Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation

C. M. Campbell, Alexis Fostier, Bernard Jalabert, B. Truscott

► To cite this version:

C. M. Campbell, Alexis Fostier, Bernard Jalabert, B. Truscott. Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation. *Journal of Endocrinology*, 1980, 85 (3), pp.371-378. 10.1677/joe.0.0850371 . hal-01601581

HAL Id: hal-01601581

<https://hal.science/hal-01601581>

Submitted on 2 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - ShareAlike| 4.0 International License

IDENTIFICATION AND QUANTIFICATION OF STEROIDS IN THE SERUM OF RAINBOW TROUT DURING SPERMATION AND OOCYTE MATURATION

C. M. CAMPBELL*†, A. FOSTIER*†, B. JALABERT*† AND
B. TRUSCOTT†

* *Laboratoire de Physiologie des Poissons, Institut National de la Recherche
Agronomique, 78350 Jouy-en-Josas, France, and †Marine Sciences Research Laboratory,
Memorial University of Newfoundland, St John's, Newfoundland, Canada*

(Received 12 June 1979)

SUMMARY

17 α -Hydroxy-20 β -dihydroprogesterone and 17 α -hydroxyprogesterone were found in higher concentrations in serum from female *Salmo gairdneri* undergoing final oocyte maturation immediately before ovulation than in serum from spermiating male trout. Other steroids (11-deoxycorticosterone, 11-deoxycortisol and progesterone) which have been implicated in oocyte maturation and/or ovulation in lower vertebrates were not identified at such high concentrations and the differences between the serum of both sexes were not so great. These results confirm that 17 α -hydroxy-20 β -dihydroprogesterone and 17 α -hydroxyprogesterone, the most potent inducers of trout oocyte maturation *in vitro*, are present in the blood when oocyte maturation occurs. The concentration of testosterone was found to be higher in serum from female than from male trout indicating that testosterone is unlikely to be the principal androgen in trout. High concentrations of 11-oxotestosterone in male and barely detectable levels in female fish support the hypothesis that 11-oxotestosterone is an important androgen in the regulation of testicular activity.

INTRODUCTION

Many steroids have been implicated in the control of oocyte maturation and ovulation but the steroids involved in spermatogenesis and spermiation remain to be identified in teleost fish. High concentrations in only one sex may indicate involvement in the reproductive processes specific to that sex at the time of sampling.

Jalabert (1976) and Jalabert, Goetz, Breton, Fostier & Donaldson (1978) suggested that 17 α -hydroxy-20 β -dihydroprogesterone (17 α ,20 β -dihydroxy-4-pregnen-3-one) might be the steroid mediator of gonadotrophin-induced oocyte maturation for *Salmo gairdneri*, *Carassius auratus*, *Esox lucius*, *Cyprinus carpio* and *Oncorhynchus kisutch*. This steroid was identified in plasma from sexually mature and spawned *Oncorhynchus nerka* and *Salmo salar* (Schmidt & Idler, 1962). Samples of plasma from *Pseudopleuronectes americanus* which induced maturation of oocytes when assayed *in vitro* contained higher concentrations of 17 α -hydroxy-20 β -dihydroprogesterone than those samples without activity (Campbell, Walsh & Idler, 1976). This steroid had not been identified previously in blood from *S. gairdneri*.

Other steroids investigated in the present study were progesterone and 17 α -hydroxyprogesterone (17 α -hydroxy-4-pregnene-3,20-dione) which are potent maturation-

† Present address: Laboratoire de Physiologie des Poissons, Avenue du Général Leclerc, B.P. 25A, 35042 Rennes, France.

inducing agents (see Jalabert, Breton & Bry, 1972), 20 β -dihydrocortisone (17 α ,20 β ,21-trihydroxy-4-pregnene-3,11-dione) found in plasma from spawned *O. nerka* (Idler, Schmidt & Ronald, 1962), and 11-deoxycortisol (17 α ,21-dihydroxy-4-pregnene-3,20-dione) and 11-deoxycorticosterone, active in oocyte maturation of *Heteropneustes fossilis* (Sundararaj & Goswami, 1977). Cortisol, a maturation-inducing steroid in catfish, was omitted from this study since the effects of stress might have obscured any differences.

Testosterone is androgenic in teleosts and has frequently been identified in body fluids and after incubation of tissues (see Ozon, 1972). Serum levels obtained by methods which may also be measuring other androgens are correlated with testicular activity (Schreck, Lackey & Hopwood, 1974; Sanchez-Rodriguez, Escaffre, Marlot & Reinaud, 1978). Wingfield & Grimm (1977) found an annual cycle of concentrations of testosterone in the plasma of plaice of both sexes but Campbell *et al.* (1976) found that levels of testosterone are higher in female than in male flounder.

11-Oxytestosterone (17 β -hydroxy-4-androstene-3,11-dione) has been implicated in the regulation of spermatogenesis and spermiation of *O. nerka* and *S. salar* (see Idler, Horne & Sangalang, 1971), *P. americanus* (Campbell *et al.* 1976) and *Salvelinus fontinalis* (Sangalang & Freeman, 1974). Idler *et al.* (1971) found that levels of 11 β -hydroxytestosterone (11 β ,17 β -dihydroxy-4-androstene-3-one) also increase with testicular development in *S. salar* but the physiological role of these androgens is unclear.

In the present study some key steroids have been identified and quantified by a double-isotope derivative assay. The assay was performed on sera from male *S. gairdneri* which were producing sperm and from female trout which were undergoing final oocyte meiotic maturation but which had not yet begun to ovulate, to compare hormone profiles in both sexes at these defined states of reproductive activity.

MATERIALS AND METHODS

Blood was collected from more than 20 hatchery-reared male rainbow trout of 1–2 kg body weight in November/December 1976. The fish were checked for the presence of ripe spermatozoa by squeezing the abdomen. A few oocytes were stripped from female fish to assess the stage of oocyte maturation. Fifteen female trout were bled by syringe from a caudal vessel. The chosen fish had oocytes in the stages named 2, 3 and 4 by Jalabert (1978), in which germinal vesicles completely touch the oocyte membrane, or have undergone germinal vesicle breakdown and where maturation has progressed to follicular preparation for ovulation. Blood was allowed to clot and then centrifuged. Serum was pooled for each sex and stored at -70°C .

17 α -Hydroxyprogesterone was isolated from 14 ml serum from male and female trout. The other steroids were assayed in 13.7 ml serum from female and 13.9 ml from male animals.

The sources and preparation of reagents were the same as those described by Truscott, Idler, Sundararaj & Goswami (1978). Radioinert and ^{14}C -labelled 11 β -hydroxytestosterone and 11-oxotestosterone were prepared by the method described by Idler *et al.* (1971). Acetates of 11 β -hydroxytestosterone, 11-oxotestosterone, 20 β -dihydrocortisone and 17 α -hydroxy-20 β -dihydroprogesterone for use in identification were prepared by the usual methods and purified by thin-layer chromatography (Idler *et al.* 1971).

Recovery tracers (purified ^{14}C -labelled steroids) were added to the samples and extraction was performed as described by Truscott & Idler (1972). Extracts were applied to silica gel thin-layer chromatography plates and developed in hexane:ethyl acetate (80:20, v/v) and chloroform:methanol:water (188:12:1, by vol.) together with steroids which were not to be assayed in order to isolate the desired steroids into five groups (Fig. 1) without the risk of any contamination. Progesterone and 17 α -hydroxyprogesterone were reduced with 20 β -hydroxysteroid dehydrogenase (Simpson, Wright & Hunt, 1964). The progesterone was isolated in fraction 1 (Fig. 1) but 17 α -hydroxyprogesterone was isolated from pooled

fraction 2 as fraction 6 (Fig. 1). The 17α -hydroxyprogesterone fractions were re-chromatographed before reduction using testosterone as a marker to reduce any possible contamination by 17α -hydroxy- 20β -dihydroprogesterone. After reduction, 17α -hydroxy- 20β -dihydroprogesterone and 20β -dihydroprogesterone were isolated using testosterone and 11-deoxycortisol as markers. The extracts and samples of tracers were acetylated using 100 mCi/mmol [^3H]acetic anhydride (6 mCi/tube), the reaction conditions being similar to those of Truscott *et al.* (1978). Radioinert corticosterone was also acetylated to determine the exact specific activity of the ^3H -labelled anhydride.

After re-extraction of the ^3H -labelled acetylation mixtures, the sample fraction groups 1, 3, 4, 5 and 6 (defined by Fig. 1) were chromatographed in System 1 (chloroform : methanol; 96 : 4, v/v) to separate individual steroid ^3H -labelled acetates. The three ^3H -labelled acetates to be isolated from group 2 were separated using chloroform : methanol : water (188 : 12 : 1, by vol.)

Fig. 1. Steroids were isolated by thin-layer chromatography in chloroform : methanol : water (188 : 12 : 1, by vol.) from pooled serum samples of both male and female trout. Steroids that were not being assayed were used as markers and were detected by absorbance of ultra-violet light and used to establish positions to elute steroid groups to be acetylated. a = corticosterone acetate; b = cortisone-21-acetate; c = cortisol-21-acetate; d = cortisone; e = cortisol; f = progesterone; g = deoxycorticosterone; h = 17α -hydroxyprogesterone; j = 20β -dihydroprogesterone; k = testosterone; m = 17α -hydroxy- 20β -dihydroprogesterone; n = 11-deoxycortisol; p = 11-oxotestosterone; q = 11β -hydroxytestosterone; r = 20β -dihydrocortisone.

^3H -Labelled acetates were further purified by thin-layer and paper chromatography (Table 1) and by crystallization from ethanol with water or acetone with pentane. In the case of samples where recovery was low, crystallization was performed but crystal samples were not taken each time. Counting was performed in a Packard 2425 using external standardization

to estimate efficiency and ^{14}C spill into the ^3H channel (spill from ^3H into ^{14}C was negligible). Samples generally contained 100 to 300 disintegrations $^{14}\text{C}/\text{min}$ and were counted to approximately 10 000.

Table 1. *Sequence of chromatography procedures used (and their composition) to purify the ^3H -labelled acetates of steroids in the double isotope derivative assay of sera from rainbow trout*

	Chromatography system						
	1	2	4	B	C	1	—
Corticosterone	1	3	A	E	—	—	—
11-Deoxycortisol	1	2	A	E	C	—	—
11-Deoxycorticosterone	1	3	5	2	C	D	—
20 β -Dihydroprogesterone	1	3	2	C	E	—	—
Testosterone	1	3	2	A	D	C	D
17 α -Hydroxy-20 β -dihydroprogesterone	1	2	4	C	A	E	—
11-Oxotestosterone	1	4	2	A	E	C	—
11 β -Hydroxytestosterone	1	2	4	E	A	—	—
20 β -Dihydrocortisone							
Thin-layer chromatography systems (by vol.)	Paper chromatography systems (by vol.)						
1. Chloroform : methanol (96 : 4)	A. Mesitylene : methanol : water (50 : 33 : 16) (reversed phase)						
2. Dichloromethane : <i>n</i> -butylacetate (70 : 30)	B. Cyclohexane : benzene : methanol : water (100 : 40 : 100 : 20)						
3. Cyclohexane : isopropanol (70 : 30)	C. Heptane : 80% methanol (50 : 50)						
4. Cyclohexane : ethyl acetate (50 : 50); irrigated twice	D. Benzene : hexane : methanol : water (33 : 66 : 80 : 20)						
5. Benzene : ethyl acetate (66 : 33)	E. Cyclohexane : dioxane : methanol : water (100 : 100 : 66 : 33)						

RESULTS

The results of the experiment are given in Table 2. Constant isotope ratios indicating purity of the ^3H -labelled acetates were attained in all cases except 11 β -hydroxytestosterone in serum from female, and 17 α -hydroxyprogesterone and 17 α -hydroxy-20 β -dihydroprogesterone in that from male trout. In these cases the values calculated should represent the maximum possible value since purification tended to reduce the ratios.

DISCUSSION

Within the limitations that these assays have measured only unconjugated steroids and that they take no account of the role of protein binding in the regulation of steroid action, it is possible to draw some conclusions based on a simplistic interpretation that high concentrations in serum may be indicative of some biological function. The samples for analysis were taken from both sexes at a physiological state which might be expected to involve changes in steroid profiles. The differences in concentrations of steroids between the sexes may be indicative of an association between high steroid concentration and oocyte maturation and ovulation in female trout, and spermiation in male trout.

In amphibia the most potent inducer of oocyte maturation is progesterone (Schuetz, 1967) but in trout the concentrations of progesterone were similar in male and female fish and the concentrations of 17 α -hydroxy-20 β -dihydroprogesterone and 17 α -hydroxyprogesterone were much higher in sera from female trout undergoing meiotic maturation. These results suggest that progesterone is not implicated in the maturation of oocytes of trout but that 17 α -hydroxy-20 β -dihydroprogesterone or 17 α -hydroxyprogesterone may be the active

Table 2. $^3\text{H} : ^{14}\text{C}$ Ratios in aliquots of purified steroid ^3H -labelled acetates after each paper chromatographic (P_1, P_2) or crystallization (C_1, C_2 , etc.) step. ^3H -Labelled acetylated steroids were isolated from pooled sera from male (M) and female (F) rainbow trout. Constant isotope ratios (*) were used to calculate the concentration of the steroid in the original serum pool. For samples which were not purified to constant ratios the calculated values are maximal

		P_1	P_2	C_1	C_2	C_3	C_4	Concentration (ng/ml)
Progesterone	M	—	2.1	1.36	1.18*	0.99*	1.06*	1.7
	F	—	2.3	1.71	1.45*	1.33*	1.57*	2.3
17 α -Hydroxyprogesterone	M	—	4.5	—	—	—	3.75	≤ 2.8
	F	—	94	—	—	99*	101*	89
17 α -Hydroxy-20 β - dihydroprogesterone	M	—	7.9	—	—	8.2	7.2	≤ 9.1
	F	—	265	346	338*	340*	336*	480
20 β -Dihydrocortisone	M	7.2	6.0	5.9*	5.7*	—	—	4.5
	F	15.6	14.4	14.1*	14.2*	—	—	12.3
11-Deoxycortisol	M	—	—	2.4*	2.4*	2.4*	2.4*	4.8
	F	—	—	6.8	6.9*	6.9*	7.0*	18.1
11-Deoxycorticosterone	M	—	—	3.4	3.0	2.9	2.8*	3.1
	F	—	—	—	—	2.1*	2.1*	2.0
Testosterone	M	31	28.8	29.5	29.8	29.7	29.7*	59.6
	F	85	85.9	90.1	89.5*	89.3*	91.6	187
11-Oxotestosterone	M	—	81	90.0*	90.2*	91.9*	92.0*	98
	F	—	1.8	1.3*	1.3*	1.3*	1.3*	0.63
11 β -Hydroxytestosterone	M	2.4	—	2.5	2.3*	2.3*	2.2*	1.8
	F	1.1	—	—	—	—	—	≤ 0.5

agent as has been suggested by in-vivo and in-vitro studies on oocyte maturation (Fostier, Jalabert & Terqui, 1973; Jalabert, Breton, Bryuska, Fostier & Wienawski, 1977). Sundararaj & Goswami (1977) suggested that 11-deoxycortisol or 11-deoxycorticosterone might be the maturation-inducing steroids of the catfish but Truscott *et al.* (1978) concluded that since secretion of these hormones into catfish plasma is stimulated neither by luteinizing hormone nor by adrenocorticotrophic hormone it is unlikely that they are involved. It has been found here, in agreement with Campbell *et al.* (1976) on flounder, that these steroids are not present in high concentrations during oocyte maturation and differences in the concentration of 11-deoxycorticosterone between male and female fish do not strongly support a hypothesis for a role for these steroids in oocyte maturation. Their function is far from clear since Colombo, Colombo-Belvedere & Arcarese (1978) have indicated that 11-deoxycorticosteroids are more important metabolites in ovaries after ovulation than in the vitellogenic ovaries of *Dicentrarchus labrax*. Several studies have shown the oocytes of brook trout, killifish and zebra fish to be sensitive to both 11-deoxycorticosteroids and progestagens (Van Ree, Lok & Bosman, 1977; Goetz & Bergman, 1978; Wallace & Selman, 1978). The higher concentrations of 11-deoxycortisol in female trout might be related to the oocyte maturation occurring in these animals. In fact Jalabert (1975) showed that treatment with cortisol and cortisone increases the sensitivity of oocytes from *S. gairdneri* to gonadotrophin and steroid *in vitro*, but 11-deoxycortisol was not tested.

Idler *et al.* (1962) found significant levels of 20 β -dihydrocortisone in the blood of *O. nerka* after spawning and we have found higher concentrations in the serum of maturing female than of ripe male rainbow trout. Unfortunately, the biological activity of this steroid has not been tested so far.

Levels of testosterone are higher in female flounder than in male flounder at the spawning period (Campbell *et al.* 1976) and we have found that testosterone was present at high concentration in female trout undergoing oocyte maturation.

11-Oxygenated androgens have been isolated from cultures of guppy and eel ovaries (Lambert & Pot, 1975; Colombo & Colombo-Belvedere, 1977) but the results of the present

study indicated that 11-oxygenated androgens were not to be found in significant quantities in sera from female fish whose oocytes were undergoing maturation. Using a radioimmunoassay, Simpson & Wright (1977) found levels of 0.95–1.82 ng 11-oxotestosterone/ml in ovulated female rainbow trout in comparison with 2–90 ng/ml in reproductively active male trout. Schmidt & Idler (1962) detected 11-oxotestosterone in the blood of female Atlantic and Pacific salmon but it is not a major steroid in the circulation of the female winter flounder and female rainbow trout undergoing normal oocyte maturation. Katz & Eckstein (1974) identified 11-oxotestosterone in higher concentrations in individual *Tilapia aurea* in which oocyte maturation was apparently blocked and suggested an inhibitory function in the regulation of maturation and ovulation.

Male Pacific and Atlantic salmon have significant concentrations of 11-oxotestosterone and Campbell *et al.* (1976) postulated that 11-oxotestosterone might be a more active androgen in flounder than testosterone, because concentrations of this steroid are higher than those of testosterone in the plasma of the male animals. The present data are in accord with this suggestion. Idler, Bitners & Schmidt (1961) found androgenic effects of 11-oxotestosterone on skin colour and thickness and some induction of spermiation in sockeye salmon and Arai (1967) showed 11-oxotestosterone to be a more potent androgen than testosterone for induction of male secondary sex characters in both sexes of *Oryzias latipes*.

11 β -Hydroxytestosterone was identified at low concentrations in both sexes of rainbow trout and has been identified as a metabolite of progesterone and androstenedione in cultures of trout ovaries (Arai & Tamaoki, 1967) but no indication of a biological function can be given.

It is possible that the importance of testosterone for the male trout has been underestimated since it has been shown that more testosterone glucuronide is produced than testosterone during in-vitro incubation of rainbow trout testis with precursors (Hews & Kime, 1978).

This possibility and the unknown importance of protein binding must impose limitations on the interpretation of the present data. This analysis does, however, show which unconjugated steroids were present during oocyte maturation in female, and during spermiation in male rainbow trout. Further studies can now evaluate the physiological significance of the high concentrations found. 17 α -Hydroxy-20 β -dihydroprogesterone, by being important in the induction of maturation of oocytes and by occurring at significant concentrations in the circulation at the appropriate time, must be proposed as the natural agent. Synthesis of the steroid during gonadotrophin-induced maturation must be demonstrated to complete the argument.

C.M.C. was supported by a Royal Society European Programme Fellowship. Our thanks to Dr D. R. Idler for providing the facilities of the Marine Sciences Research Laboratory, Memorial University of Newfoundland, St John's, Newfoundland and for supporting the study during a visit by C. M. C. The study was funded from a grant from 'Le Ministere de l'Environnement et de la Culture'. MSRL Contribution number 367.

REFERENCES

- Arai, R. (1967). Androgenic effects of 11-ketotestosterone on some sexual characteristics in the teleost, *Oryzias latipes*. *Annotationes Zoologicae Japonenses* **40**, 1–5.
- Arai, R. & Tamaoki, B. I. (1967). Steroid biosynthesis *in vitro* by testes of rainbow trout, *Salmo gairdneri*. *General and Comparative Endocrinology* **8**, 305–313.
- Campbell, C. M., Walsh, J. M. & Idler, D. R. (1976). Steroids in the plasma of the winter flounder (*Pseudopleuronectes americanus* Walbaum). A seasonal study and investigation of steroid involvement in oocyte maturation. *General and Comparative Endocrinology* **29**, 14–20.
- Colombo, L. & Colombo-Belvedere, P. (1977). Gonadal steroidogenesis in teleost fishes. *Investigación Pesquera* **41**, 147–164.

- Colombo, L., Colombo-Belvedere, P. & Arcarese, G. (1978). Emergence of ovarian 11-deoxycorticosteroid biosynthesis at ovulation time in the sea bass, *Dicentrarchus labrax* L. *Annales de Biologie Animale, Biochimie et Biophysique* 18, 937-941.
- Fostier, A., Jalabert, B. & Terqui, M. (1973). Action prédominante d'un dérivé hydroxylé de la progestérone sur la maturation in-vitro des ovocytes de la truite arc-en-ciel *Salmo gairdneri*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 277D, 421-424.
- Goetz, F. W. & Bergman, H. L. (1978). The effects of steroids on final maturation and ovulation of oocytes from brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca florescens*). *Biology of Reproduction* 18, 293-298.
- Hews, E. A. & Kime, D. E. (1978). Formation of testosterone glucuronide by testes of the rainbow trout, *Salmo gairdneri*. *General and Comparative Endocrinology* 34, 116-119.
- Idler, D. R., Bitners, I. I. & Schmidt, P. J. (1961). 11-Ketotestosterone: an androgen for sockeye salmon. *Canadian Journal of Biochemistry and Physiology* 39, 1737-1742.
- Idler, D. R., Horne, D. A. & Sangalang, G. B. (1971). Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (*Salmo salar*) during sexual maturation. *General and Comparative Endocrinology* 16, 237-267.
- Idler, D. R., Schmidt, P. J. & Ronald, A. P. (1962). Isolation of 20 β -dihydrocortisone from sockeye salmon (*Oncorhynchus nerka*) plasma. *Canadian Journal of Biochemistry and Physiology* 40, 549-553.
- Jalabert, B. (1975). Modulation par différents stéroïdes non-maturants de l'efficacité de la 17 α -hydroxy-20 β -dihydroprogestérone ou d'un extrait gonadotrope sur la maturation intrafolliculaire in vitro des ovocytes de la truite arc-en-ciel *Salmo gairdneri*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 281D, 811-814.
- Jalabert, B. (1976). In-vitro oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*) and goldfish (*Carassius auratus*). *Journal of the Fisheries Research Board of Canada* 33, 974-988.
- Jalabert, B. (1978). Production of fertilizable oocytes from follicles of rainbow trout (*Salmo gairdneri*) following in vitro maturation and ovulation. *Annales de Biologie Animale, Biochimie et Biophysique* 18, 977-984.
- Jalabert, B., Breton, B. & Bry, C. (1972). Maturation et ovulation in-vitro des ovocytes de la truite arc-en-ciel, *Salmo gairdneri*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 275D, 1139-1142.
- Jalabert, B., Breton, B., Bryuska, E., Fostier, A. & Wienawski, J. (1977). A new tool for induced spawning: the use of 17 α -hydroxy-20 β -dihydroprogestérone to spawn trout at low temperature. *Aquaculture* 10, 353-364.
- Jalabert, B., Goetz, F. W., Breton, B., Fostier, A. & Donaldson, E. M. (1978). Precocious induction of oocyte maturation and ovulation in coho salmon, *Oncorhynchus kisutch*. *Journal of the Fisheries Research Board of Canada* 35, 1423-1429.
- Katz, Y. & Eckstein, B. (1974). Changes in the steroid concentration in blood of female *Tilapia aurea* (Teleostei, cichlidae) during initiation of spawning. *Endocrinology* 95, 963-967.
- Lambert, J. G. D. & Pot, M. G. E. (1975). Steroidogenesis in ovarian tissue of a viviparous teleost, the guppy *Poecilia reticulata*. *Comparative Biochemistry and Physiology* 50B, 585-589.
- Ozon, R. (1972). Androgens in fishes, amphibians, reptiles and birds. In *Steroids in non-mammalian vertebrates*, pp. 328-389. Ed. D. R. Idler. New York: Academic Press.
- Sanchez-Rodriguez, M., Escaffre, A. M., Marlot, S. & Reinaud, P. (1978). The spermiation period in the rainbow trout (*Salmo gairdneri*). Plasma gonadotropin and androgen levels, sperm production and biochemical changes in the seminal fluid. *Annales de Biologie Animale Biochimie et Biophysique* 18, 943-948.
- Sangalang, G. B. & Freeman, H. C. (1974). Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production in vivo in brook trout. *Biology of Reproduction* 11, 429-435.
- Schmidt, P. J. & Idler, D. R. (1962). Steroid hormones in the plasma of salmon at various stages of maturation. *General and Comparative Endocrinology* 2, 204-214.
- Schreck, C. B., Lackey, R. T. & Hopwood, M. L. (1974). Seasonal androgen and estrogen patterns in the goldfish *Carassius auratus*. *Transactions of the American Fisheries Society* 103, 375-378.
- Schuetz, A. W. (1967). Effect of steroids on the germinal vesicles of the frog (*Rana pipiens*) oocytes. *Proceedings of the Society for Experimental Biology and Medicine* 124, 1307-1310.
- Simpson, T. H. & Wright, R. S. (1977). A radioimmunoassay for 11-oxotestosterone: its application in the measurement of levels in blood serum of rainbow trout (*S. gairdneri*). *Steroids* 29, 383-398.
- Simpson, T. H., Wright, R. S. & Hunt, S. V. (1964). Steroid biosynthesis in the testis of dogfish (*Squalus acanthias*). *Journal of Endocrinology* 31, 20-38.
- Sundararaj, B. I. & Goswami, S. V. (1977). Hormonal regulation of in vivo and in vitro oocyte maturation in the catfish, *Heteropneustes fossilis* (Bloch). *General and Comparative Endocrinology* 32, 17-28.
- Truscott, B. & Idler, D. R. (1972). Corticosteroids in plasma of elasmobranchs. *Comparative Biochemistry and Physiology* 42A, 41-50.
- Truscott, B., Idler, D. R., Sundararaj, B. I., & Goswami, S. V. (1978). Effects of gonadotropins and adrenocorticotropin on plasmatic steroids of the catfish, *Heteropneustes fossilis* (Bloch). *General and Comparative Endocrinology* 34, 149-157.
- Van Ree, G., Lok, D. & Bosman, G. J. C. G. M. (1977). In vitro induction of nuclear breakdown in oocytes of the zebra fish, *Brachydanio rerio* (Hamm. Buch). Effects of the composition of the medium and of protein and steroid hormones. *Proceedings. Koninklijke Nederlandse Akademie van Wetenschappen* 80C, 353-371.

- Wallace, R. A. & Selman, K. (1978). Oogenesis in *Fundulus heteroclitus*. I. Preliminary observations on oocyte maturation *in vitro*. *Developmental Biology* **62**, 354–369.
- Wingfield, J. C. & Grimm, A. S. (1977). Seasonal changes in plasma cortisol, testosterone and oestradiol-17 β in the plaice *Pleuronectes platessa*. *General and Comparative Endocrinology* **31**, 1–11.