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Steroid conversion by oocytes and early embryos of Salmo gairdneri

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Oocytes obtained freely from coeloma of breeding *Salmo gairdneri* and embryos from cleavage to early somite stages (aged between 0 and 8 days at 10 C, stages 2-11) were incubated for 2, 4 or 15 hours with radioactive pregnenolone, progesterone, dehydroepiandrosterone or estradiol-17β. The accumulation of radioactivity in embryos increased during incubation to over 50 % of the dose (about over 0.08 nmol/embryo. The yolk and chorion did not affect this accumulation, as shown by comparison of intact to yolk-free embryos. In all incubations the radioactivity was retained by oocytes more effectively than by embryos. Thin layer chromatographic analysis from embryos and medium showed minor conversion to metabolites in all cases. In embryos pregnenolone was slowly converted to progesterone. Progesterone was further reduced to 5α- and 5β-pregnanedione by oocytes as well as by embryos. In contrast to earlier results obtained with other vertebrates, rainbow trout embryos appeared to lack Δ^4-steroid hydroxylases.

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1. Introduction

The ovary of teleost fishes is a rich source of a variety of steroids besides estrogens. Recently attention has been focused on the hormonal regulation of oogenesis and spawning, especially in the rainbow trout, *Salmo gairdneri*. During the maturation period three days before ovulation the plasma levels of androgens and their reduced derivatives are high (27.6-167.3 ng/ml), while the levels of estradiol-17β, pregnenolone, progesterone and 17α-hydroxy-20β-dihydroprogesterone are low (1.2-6.6 ng/ml). The amounts of these steroids, obtained after ovulation, are significantly higher (42.2-245.7 ng/ml) (Diederik & Lambert 1982). The maintenance of high levels of androgens and low levels of estradiol-17β might contribute to the establishment of an adequate steroid environment for oocyte maturation (Zohar et al. 1982).

Steroidogenesis is known to take place in the granulosa and stromal cells in the ovary of *Salmo gairdneri* (Lambert et al. 1978). The definitive anatomical proximity to developing oocytes might answer a need for functional coordination. Indeed, control over meiotic maturation and ovulation has been shown to be a function of gonadal steroids (Colombo et al. 1979).

Histochemical studies of the oocytes have indicated intracellular conversion of steroids. Lambert (1970) has demonstrated 3α- and 17β-hydroxysteroid dehydrogenase (HSD) activities on the peripheral ooplasm of older yolk-loaded oocytes of the guppy, *Poecilia reticulata*. Biochemical studies with amphibian oocytes have demonstrated 5α-reductase, 3βHSD and 20αHSD in *Rana pipiens* (Reynhout & Smith 1973) and 5α-reductase, 3αHSD and 3βHSD in *Pleurodes waltlii* (Ozon et al. 1975). The question as to whether these enzymes are significant for the developing oocyte has not been answered. It is noteworthy that steroid metabolism is also found in mammalian spermatozoa (Rajalakshmi et al. 1983) and in germ line tumor cells (Antila & Wartiovaara 1980, O'Hare et al. 1981, Antila et al. 1983). Not only the oocytes but also the early embryos of amphibians (Antila 1977), blastoderms of the chick (Parsons 1970, Antila et al. 1984) and the blastocysts of some mammals (Bullock 1977), metabolize steroids.

Oocytes and embryos of *Salmo gairdneri* provide a useful tool for testing *in vitro* whether steroids are metabolized during early

development in fishes. We therefore incubated follicle-free oocytes recovered from coeloma and early embryos from the cleavage stages to the 20 pair-of-somites stage with various ^{14}C-labeled steroids.

2. Material and methods

2.1. Animals

2- to 3-year-old rainbow trout, *Salmo gairdneri* strain A13, KLK or KLT bred at the Laukaa Fish Culture Research Station in Central Finland were ovulated and the eggs fertilized in a hatchery, in February or in May. The eggs and early embryos were cultured in flowing natural lake water the temperature of which was 10° C. Eggs were collected manually using a glass pipette. The developmental stages used were freshly ovulated oocytes obtained from coeloma, and embryos from early cleavage stages (stages 2–5) to the 20 pair-of-somites stage (stage 11). The stages were determined under a dissecting microscope in accordance with the normal embryonic stages for salmonid fishes described by Ballard (1973).

2.2. Chemicals

All solvents used were of analytical grade and they were redistilled before use. Cold reference and carrier steroids were purchased from Sigma Chemical Co. (St. Louis, Mo.), Research Plus Inc. (Bayonne, N.J.) Steraloids Inc. (Croydon, England) or were obtained as gifts from the M. R. C. Steroid Reference Collection (London, England) by courtesy of Prof. D. N. Kirk. The following tritium labeled steroids were obtained from the Radiochemical Centre (RC, Amersham, England) or from New England Nuclear (NEN, Boston, Mass.): [7-^{3}H]pregnenolone, 10 Ci/mmol, [1,2,6,7-^{3}H]progesterone, 81 Ci/mmol, [1,2-^{3}H]5α-pregnanedione, 55.7 Ci/mmol, [1,2,6,7-^{3}H]androstenedione, 87 Ci/mmol, [1,2-^{3}H]dehydroepiandrosterone, 40.2 Ci/mmol, and [2,4,6,7-^{3}H]estradiol-17β, 115.0 Ci/mmol. The 4-^{14}C-labeled steroids were: pregnenolone, 57.2 mCi/mmol (NEN), progesterone, 57.2 mCi/mmol (NEN), dehydroepiandrosterone, 55,0 mCi/mmol (RC) and estradiol-17β, 52.0 mCi/mmol. The purity of labeled steroids was checked before use by thin layer chromatography.

The trivial and IUPAC names of the steroids used were as follows:

androstenedione	4-androstene-3,17-dione
dehydroepiandrosterone (DHA)	3β-hydroxy-5-androsten-17-one
estradiol-17β	1,3,5,(10)-estratriene-3,17β-diol
5α-pregnanedione	5α-pregnane-3,20-dione
5β-pregnanedione	5β-pregnane-3,20-dione
pregnenolone	3β-hydroxy-5-pregnen-20-one
progesterone	4-pregnene-3,20-dione
testosterone	17β-hydroxy-4-androsten-3-one

2.3. Incubation

Oocytes obtained freely from coeloma and embryos were incubated in groups of 30 intact individuals per 10 ml of Cortlands saline and of lake water, respectively, or 10 embryos, prepared free from membranes and yolk, per 10 ml of Cortlands solution. Incubations with ^{14}C-labeled steroids were carried out for 2, 4 or 15 hours at 10° C under oxygen saturation. The substrates were dissolved in 50 μl of ethanol-propylene glycol solution (1:1, v/v). The final concentration of substrates in incubations was 3.8–5.0 nmol/10 ml. Controls were established by incubating lake water alone in the same way with substrates, but with a doubled incubation time. Ovarian follicles were incubated as a source control for oocyte metabolism. After incubation, medium and embryos/oocytes were separated and embryos/oocytes were washed three times with 2 ml of Cortlands solution. Finally, 1.5 ml acetone was added to both medium and embryos/oocytes.

2.4. Extraction, purification and characterization of steroids

Radioactive steroids were extracted with diethyl ether (Antila 1977). The lipids derived from embryos were precipitated in cold 70 % methanol as described by Saure (1973). The supernatant liquid was evaporated and dissolved in eluting solvent, methanol-water-chloroform (9:1:2) for further purification in a Lipidex-5000 column. This method was slightly modified after Axelson et al. (1974). The recovery of radioactivity extracted from the embryos was measured from aliquotes by liquid scintillation counting using a Wallac Decem NTL^{314} counter (Turku, Finland) before precipitation and after column chromatography. The recovery of radioactivity extracted from the medium was similarly measured after extraction. Both extracts were submitted to thin layer chromatography (TLC) for the separation of metabolites (Saure 1973). Lipids still remaining in samples were first eluted on the same TLC plates by toluene-cyclohexane, 1:1 (Lambert & van Bohemen 1979). The solvent system for the first separation in TLC was acetone-chloroform, 15:85 for pregnanes and diethyl ether-chloroform, 1:3 for DHA and estradiol-17β incubations. The radioactivity was detected and quantified on TLC-plates by a TLC-scanner (LB 2721, Berthold Co., Wildbad, G.F.R). Reference steroids were visualized by their U.V.-absorption (240 nm) and/or as fluorescence after spraying with *p*-toluene sulfonic acid (20 % in ethanol).

The metabolites on TLC-plates were divided into fractions which were numbered in order of decreasing polarity. The radioactive metabolites were further characterized by rechromatography of pooled fractions in different solvent systems. Identifications were accomplished by recrystallization (Axelrod et al. 1965) or by TLC to constant specific activity (CSA) or constant isotope ratio (CIR), as previously described (Antila 1977).

The solvent systems used in TLC were as follows:
A acetone:chloroform, 15:85
B benzene:ethanol, 9:1
C cyclohexane:ethyl acetate:ethanol, 45:45:10
D chloroform:ethanol, 9:1
E ethyl acetate:*n*-hexane:ethanol:acetic acid, 144:27:9:20
F chloroform:ethanol, 19:1
G benzene:ethanol, 19:1
M chloroform-diethyl ether, 3:1
P cyclohexane:ethyl acetate, 1:1
Q benzene:ethanol, 4:1

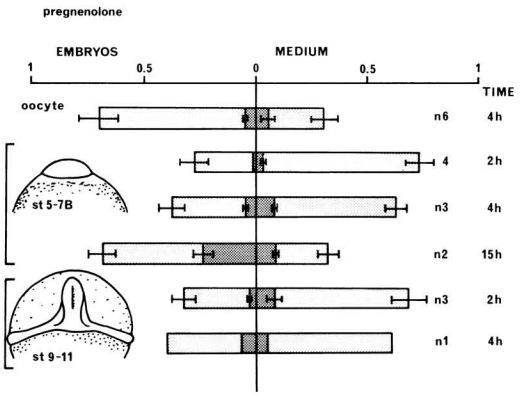

Fig. 1. The relative distribution of recovered radioactivity in pregnenolone incubations with follicle free oocytes (st O) and intact embryos. Radioactivity accumulated in yolk-free embryos (10/incubation, in 4 h) 14.2±3.6 % of the dose ($n = 5$, about 0.07 nmol/embryo). Accumulation here in intact embryos 35.3±7.7% of the dose ($n = 4$, about 0.06 nmol/embryo in 4 h). Symbols: thick dots = the metabolite, thin dots = the substrate, bars = standard deviation of the mean relative distribution on TLC.

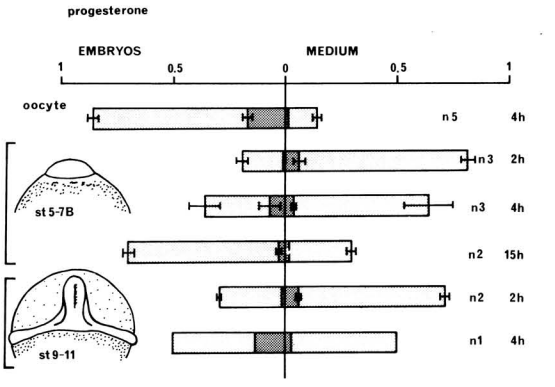

Fig. 2. The relative distribution of recovered radioactivity in progesterone incubations with follicle-free oocytes (st O) and intact embryos. Radioactivity accumulated in yolk-free embryos (10/incubation 4 h) 18.8 % of the dose ($n = 1$, about 0.09 nmol/embryo). Accumulation here in intact embryos 34.3±11.7 of the dose ($n = 3$, about 0.05 nmol/embryo in 4 h). Symbols: see legend in Fig. 1.

3. Results

Recoveries as a percentage of dose in the diethyl ether phase (free phenolic and neutral steroids) after extraction with pregnenolone as substrate were 82.9±10.5, with progesterone 85.0±9.2, with DHA 77.6±12.1 and with estradiol-17β 87.8±6.9 respectively. Radioactivity recovered from oocytes and embryos decreased by about 15 % after purification with cold methanol and the Lipidex-column. The precipitated residues studied in control TLC showed a similar distribution of radioactivity similar to the purified sample but with poorer separation into fractions. Radioactivity recovered in the water phase was found to be below 3 %.

3.1. Pregnenolone incubations

The following incubations were carried out: 13 with intact embryos ranging from stage 5 to stage 11, 5 with yolk-free stage 2–5 embryos, 6 with coelomic oocytes and 2 with follicles as source controls for early intact embryos.

The analysis of metabolism in embryos (Figs. 1 and 4) showed that pregnenolone is slowly converted to apolar metabolites and to two metabolite fractions more polar than itself. Two-dimensional and repeated TLC with authentic carriers revealed that only part of the apolar metabolite peak was progesterone. This was confirmed by TLC to CIR, as shown in Table 1. Neither of the two polar metabolite fractions were 17α-hydroxylated derivatives of pregnenolone (3β,17α-dihydroxy-5-pregnen-20-one, 3β,17α-dihydroxy-5α-pregnan-20-one, 3β,17α-dihydroxy-5β-pregnan-20-one). Pregnenolone itself was found in fraction 4 which was chromatographed to CIR with authentic tritiated carrier (Table 1). Yolk-free embryos showed similar patterns of metabolism. The accumulation of radioactivity per individual was still of the same order as in intact embryos. Metabolites in oocyte incubations remained unidentified. In follicles metabolism was very marked, the main metabolite being a very polar one which cochromatographed with that found in incubations with embryos.

3.2. Progesterone incubations

The following incubations were carried out: 11 with intact embryos ranging from stage 5 to stage 11, 1 with yolk-free stage 2–5 embryos and 5 with coelomic oocytes.

Progesterone was converted to metabolites rather ineffectively although it was incorporated into embryos fairly well. Radioactivity

Table 1. Identification of TLC fractions by repeated chromatographies to constant isotope ratio. For chromatography systems see Material and methods.

Substrate	Fraction		Carrier/derivatization	Before	After	Chromatography Isotope ratio $^3H/^{14}C$				Systems
	No	$\times 10^3$ dpm		$\times 10^3$	3rd dpm	0	1st	2nd	3rd	
pregnenolone	6	30.0	progesterone	43.5	10 .8	1.356	1.207	1.248	1.164	B,C,D
pregnenolone	4	1187	pregnenolone	2159	1335	0.882	0.934	0.934	0.971	C,B,D
progesterone	6	2829	progesterone	4347	2218	0.792	0.819	0.792	0.790	C,B,D
progesterone	6	90.7	reduction	142	25	0.805	0.707	0.715	0.718	D,B,G
progesterone	5-6	498	progesterone	1243	690	1.068	0.959	0.889	0.894	C,B,D
progesterone	7	5.3	5α-pregnanedione	24.7	13.9	1.525	1.204	1.251	1.242	P,D,C
DHA	3	427	DHA	943	669	0.992	1.022	1.031	1.020	C,E,Q
DHA	5	58.9	androstenedione	112.3	86.3	1.16	0.911	0.913	0.883	C,E,Q
			reduction	82.6	28.1	0.882	0.836	0.815	0.837	C,E,Q
estradiol-17β	3	160	estradiol-17β	625	257	1.376	2.087	1.949	1.899	Q,C,E

accumulation in embryos in 15 hours was up to 70.5 % of the dose, which means about 0.1 nmol/embryo (Figs. 2 and 5). The only metabolites found were more apolar than progesterone. The apolar fraction 7 was divided into two subfractions, 7a and 7b, which cochromatographed in repeated and two-dimensional TLC with authentic 5α- and 5β-pregnanedione. 7a was chromatographed to CIR with tritiated 5α-pregnanedione and 7b was recrystallized to CSA with authentic 5β-pregnanedione (Table 1 and 2). The main fraction of all incubates, fraction 6, was chromatographed to CIR with authentic tritiated progesterone (Table 1). Coelomic oocytes and yolk-free embryos showed a similar pattern of metabolism. The accumulation of radioactivity per individual was still of the same order in yolk-free embryos as in those with yolk (intact embryos). However, the accumulation of radioactivity in coelomic oocytes was more than twofold compared to stage 5-7B.

3.3. DHA incubations

Four incubations were carried out with intact embryos of stages 5 to 7B. One incubation was performed using coelomic oocytes and one using follicles. DHA was converted to metabolites very ineffectively although it was incorporated into the embryos. A similar pattern of metabolism was obtained in the coelomic oocytes, but here too the accumulation of radioactivity was higher than in embryos (Fig. 3). In contrast to these, follicles effectively converted DHA to androstenedione (Table 1) and to other metabolites.

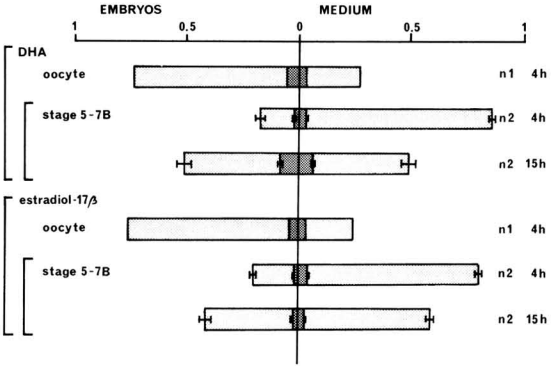

Fig. 3. The relative distribution of recovered radioactivity in DHA and estradiol-17β incubations with follicle free oocytes (st O) and intact embryos. Symbols: see legend in Fig. 1.

3.4. Estradiol-17 β incubations

Four incubations were made using intact embryos of stages 5 to 7B and one incubation with coelomic oocytes.

Practically no conversion of estradiol-17β to metabolites was obtained. This was not due to poor incorporation into embryos, as can be seen from Fig. 3.

4. Discussion

The results show that oocytes and early embryos (from cleavage to 20 pair-of-somites stage) of *Salmo gairdneri* are able to accumu-

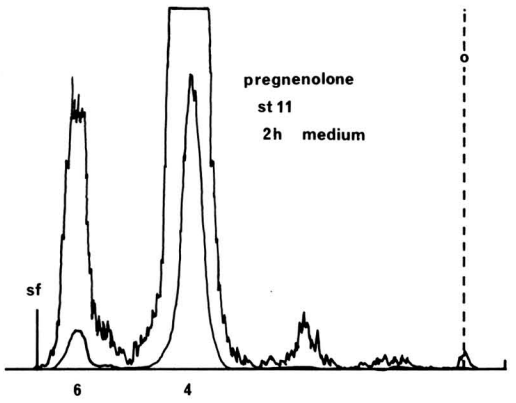

Fig. 4. A TLC-gram showing the distribution of radioactivity recovered from the incubation medium of stage 11 embryos after incubation of 2 h with pregnenolone. Sf = solvent front, striped line = origin, 4 = pregnenolone.

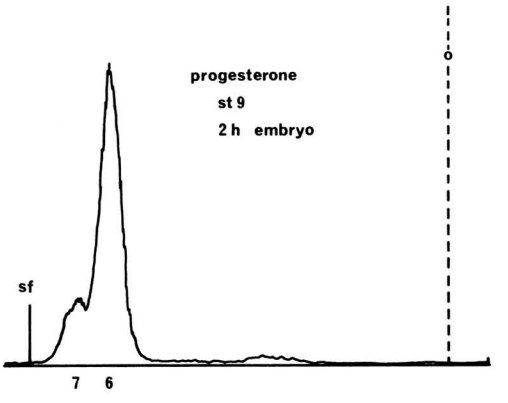

Fig. 5. A TLC-gram showing the distribution of radioactivity recovered from embryos of stage 9 after incubation of 2 h with progesterone. Sf = solvent front, striped line = origin, 6 = progesterone, 7 = 5α- and 5β-pregnanedione.

late from the environment and convert to metabolites a variety of steroids. Accumulation was apparently independent of yolk and chorion, as shown by studies with yolk-free embryos. Intact embryos showed patterns of accumulation and metabolism similar to those of yolk-free embryos.

Radioactivity was increased in embryos during incubation to over 50 % of the dose, which means an increase of about 0.08 nmol/embryo. With all substrates (pregnenolone, progesterone, DHA and estradiol-17β) radioactive steroids were retained more effectively by coelomic oocytes than by the later stages studied. This difference could reflect the possible importance of maternal steroids to the maturing oocytes.

Lambert (1970), who suggested that steroids synthesized in follicle cells may be transported to the oocyte, found at the periphery of yolk-laden oocytes of the guppy (*Poecilia reticulata*) 3α- and 17βHSD-activities which could be involved in the intermediary metabolism of steroids. He stated that the absence of $\Delta^{4}3\beta$HSD seems to exclude the possibility of steroid hormone synthesis in the oocyte. Similarly, in the present study low $\Delta^{4}3\beta$HSD activity was demonstrated only in the embryos of the rainbow trout at the late cleavage to the early somite stage (stages 5–11 by Ballard 1973), not in the oocytes. Although this difference may be related to the sensitivity of the methods, it suggests that this key enzyme in steroidogenesis is expressed during early development well before gonadal differentiation. A similar situation is found in blastoderms of the chick embryo (Antila et al. 1984) and in the blastulae of amphibians (Antila 1977). The occurence of $\Delta^{4}3\beta$HSD in early mammalian embryos is more complicated — $\Delta^{4}3\beta$HSD may be involved in implantation and maternal recognition of pregnancy (Dickmann et al. 1976, Bullock 1977, Flint et al. 1979, Heap et al. 1979).

The role of steroids in the preimplantation embryo and at the time of implantation has been an issue of considerable debate and it has become evident that differences between species exist in the origin and metabolism of steroids (Antila et al. 1977, Dickmann et al.

Table 2. Identification of 5β-pregnandione by recrystallizations to csa. Solvent system in recrystallizations: methanol-water

Substrate	Fraction No.	$\times 10^3$ dpm	Carrier	mg before	After 3rd	Crystallizations 0	1st	2nd	3rd
progesterone	7b	14.8	5β-pregnanedione	12.8	5.9	1.160	1.345	1.230	1.246

1976, Flint et al. 1979). However, biochemical evidence suggests the presence of aromatase enzyme systems in rabbit blastocysts (George & Wilson 1978, Wu & Lin 1982). Studies by Paria et al. (1984) have indicated that embryonic estrogen might be required for supporting differentiation and metabolic functions in blastocysts, but steroidal precursors may have a maternal origin, as shown for rabbit blastocyst progesterone (Fujimoto & Sundaram 1978).

In the present study 5α- and 5β-reduction is demonstrated as the only progesterone processing activity in the oocytes and embryos of the rainbow trout. Activities found in embryos could be directly derived from the oocyte stages, but they persist during the whole embryonic period studied. Reductions of the A-ring are known to alter the biological activity of a steroid. Ozon et al. (1975) have suggested that the 5α-reductive pathway of progesterone in oocytes of *Pleurodeles waltlii* can be interpreted as an inactivation process which functions as an intracellular regulation mechanism. Levere et al. (1967) have earlier observed that certain 5β-reduced androstanes and pregnanes stimulate hemoglobin synthesis in chick blastoderms *in vitro* but that 5α-reduced derivatives have only a weak or no effect at all. It is supposed that the ratio of 5α/5β-reduction shares in regulation of the function of a particular steroid. It is also known that during development this ratio and predominancy are constantly changing (Lantos 1981, Conzales et al. 1983). In the present study possible changes in the ratio of 5α/5β-reduction were not analyzed. 5β-reduction of C_{19}-steroids has been shown to predominate in bovine blastocysts (Chenault 1980) and in chick blastoderms (Parsons 1970) but in rabbit blastocysts androgens were 5α-reduced and progesterone was readily reduced to 5β-metabolites (Singh & Booth 1978). However, 5α-reduction of progesterone predominates in chick blastoderm (Antila et al. 1984). There always remains the possibility that the 5α/5β-ratio might be induced by the *in vitro* conditions imposed. Hence, we have not paid attention to this aspect.

In contrast to amphibian (Antila 1977) and avian embryos (chick, Antila et al. 1984) the fish embryos (rainbow trout, *S. gairdneri*) seem to lack Δ^4-steroid hydroxylases, specially 17α-hydroxylase. This could mean that the genetic program for steroid metabolizing enzymes is expressed, in this respect, in the early development of fishes to a different extent than in other orders of vertebrates. In the mature gonads of all vertebrates the genetic program, for instance for steroid 17α-hydroxylase, is always expressed (Colombo et al. 1979).

Proposals for the physiological role, if any, of the early steroid metabolism in embryos of the rainbow trout can be summarized for all vertebrates, fishes, amphibians and amniotes alike, as follows:

— a remnant of the oocyte stages, significant in intermediary metabolism of steroids during oocyte growth and maturation (intracellular regulation, Ozon et al. 1975)

— production of biologically active compounds acting during differentiation (5β-reduced pregnanes and androstanes in hemoglobin synthesis in chick blastoderm, Levere et al. 1967, estradiol-17β in differentiation of rabbit blastocyst, Paria et al. 1984)

— inactivation of harmful steroids (toxic levels of progesterone, Singh & Booth 1978)

— insignificant background activity representing basal levels of genetic expressions (Antila 1977, Antila et al. 1984).

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References

Antila, E. 1977: Early steroid metabolism in Xenopus laevis, Rana temporaria and Triturus vulgaris embryos. — Differentiation 8:71-77.

Antila, E., Fotsis, T., Wartiovaara, J. & Adlercreutz, H. 1983: Steroid metabolism in human teratocarcinoma cell line PA 1. — J. Steroid Biochem. 19:1583-1590.

Antila, E., Koskinen, J., Niemelä, P. & Saure, A. 1977: Steroid metabolism by mouse preimplantation embryos in vitro. — Experientia 33:1374-1375.

Antila, E., Leikola, A. & Tähkä, S. 1984: Early steroid metabolism by chick blastoderm in vitro. — Steroids 43(3).

Antila, E. & Teräväinen, T. 1974: Metabolism of exogenous 4-^{14}C-progesterone during early development of Xenopus laevis. — Differentiation 2:137-141.

Antila, E. & Wartiovaara, J. 1980: Metabolism of steroids by mouse teratocarcinoma cells. — J. Steroid Biochem. 13:431-437.

Axelrod, L. R., Matthijssen, C., Goldzieher, J. W. & Pul-

liam, J. F. 1965: Definitive identification of microquantities of radioactive steroids by recrystallisation to constant specific activity. — Acta Endocrinol., Copenhagen, Suppl. 99:7-65.

Axelson, M., Schumacher, G. & Sjövall, I. 1974: Analysis of tissue steroids by liquid-gel chromatography by computerized gas chromatography-mass spectrometry. — J. Chromatogr. Sci. 12:535-540.

Ballard, W. W. 1973: Normal embryonic stages for salmonid fishes based on Salmo gairdneri (Richardson) and Salvelinus fontinalis (Mitchill). — J. Exp. Zool. 184:7-26.

Bullock, D. W. 1977: Steroids from the pre-implantation blastocyst. — In: Johnson, M. H. (ed.), Development in mammals: 199-208. North-Holland, Amsterdam.

Chenault, J. R. 1980: Steroid metabolism by the early bovine conceptus. — I. 5β-reduction of neutral C_{19}-steroids. — J. Steroid Biochem. 13:499-506.

Colombo, L., Belvedere, P. C. & Marconato, A. 1979: Biochemical and functional aspects of gonadal biosynthesis of steroid hormones in teleost fishes. — Proc. Indian Natn. Sci. Acad. B 45:443-451.

Conzalez, C. B., Cozza, E. N., de Bedners, E. O., Lantos, C. P. & Aragones, A. 1983: Progesterone and its reductive metabolites in steroidogenic tissues of the developing hen embryo. — Gen. Comp. Endocrinol. 51:384-393.

Dennis, S. L. & Ecker, R. E. 1971: The interaction of steroids with Rana pipiens oocytes in the induction of maturation. — Develop. Biol. 25:232-247.

Dickmann, Z., Dey, S. K. & Sen Gupta, J. 1976: A new concept: control of early pregnancy by steroid hormones originating in the preimplantation embryo. — In: Munson, P. L., Diczfalusy, E., Glover, J. & Olson, R. E. (eds.), Vitamins and Hormones 34:215-242.

Diederik, H. & Lambert, J. G. D. 1982: Steroids in plasma of the female rainbow trout before and after ovulation by NCI-GCMS. — In: Richter, C. J. J. & Goos, H. J. Th. (eds), Proc. Int. Symp. Reproductive Physiol. Fish, Wageningen, the Netherlands, 2-6 August 1982: 107-108. Centre for Agricultural Publishing and Documentation, Wageningen.

Flint, A. P. F., Gadsby, J. E. & Heap, R. B. 1979: Blastocyst steroids; their synthesis and action. — In: Klopper, A., Lerner, L., van der Molen, H. J. & Scierra, F. (eds.), Research on Steroids VIII: 3-9. Academic Press, London.

Fujimoto, S. & Sundaram, K. 1978: The source of progesterone in rabbit blastocysts. — J. Reprod. Fert. 52:231-233.

George, F. W. & Wilson, J. D. 1978: Estrogen formation in the early rabbit embryo. — Science, N.Y. 199:200-201.

Lambert, J. G. D. 1970: The ovary of the guppy Poecilia reticulata. The granulosa cells as sites of steroid biosynthesis. — Gen. Comp. Endocr. 15:464-476.

Lambert, J. G. D. & van Bohemen, Ch. G. 1979: Steroidogenesis in the ovary of the rainbow trout, Salmo gairdneri, during the reproductive cycle. — Proc. Indian Natn. Sci. Acad. B 45:414-420.

Lambert, J. G. D., Bosman, G. I. C. G. M., van den Hurk, R. & van Oordt, P. G. W. J. 1978: Annual cycle of plasma oestradiol-17β in the female trout Salmo gairdneri. — Ann. Biol. Anim. Bioch. Biophys. 18:923-927.

Lantos, C. P. 1981: Corticosteroids as hormones and metabolic precursors in normal and abnormal tissues. — In: Fotherby, K. & Pal, S. B. (eds), Hormones in normal and abnormal human tissues. II. Walter de Gruyter, Berlin.

Levere, R. D., Kappas, A. & Granick, S. 1967: Stimulation of hemoglobin synthesis in chick blastoderms by certain 5β androstane and 5β pregnane steroids. — Proc. Natl. Acad. Sci. 58:985-990.

Ozon, R., Bell, R., Serres, C. & Fouchet, C. 1975: Mechanism of action of progesterone on amphibian oocytes. A possible role for progesterone metabolism. — Molec. Cell Endocrinol. 3:221-231.

Parsons, I. C. 1970: The metabolism of testosterone by early chick embryonic blastoderm. — Steroids 16:59-65.

Rajalakshmi, M., Sehgal, A., Pruthi, J. S. & Anand Kumar, T. C. 1983: Steroid metabolism by monkey and human spermatozoa. — Steroids 41:587-595.

Reynhout, J. K. & Smith, L. D. 1973: Evidence for steroid metabolism during the in vitro induction of maturation in oocytes of Rana pipiens. — Develop. Biol. 30:392-402.

Saure, A. 1973: The biogenesis and metabolism of steroids in the human placenta of 9-14 weeks in vitro. — Ann. Acad. Scient. Fennicae (A5) 159:1-131.

Singh, M. M. & Booth, W. D. 1978: Studies on the metabolism of neutral steroids by preimplantation rabbit blastocysts in vitro and the origin of blastocyst oestrogen. — J. Reprod. Fert. 53:297-304.

Wu, J. T. & Lin, G.-M. 1982: Effect of aromatase inhibitor on oestrogen production in rabbit blastocysts. — J. Reprod. Fert. 66:655-662.

Zohar, Y., Breton, B. & Fostier, A. 1982: Gonadotropic function during the reproductive cycle of the female rainbow trout, Salmo gairdneri, in relation to ovarian steroid secretion: in vivo and in vitro studies. — In: Richter, C. J. J. & Goos, H. J. Th. (eds), Proc. Int. Symp. Reproductive Physiol. Fish, Wageningen, the Netherlands, 2-6 August 1982: 14-18. Centre for Agricultural Publishing and Documentation, Wageningen.

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