H]A₄ and [³H]E₂, 40,000 cpm; [³H]T, 20,000 cpm). After final dichloromethane extraction, an aliquot of each sample was counted in order to measure total solvent extractable radioactivity corresponding to unconjugated steroids. Extracts were then chromatographed on Sephadex LH 20 (φ: 1 cm, h: 12 cm). Further purification was obtained by reversed-phase high-pressure liquid chromatography (HPLC, Waters) on a µBondapack-C18 column (φ: 0.4 cm, h: 30 cm) eluted with methanol: water 60:40 for androgens and 55:45 for oestrogens (2 ml/min; temperature, 18-20°). Collected fractions corresponding to each steroid were extracted with dichloromethane. After evaporation under air, dry residues were dissolved in 0.5 ml ethanol and aliquots were sampled for liquid scintillation counting (OCS Amersham, Intertechnique SL 4000 spectrophotometer). Counting yields were estimated by an internal standard method. Data were corrected for recovery. Aromatase activity was appraised from [¹⁴C]A₄ metabolism analysis by the ratio defined by Sire and Dépêche (1981): oestrogen (E₁ + E₂) associated radioactivity × 100/total solvent extractable radioactivity. This took into account E₂ but not E₁ recovery yield.

Data analysis. Mean values were given ± SE (SD_{n-1}/√(n - 1)). For testing correlation significance, percentages were transformed following X' = arcsin √(X × 100). Student's *t* tests were then performed. The relationship between parameters was estimated by calculation of the correlation coefficient (*r*), and its statistical significance was tested (Zar, 1984).

RESULTS

Analysis of Ovarian Development

Experiments were performed 5 to 50 days after ovulation. Maximal oocyte diameter measured from the histological sections for each female ranged between 0.64

and 1.2 mm and increased with time since ovulation ($r = 0.44$, $P < 5\%$). The ODI ranged between 14 and 61% and was highly correlated with time postovulation ($r = 0.82$, $P < 0.05\%$) and with maximal oocyte diameter ($r = 0.76$, $P < 0.05\%$) (Fig. 1). In contrast, no relation could be found between the gonadosomatic index (GSI: ratio of total gonadal weight to eviscerated body) and ODI or time postovulation.

Yolk globules (lipoprotein) were observed in some females, in one of them as early as 1 week postovulation, and were present in greater quantities in the ovaries showing the four highest ODI.

Evolution of Oestradiol and Androstenedione Concentration

In all females ($n = 16$), significant E_2

FIG. 1. Relation between ovarian development index and time postovulation or maximal oocyte diameter in spring-spawning rainbow trout. Ovarian development index corresponds to the relative volume occupied in the ovary by oocytes in an advanced stage of differentiation, defined as oocytes containing cortical alveoli.

plasma levels were measured in the first month postovulation. Individual maximal concentrations ranged from 0.9 to 5.5 ng/ml (mean, 2.1 ± 0.4 ng/ml). In 10 animals, blood was sampled during more than 2 weeks (more than six sampling). For 3 of them (ODI: 54 to 59%, evaluated 35 to 50 days PO), a clear-cut peak was observed 15 to 20 days postovulation (examples in Fig. 2A), whereas for the others (ODI: 37 to 61%; mean, 44%, evaluated 23 to 51 days PO), E_2 levels decreased during that period (examples in Fig. 2B). For all females, the E_2 plasma concentrations subsequently reached basal values (<0.5 ng/ml, often undetectable) maintained for a few days. They started rising again about 1 month postovulation. Correlation of individual maximal E_2 levels with maximal oocyte diameter and with ovarian development index were similar ($r = 0.62$ and 0.59 ; $P < 1\%$).

When analyzed (three fish, 29 samples), A_4 patterns were parallel to those of E_2 ($r = 0.92$; $P < 0.05\%$) (examples in Fig. 2, bottom graphs).

At the time of ovary removal, E_2 plasma levels were lower than 1 ng/ml in all the animals. E_2 in unincubated ovarian lamellae was detectable except in one case (ovaries showing the lowest ODI), ranging from 5 to 86 pmol/g tissue (mean, 18 ± 5 pmol/g tissue; corresponding to 0.22 ± 0.06 nmol/total developing tissue) and were positively correlated with the ODI ($r = 0.62$; $P < 1\%$) but not with maximal oocyte diameter ($r = 0.38$, $P > 5\%$).

Conversion of Unlabeled Androstenedione

E_2 net production from A_4 (concentration after incubation minus concentration in unincubated ovarian lamellae) was 62 ± 17 pmol/g tissue corresponding to 0.67 ± 0.16 nmol/total developing ovarian tissue ($n = 15$) (Table 1). It increased significantly with ovarian development, assessed by maximal oocyte diameter or by ODI (E_2 net production per gram of tissue: respectively, $r =$

FIG. 2. Evolution of oestradiol (E₂) and androstenedione (A₄) levels in the plasma of spring-spawning rainbow trout during the postovulation period. (A) Example of two animals in which E₂ levels peak 15 to 20 days postovulation; (B) example of three animals for which E₂ levels decrease during the same period. The smallest significant differences between concentrations were 0.2 ng/ml for E₂ and 0.7 ng/ml for A₄, as estimated from Solari (1983). The asterisks indicate the date of the last blood sampling and of removal and fixation of the ovaries for the evaluation of the ovarian development index (ODI; see Fig. 1).

0.53, $P < 2.5\%$ and $r = 0.66$, $P < 1\%$; E₂ net production per total developing ovarian tissue: $r = 0.73$ and 0.75 , $P < 1\%$).

Conversion of ¹⁴C-Labeled Androstenedione

In all but one case, less than 1% of initially added androstenedione was recovered after 18 hr of incubation (Table 1), indicating that the androgen was actively metabolized. Conversion to testosterone was generally less than 5%. Oestradiol and oestrone production ranged respectively between 0.3 and 2.6% and 0.1 and 0.4% of initial A₄. Oestrone was 7 to 40% of oestradiol (that is, 6 to 29% of E₂ + E₁) and this proportion tended to decrease with ovarian development ($r = 0.72$; $P < 0.25\%$). Aromatase activity was low (1 to 5%) but significant ($P < 0.05\%$; $n = 15$). Furthermore,

it was correlated with ovarian development index ($r = 0.71$; $P < 0.25\%$) and with maximal oocyte diameter ($r = 0.65$; $P < 0.5\%$).

Conjugation (estimated by the loss of ¹⁴C-radioactivity in organic phase after dichloromethane extraction) varied generally between 20 and 60% of initial A₄ but reached 80% (Table 1). It decreased with increased time from ovulation ($r = -0.65$; $P < 0.5\%$) and with ovarian development ($r = -0.53$; $P < 2.5\%$).

Comparison between the Two Methods for Aromatase Activity Evaluation

Radiolabeled and inert precursor metabolism analysis generally gave close estimates of E₂ production (Table 1). In 9 cases (out of 14) the ratio of the E₂ production estimate obtained from [¹⁴C]A₄ metabolism analysis to that obtained from unlabeled A₄

TABLE 1
METABOLISM OF ^{14}C -LABELED OR UNLABELED ANDROSTENEDIONE (A_4 , 2.10^{-6} M) BY POSTOVULATORY OVARIAN TISSUES OF 16 FEMALES, AFTER 18 hr OF INCUBATION

ODI (%)	Conjugation (%)	Precursor: [^{14}C] A_4						Precursor: A_4
		A_4 (%)	T (%)	E_1 (%)	E_2 (%)	Aromatase activity (%)	[^{14}C] E_2 production (nmol/total developing tissue)	E_2 net production (nmol/total developing tissue)
14.2	57.3	0.43	1.81	0.12	0.30	1.00	0.82	0.11
16.8	—	—	—	—	—	—	—	0.00
24.6	61.7	0.26	1.39	0.15	0.65	2.07	0.55	0.67
27.9	19.9	0.30	4.01	0.13	1.07	1.51	0.92	0.14
30.4	65.0	0.18	0.98	0.10	0.28	1.11	0.20	0.06
31.0	80.7	0.15	0.56	0.09	0.33	2.16	0.29	0.04
37.0	63.5	0.24	1.42	0.21	0.91	3.05	0.73	0.77
39.0	32.1	0.37	2.39	0.35	1.22	2.30	0.67	0.37
45.4	32.0	3.34	16.8	0.28	1.39	2.46	1.26	1.31
47.9	48.7	0.77	4.51	0.30	1.90	4.28	1.57	1.07
51.5	35.5	0.27	0.98	0.25	1.10	2.09	0.94	0.61
54.0	30.0	0.49	5.08	0.13	0.96	1.56	0.91	1.27
56.8	29.5	0.48	3.64	0.39	2.97	4.77	1.53	0.60
57.8	30.2	0.55	4.57	0.34	1.95	3.28	1.79	—
60.5	37.4	0.61	0.82	0.17	2.55	4.33	2.59	2.14
—	22.9	0.97	2.35	0.43	2.11	3.30	1.31	0.88

Note. Ovarian development of each animal was assessed by the ovarian development index (ODI; see Fig. 1). The conjugation and quantity of steroids isolated in the incubates (androstenedione, testosterone, T; oestrone, E_1 ; oestradiol, E_2) were expressed as a percentage of initially added [^{14}C] A_4 and aromatase activity as a percentage of extractable radioactivity. [^{14}C] E_2 and E_2 net production corresponds to the quantity of E_2 which would have been produced by total developing ovarian tissue (ovaries freed from large unovulated and atretic follicles) as estimated from ^{14}C -labeled and inert A_4 metabolism analysis.

metabolism analysis ($[\text{C}] \text{E}_2/\text{E}_2$) ranged between 0.7 and 1.8. In the 5 other cases, HPLC analysis of [^{14}C] A_4 incubates led to estimates more than twice the RIA estimates, the [^{14}C] E_2/E_2 ratio reaching 2.6 to 7.7. The correlation between the two series of estimates was high ($r = 0.82$; $P < 0.05\%$).

DISCUSSION

In the present study, ovarian development and oestradiol production were followed in spring strain rainbow trout during the first 2 months PO. Ovarian development was evaluated using different criteria: GSI, maximal oocyte diameter, and relative volume of the gonad occupied by oocytes in an advanced stage

of differentiation (containing cortical alveoli), assessed by the ODI. GSI could neither be correlated to any histological, metabolic, or endocrinological criterion nor to the time since ovulation confirming its unreliability for assessment of gonadal development, at least during this period of the cycle. Maximal oocyte diameter and ODI evolved concurrently. However, correlation of endocrinological criteria with ODI was almost always higher than with maximal oocyte diameter. The former was also significantly more correlated to the time postovulation. Thus, though only one ovarian lamella per animal was fixed, which may result in any heterogeneity in the ovaries being missed, ODI appears to be the best indicator of ovarian development during this period, presumably be-

cause it takes into account qualitative (oocyte type) as well as quantitative aspects.

Oogenesis in the animals we used was much more advanced than reported in other works for the same or related species after a similar time postovulation (*Salmo gairdneri*, Lambert *et al.*, 1978; *S. trutta*, Breton *et al.*, 1983): maximal oocyte diameter was quite elevated (0.6 to 1.2 mm) and vitellus (lipoprotein) was incorporated in oocytes very soon after ovulation (in one female, as early as 1 week PO). In contrast, Lambert and colleagues (1978) report a maximal oocyte diameter of 0.3 mm during the first 6 months PO and observe yolk globules in the ovaries only 1 month later. Similarly, in *S. trutta*, no vitellus was seen before 5 months PO (Breton *et al.*, 1983). Characteristics of our strain could be responsible for these differences in oogenesis kinetics since animals studied by Lambert and colleagues and Breton and colleagues were winter strain trouts. Indeed, Jalabert and Fostier (unpublished results), working on autumn and spring strain animals, measured larger follicles in the latter after a similar time postovulation. However, high maximal oocyte diameters (0.4 to 0.8 mm) were also measured in winter strain animals 0 to 2 months PO (Sire and Dépêche, 1981), indicating that other factors are involved.

The fact that developed follicles can be observed almost immediately after ovulation suggests that oocytes have started their growth before ovulation. Several observations corroborate this idea. Billard (1987) notes, in brown trout, an increase in the absolute volume of the population of oögonia and small oocytes (before the appearance of lipid globules) starting before ovulation. Whitehead and Bromage (Bromage *et al.*, 1982) report an influence of photoperiod during one cycle on the ovarian development during the next cycle. Finally, Busson-Mabillot (1984) indicates that full cytoplasmic differentiation of brown trout oocytes takes about 2 years (which could

explain why females mature 1 year later than males).

As in a previous preliminary study in which 50% of exogenous testosterone was conjugated by follicle envelopes from almost mature oocytes (stage: peripheral germinal vesicle as defined by Jalabert and Fostier, 1984) (de Monès, 1987), conjugation of A₄ or its metabolites by postovulatory ovarian tissue was very important. Testosterone glucuronide (TG) has been identified and appears to be the prevailing steroid conjugate in Atlantic salmon serum (So *et al.*, 1985) as well as in ovarian incubates from African catfish (Lambert and van den Hurk, 1982) and common carp (Manning and Kime, 1984). E₂ glucuronide is not reported in salmonids, as far as we are aware, and its production in carp is often low. The highest levels of TG were reported in the last stages of ovarian development or shortly after ovulation. Moreover, we show here that conjugation diminishes with time since ovulation. Thus, in trout, glucuronosyl transferase possibly appears in follicles near the end of vitellogenesis and persists in postovulatory follicles (POFs), progressively disappearing with the latter. Such a pattern would support the hypothesis of a pheromonal role of glucuronides (Colombo *et al.*, 1982; van den Hurk *et al.*, 1982).

High levels of conjugates implied that only part of the A₄ was available for aromatization. Expressing aromatase activity as a percentage of initially added A₄ would then have led to a bias. That is why, to limit error, we expressed it as a percentage of total extractable radioactivity, as proposed by Sire and Dépêche (1981).

Both labeled and unlabeled androstenedione were used for aromatase activity evaluation. Radioactive precursor metabolism analysis (by chromatography including reverse-phase HPLC) gave similar or higher estimates than did unlabeled androstenedione metabolism analysis (by RIA following one chromatographic step). This

result is opposite to those reported in other studies (Tcholakian and Steinberger, 1979; Ursely *et al.*, 1981) where RIA appeared to overestimate aromatase activity, possibly by lack of specificity. In our work, interference of lipids in RIA (Jawad *et al.*, 1981) and isotope effect on metabolism and/or analysis (Kelly *et al.*, 1979) might be responsible for the discrepancy between some of the estimates.

We show here the presence of an aromatase activity in the postovulatory ovary. Measured percentages of A_4 conversion to E_1 and E_2 were low but significant and were in keeping with those reported by Sire and Dépêche (1981). Moreover, preliminary results (data not shown) indicate that the gonad contains, at that period, all the enzymes necessary to produce oestradiol from pregnenolone. Oestradiol production from both ^{14}C -labeled and radioinert androstenedione was correlated with ODI indicating that aromatization potential increases concurrently with ovarian development.

In fish in which oocytes develop synchronously, low and stable E_2 plasma levels are generally reported for several weeks or even for a few months after ovulation (Scott *et al.*, 1980; Tam *et al.*, 1986; Singh and Singh, 1987). However, the sampling rate in those studies was usually about one per month and E_2 levels were measured throughout the year leading to a wide range of concentrations that does not reveal small peaks or depressions. Furthermore, data reported are almost exclusively means of measurements from several fish. However, Whitehead and colleagues (1983) demonstrated a slight elevation of E_2 (3.6 ng/ml) about 1 month PO in rainbow trout. Using individualized and more frequent sampling over a short period of time, we also observed, in some animals, a transient increase of E_2 plasma concentration within 1 month PO. This rise, though of about the same amplitude as the one reported by Whitehead and colleagues, was far less important than the one detected by Jalabert

and Fostier, reaching about 50 ng/ml (unpublished data). This could be accounted for by our and Whitehead and colleagues use of animals spawning at a period when photophase increases whereas Jalabert and Fostier dealt with a strain of fish spawning in November–December. Bromage *et al.* (1982) showed that acceleration of photoperiodic cycle determined lower E_2 peak concentrations. Such an effect of photoperiod could be responsible for the discrepancy between our data and those of Jalabert and Fostier. Yet we cannot exclude variations from 1 year to another (see for example Lamba *et al.*, 1983), simple individual variations (in our study, E_2 peak was indeed an irregular phenomenon), and variations due to sampling frequency. Zohar *et al.* (1986) showed short term fluctuations of E_2 plasma levels during advanced exogenous vitellogenesis. Though such fluctuations were not detected during early exogenous vitellogenesis, it must be underlined that only a few winter strain females were studied for that period, which was located 2 to 3 months PO.

In our animals, a constant feature was the decrease of E_2 concentrations which reached basal levels 20 to 30 days PO. Contrast between increasing aromatization potential, rise of E_2 ovarian concentrations, and evolution of plasma E_2 levels could have several origins. The increase of oocyte cytoplasm relative volume, part of which is lipidic and can retain weakly polar steroids (Colombo and Colombo Belvedere, 1976; Antila, 1985; de Monès, 1987), could favor E_2 sequestration in the ovary and could explain the increase of E_2 tissue concentrations. E_2 plasma levels are regulated by the peripheral metabolism. The oestradiol metabolic clearance in rainbow trout is higher during previtellogenesis than later during advanced vitellogenesis (Baroiller *et al.*, 1987). Finally, E_2 production is modulated by precursor availability. Indeed, E_2 levels were strongly correlated with those of androstenedione. Postovulatory follicles (POFs) synthesize very little

or no E₂ but produce androgens, especially as vitellogenic follicles, under GtH stimulation (Kagawa *et al.*, 1983; van der Kraak and Donaldson, 1986). The decrease of A₄ and E₂ plasma levels could be explained by the drop of GtH plasma levels after their PO peak (Jalabert and Breton, 1980; Fostier *et al.*, 1981) and by the short life span of POFs, if they are the major androgen producers.

E₂ produced early and accumulating in the ovary could act at several levels to favor oogenesis. The *in vivo* positive effect of oestrone on oogonia proliferation was recorded (Bullough, 1942; Mackay, 1973). A steroid action on oogonia proliferation was proposed again in goldfish by Khoo (1975). However, such an effect is probably indirect since E₂ does not stimulate oogenesis in goldfish ovarian explants (Remacle *et al.*, 1976). Moreover, in our study, though oestradiol was present in ovaries, no mitosis could be observed. In addition, preliminary experiments with colchicine (blocking mitosis in metaphase) on the same gonads failed to show cell divisions (data not shown). Apart from an effect on mitosis, a specific action of E₂ on oocyte development is possible. Indeed, Khoo (1979) showed that oestrogens control the formation of cortical alveoli (which he calls yolk vesicles) in hypophysectomized goldfish. Furthermore, Sundararaj and Nath (1981) showed that repeated injections of E₂ evoke an amplification of vitellogenin synthesis stimulation. Thus, plasma E₂, especially when a transient rise is observed, could prime the liver for vitellogenin production. It might also have had a direct stimulatory effect since we observed yolk globules in the oocytes of some animals, especially those having shown the highest maximal E₂ levels and displaying the highest ODI. Finally, E₂ could indirectly participate in ovulated egg survival in the abdominal cavity (Lam *et al.*, 1978, 1979).

In conclusion, the first months following ovulation are a period of endocrinological and morphological events that must not be

underestimated. Oogenesis in our animals was very advanced, even a few days after ovulation. This suggests that young follicles have started their development prior to ovulation, as has been proposed by other authors. The discrepancy in ovarian stage and activity between our study and those reported by other authors might be linked with the use of spring strain animals whose spawning time is shifted with regard to photoperiodic cycle in comparison with standard winter strain animals. However, other internal and external factors are probably involved.

The oestradiol production potential in the ovary was demonstrated from the first days PO. It was low but increased with ovarian development. Though E₂ plasma levels did not increase with time, the oestrogen appeared to accumulate in the gonads. Conjugation of A₄ was important but decreased with time postovulation. It is inferred that, while conjugates and part of oestrogen precursors are produced by POFs, oestradiol is synthesized in developing follicles.

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