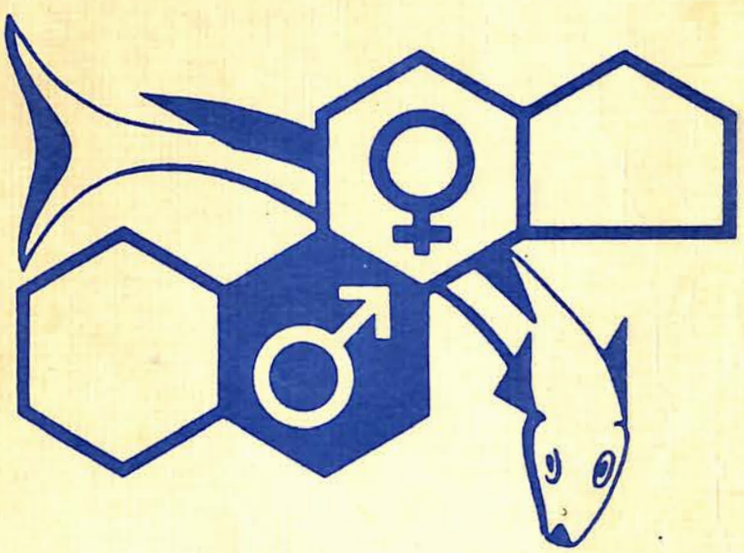
A. FOSTIER

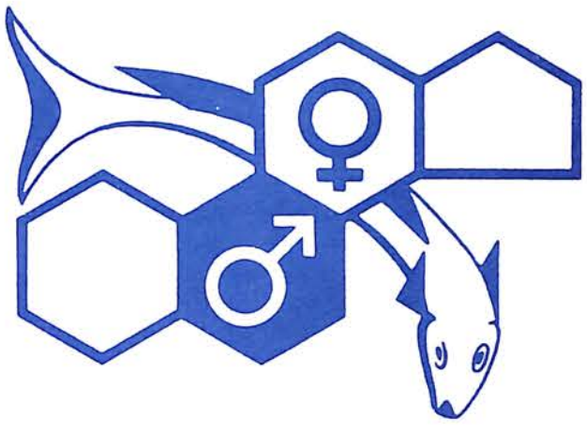
THIRD INTERNATIONAL SYMPOSIUM ON REPRODUCTIVE PHYSIOLOGY OF FISH



AUGUST 2 - 7, 1987
ST. JOHN'S, NEWFOUNDLAND
CANADA

PROGRAMME
AND
ABSTRACTS OF PAPERS AND POSTERS

THIRD INTERNATIONAL SYMPOSIUM ON REPRODUCTIVE PHYSIOLOGY OF FISH



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marine sciences research laboratory

MARINE SCIENCES RESEARCH LABORATORY
MEMORIAL UNIVERSITY OF NEWFOUNDLAND
ST. JOHN'S, NEWFOUNDLAND, CANADA

ST. JOHN'S, AUGUST 1987

THIRD

INTERNATIONAL SYMPOSIUM ON

REPRODUCTIVE PHYSIOLOGY OF FISH

2 - 7 August 1987

ST. JOHN'S, NEWFOUNDLAND

CANADA

greatly acknowledges the generous support provided by:

- The Government of Newfoundland and Labrador
- Memorial University of Newfoundland
- Department of Regional Industrial Expansion
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Session I in the chair Zvi Yaron
Abstract pages in this session

3, 9, 16, 17, 21, 27, 36, 37, 39, 43, 50, 53, 57, 68, 73, 85, 90, 120, 121, 126, (140), (145), 153, (156), 157, 166

140 Goos talk; 145 Sherwood talk; 156 Crim talk

Session II in the chair Peter Thomas
Abstract pages in this session

35, (40), 41, 42, 80, (86), 89, 104, 113, 117, 119, 123, 127, 146, 155, 159, (162)

40 Fontaine talk; 86 Ishii talk; 162 Idler talk

Session III in the chair Tillman J. Benfey
Abstract pages in this session

14, 22, 26, 44, 45, 47, 70, 72, 77, (88), (139), 148, (158)

88 Naas talk; 139 Paulsen talk; 158 Marte talk

Session IV in the chair Carel J.J. Richter
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Session V in the chair Jan W. Resink
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24 Satou talk; 92 Stacey talk; 125 Liley talk

Session VI in the chair John P. Sumpter
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Session VII in the chair Keiji Hirose
Abstract pages in this session

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56 Goetz talk; 101 Scott talk; 116 Fostier talk

Session VIII in the chair Niall R. Bromage
Abstract pages in this session

1, 11, 13, 46, 59, 81, 100, 107, 111, (114), 129, 131, 132, 144, (150), 154, (164)

114 Sumpter talk; 150 Bye talk; 164 Lam talk

Saturday August 1st.

Registration at the Littledale Conference Centre.

Posters to be set up on the display boards.

Sunday August 2nd.

Registration at the Littledale Conference Centre.

Posters to be set up on the display boards.

14:00 Tour to Cape Spear - most easterly point of land in North America and a National Historic Site.

20:00 Informal Reception at Littledale hosted by Dr. Leslie Harris, President of Memorial University of Newfoundland.

SITES OF INTEREST

1. Oxen Pond Botanical Gardens - Mount Scio Road
2. Bird Island Charters - St. John's
3. Salmonier Nature Park - Outside St. John's (40 miles)
4. St. Mary's Bird Sanctuary - Outside St. John's (100 miles)
5. The St. John's Regatta - Oldest and Continuous Sporting Event (rowing) in North America, held on 1st. Wednesday in August

LOCAL CRAFTS

1. Winmill Woollens - Murray Premises
2. Nonia - Water Street across from Murray Premises
3. Saltbox - Duckworth Street
4. Newfoundland Weavery - Duckworth Street

MUSEUMS

1. Newfoundland Museum - Murray Premises
2. Newfoundland Museum - Duckworth Street
3. Memorial University Art Gallery - Arts & Culture Center

FINE RESTAURANTS

1. Fishing Admiral - Water Street
2. King Cod - Duckworth Street
3. Flake House - Quidi Vidi Village
4. Jade Garden - Kenmount Road
5. Kenmount Restaurant - Kenmount Road

Monday August 3rd. - Morning

08:30-08:35 Seating

08:35-08:40 Opening remarks by David R. Idler (Canada), the President of the Organizing Committee.

08:40-08:55 Introductory lecture by Piet G.W.J. van Oordt (Netherlands), the Honorary President of the Symposium.

Session I - Hypothalamo - Hypophysial Axis
in the chair: Zvi Yaron

08:55-09:30 Nancy M. Sherwood (Canada):
Current status of brain peptide releasing hormones.

09:30-09:45 Discussion

09:45-10:05 Henk J. Th. Goos (The Netherlands):
Steroid hormone feedback on the pituitary.

10:05-10:15 Discussion

10:15-11:30 Poster Session with Coffee Break.

11:30-11:50 Laurence W. Crim (Canada):
LHRH receptor characteristics in the pituitary.

11:50 - 12:00 Discussion.

12:00-13:30 Lunch.

Monday August 3rd. - Afternoon

Session II - Gonadotropins
in the chair: Peter Thomas

13:45-13:55 Seating

13:55-14:30 Yves A. Fontaine (France):
Current status of LH-FSH-like-GTH.

14:30-14:45 Discussion

14:45-15:05 David R. Idler (Canada):
Carbohydrate-poor GtH's.

15:05-15:15 Discussion

15:15-16:30 Poster Session with Coffee Break.

16:30-16:50 Susumu Ishii (Japan):
Biological effects and bioassays of GtH.

16:50-17:00 Discussion.

Suppertime.

Tuesday August 4th. - Morning

Session III - Practical fish culture, broodstock care and larval rearing
in the chair: Tillman J. Benfey

08:45-08:55 Seating

08:55-09:30 Kjell Naas (Norway):
Current status of cod culture.

09:30-09:45 Discussion

09:45-10:05 Helge Paulsen (Denmark):
Turbot culture.

10:05-10:15 Discussion

10:15-11:30 Poster Session with Coffee Break.

11:30-11:50 Clarissa L. Marte (Philippines):
Milkfish culture.

11:50-12:00 Discussion.

12:00-13:30 Lunch.

Tuesday August 4th. - Afternoon

Session IV - Sex manipulation and induced breeding
in the chair: Carel J.J. Richter

13:45-13:55 Seating

13:55-14:30 Edward M. Donaldson (Canada):
Current status of induced sex manipulation.

14:30-14:45 Discussion

14:45-15:05 Richard E. Peter (Canada):
Drug/hormone induced breeding of Chinese teleosts.

15:05-15:15 Discussion

15:15-16:30 Poster Session with Coffee Break.

16:30-16:50 Rudolf Reinboth (West Germany):
Natural sex reversal.

16:50-17:00 Discussion.

Suppertime.

Wednesday August 5th. - Morning (Regatta Day)

Session V - Reproductive behavior in fish
in the chair: Jan W. Resink

08:45-08:55	Seating
08:55-09:30	N. Robin Liley (Canada): Current status of hormones and sex behavior.
09:30-09:45	Discussion
09:45-10:05	Norman E. Stacey (Canada): Reproduction and pheromones.
10:05-10:15	Discussion
10:15-11:30	Poster Session with Coffee Break.
11:30-11:50	Masahiko Satou (Japan): Neural substrates for reproductive behavior.
11:50-12:00	Discussion.
12:00-13:30	Lunch.
14:00	Short scenic trip with tour of the Marine Sciences Research Laboratory facilities.
17:00-	Supertime. Barbeque planned.
20:00-	SCREECH-IN

ANOTHER LOOK

Everything is farther away than it used to be. It is twice as far to the corner, and they have added a hill that I never noticed before. I have given up running for the bus, it leaves much faster now than it did before.

It seems to me that they are making steps steeper than they used to in the good old days. And have you noticed the small print that the newspapers are using now?

There's no use in asking anyone to read aloud anymore. Everyone speaks in such a low voice that I can hardly hear what is said.

The material in dresses is getting skimpy, especially around the waist and hips. Really cheap, if you ask me.

Even people are changing. They are so much younger than they used to be when I was their age. On the other hand, people my age are so much older than I am.

I ran into an old friend the other day and she had aged so much that she didn't even recognize me any more.

I got to thinking about the poor thing while I was combing my hair this morning, and in doing so, I glanced at my reflection, and confound it, they don't even make good mirrors anymore either.

Thursday August 6th. - Morning

Session VI - Gonadal Growth/Recrudescence
in the chair: John P. Sumpter

08:45-08:55 Seating

08:55-09:30 Robin A. Wallace (U.S.A.):
Current status of oocyte growth.

09:30-09:45 Discussion

09:45-10:05 Catherine B. Lazier (Canada):
Gene regulation of vitellogenesis, liver estrogen receptors.

10:05-10:15 Discussion

10:15-11:30 Poster Session with Coffee Break.

11:30-11:50 Roland Billard (France):
Male gonadal growth and spermatogenesis.

11:50-12:00 Discussion.

12:00-13:30 Lunch.

Thursday August 6th. - Afternoon

Session VII - Maturation of Gonads/Gonadal Steroid Hormones
in the chair: Keiji Hirose

13:45-13:55 Seating

13:55-14:30 A.P. (Sandy) Scott (United Kingdom):
Current status of ovarian steroids.

14:30-14:45 Discussion

14:45-15:05 Frederick W. Goetz (U.S.A.):
Mechanism & hormonal regulation of ovulation.

15:05-15:15 Discussion

15:15-16:30 Poster Session with Coffee Break.

16:30-16:50 Alexis Fostier (France):
Steroids in male reproduction.

16:50-17:00 Discussion.

Suppertime.

Friday August 7th. - Morning

Session VIII - Environmental factors and reproduction
in the chair: Niall R. Bromage

08:45-08:55 Seating

08:55-09:30 Tom J. Lam (Singapore):
Current status of environmental influences on gonadal activity.

09:30-09:45 Discussion

09:45-10:05 Victor J. Bye (United Kingdom).
Environmental management of marine fish reproduction.

10:05-10:15 Discussion

10:15-11:30 Poster Session with Coffee Break.

11:30-11:50 John P. Sumpter (United Kingdom):
Stress and reproduction.

11:50-12:00 Discussion.

12:00- Roland Billard (France):
Concluding Remarks.

Lunch.

Friday Evening

19:30- Banquet and closings.

EFFECT OF THYROID HORMONES ON THE GROWTH, FOOD CONVERSION AND TISSUE CHEMISTRY OF THE CARP, CYPRINUS CARPIO

Hanuman Singh

Laboratory of Molecular Endocrinology and Toxicology, Department of Life Sciences, Manipur University, Imphal 795003, India

Changes in rate of growth, food conversion efficiency and proximate composition of muscles have been analysed over a priod of one year in juvenile common carp, Cyprinus carpio communis after treatment with thyroid hormones (L-T_3 and T_4). Hormones were administered to fish either by injection (I/P and I/M) or orally. Oral administration of hormones produced the best response followed by I/M and then I/P injections. Results of only oral administration of hormones will be discussed here. Duplicate groups of juvenile carp were fed daily to satiation their normal diet (5% of body weight, rice bran and oil cake in the ratio of 2 : 1) and diet supplemented with 1, 5 and 10 mg/kg body weight of T_3 or 5, 25 and 50 mg/kg body weight of T_4. All the groups received the treatment for one year. Treatment period from April 1984 to March 1985 was divded into four phases. Phase I was from April to June, Phase II from July to September, Phase III from October to December and Phase IV was from January to March. At the end of each phase ten fish were randomly selected from each treatment group, their growth rate, food conversion efficiency and proximate composition of muscle were analysed. When the effects of T_3 and T_4 supplemented diets were compared 5 mg/kg of T_3 and 25 mg/kg of T_4 were found to be most efective growth promoters, therefore, only the results obtained with these dosages of hormones have been discussed here. Both T_3 and T_4 have significantly induced the growth rate (% gain in body weight over control) and condition factor, however maximum induction was achieved during Phase I and first half of Phase II. Feeding of the hormones also increased the food conversion efficiency of the experimental groups. Food consumption was de-creased during latter part of the phase III and Phase IV. Protein efficiency ratio (PER) was minimum during Phase I and Phase II whereas during Phase III and Phase IV increased PER (in most cases) was encountered. Total proteins increased in liver, kidney, and muscle. RNA, RNA/DNA and protein/DNA ratio were generally increased in all tissues studied. No change in moisture content of muscles was noticed in hormone treated groups, except in early part of Phase IV where it was decreased. Total lipid in liver and muscle were high in Phase I and then decreased slightly in latter part of Phase II followed by another increase in their level in Phase III and finally the lowest level in Phase IV. Ash contents were significantly different in all treated groups. When the results of T_3 supplemented diets were compared with that of T_4; T_3 was found to have elicited better response.

Acknowledgements

Financial assistance from Department of Science and Technology, Government of India is gratefully acknowldged. I am thankful to Ms Archana Srivastav for technical assistance.

EVIDENCE OF STEROIDOGENESIS IN POSTOVULATORY FOLLICLES OF THE TILAPIA, OREOCHROMIS MOSSAMBICUS

C.J. Smith, S.R. Haley

Department of Zoology, University of Hawaii, Honolulu, Hawaii, U.S.A.

Summary

Developing oocytes become surrounded by two or more layers of cells which comprise the follicle. The outermost layer is the thecal layer while the innermost is the granulosa layer. No endocrine function has yet been demonstrated for postovulatory follicles in oviparous teleosts, although appropriate ultrastructure and steroid converting enzymes appear in the ovaries of many of the species examined. This study is the first of several designed to investigate a possible endocrine role for postovulatory follicles in an oviparous teleost, the tilapia, Oreochromis mossambicus. The tilapia has two characteristics that make it an especially good candidate for this project. First, it breeds continuously in warm water, so that the next clutch of eggs will begin development in the presence of postovulatory follicles. Second, female tilapia are mouthbrooders. After spawning, she gathers the eggs into her mouth and holds them until after the yolk sacs are resorbed. In our system, the water was maintained at 27°C with a photoperiod of 12hr light/12hr dark, in which the females mouthbrooded continuously for 12-15 days after spawning. Subsequently, the fry were gathered up whenever the female sensed danger or darkness. This discontinuous mouthbrooding continued for up to 25 days after spawning.

Ovarian tissue was examined by electron microscopy and enzyme histochemistry for evidence of steroid hormone production. Light microscopy was also used to examine changes in the ovary with time after spawning. Two groups of females were examined at various times after spawning. The first group consisted of females that mouthbrooded. The second group included females that swallowed the zygotes within 1 day after spawning, and females in which the zygotes were manually removed within 1 day after spawning. Since there were no differences found in the ovaries between the two groups of females in which mouthbrooding did not occur, they were pooled together as non-mouthbrooders. Tissue from the mouthbrooders was taken 1, 3, 4, 5, 6, 7, 10, 20, and 25 days after spawning. Ovarian tissue from non-mouthbrooders was examined 3, 5, 7, 10, 20, and 25 days after spawning. Enzyme histochemistry was performed only on the tissue from mouthbrooders.

Electron microscopy detected the presence of smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubular cristae in certain cells of the theca interna of both pre- and postovulatory follicles. These structures are found in cells that synthesize steroid hormones. Granulosa cells also contained some smooth endoplasmic reticulum, but only in postovulatory follicles. The granulosa cells of postovulatory follicles also showed an augmentation of Golgi complexes, vesicles, microvilli, and microfilaments within 5-7 days after spawning. Enzyme histochemistry demonstrated an intense reaction of $\Delta 5$, 3B-hydroxysteroid dehydrogenase (3B-HSD) in variably placed thecal cells of postovulatory follicles from 1-7 days after spawning. During this time, there was an increase in young primary oocytes and recruitment of some of these to vitellogenic oocytes. Electron microscopy also showed that the follicles of oocytes in the yolk vesicle stage were more developed at regions where postovulatory follicular tissue directly contacted it. This may allow a localized stimulation of folliculogenesis, may aid in the transport of materials between the two follicular structures, or may be a method by which the postovulatory follicle donates cells to a developing follicle.

At 6-7 days after spawning, 3B-HSD activity became equally intense in sporadic thecal cells of both postovulatory follicles and the follicles of vitellogenic oocytes. By 10 days after spawning, the 3B-HSD activity was more intense in the follicles around vitellogenic oocytes. This indicates a shift in the dominant steroid-producing structure in the ovary.

Postovulatory follicles began to degenerate 10 days after spawning in non-mouthbrooders and 25 days after spawning in mouthbrooders. Also, the next clutch of eggs developed at a much higher rate in non-mouthbrooders. The ovarian cycle for non-mouthbrooders was about 25 days, while that for mouthbrooders was about 40 days. This would suggest that the presence of postovulatory follicular tissue may inhibit further oocyte development and/or be involved in the parental behavior of the female tilapia.

"Complete manuscript published in Cell and Tissue Research".

I 3

CHARACTERIZATION OF DOPAMINE RECEPTORS WITH REGARD TO GONADOTROPIN RELEASE IN THE AFRICAN CATFISH CLARIAS GARIEPINUS

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Introduction

In the African catfish Clarias gariepinus gonadotropin release is regulated by at least two hypothalamic factors. It is stimulated by gonadotropin releasing-hormone (GnRH), and this stimulation is inhibited by dopamine (DA; de Leeuw et al., 1986). A similar situation has been observed in other teleosts. Most indications regarding the dopaminergic inhibition were obtained using DA antagonists and agonists. In the African catfish also DA itself proved to inhibit GnRH induced GTH release (effective dose 5x 10^{-9} mole/kg body wt). The aim of the present communication is to characterize the DA receptors involved in the GTH release by means of administration in vivo of specific DA antagonists and agonists.

Materials and Methods

Sexually mature female African catfish with an average body wt of 595 g were used for the experiments. Groups of 10 animals each were injected with des-Gly^{10}-/D-ala^{6}/ LHRH ethylamide (LHRHa, Sigma, 10^{-7}/mole/kg body wt) alone or in combination with resp. sulpiride (D2 antagonist, Pharmexport B.V.), SCH 23390 (D1 antagonist, Schering Corp.), bromocryptine (D2 agonist, Sigma) and SKF 38393A (D1 agonist, Smith, Kline and French). The dose of all drugs was 10^{-6} mole/kg body wt. Bloodsamples were taken at t=0, 1, 2, 3, 4, 8 and 24 hr postinjection. After centrifugation, plasma samples were stored at -40°C until GTH measurements.

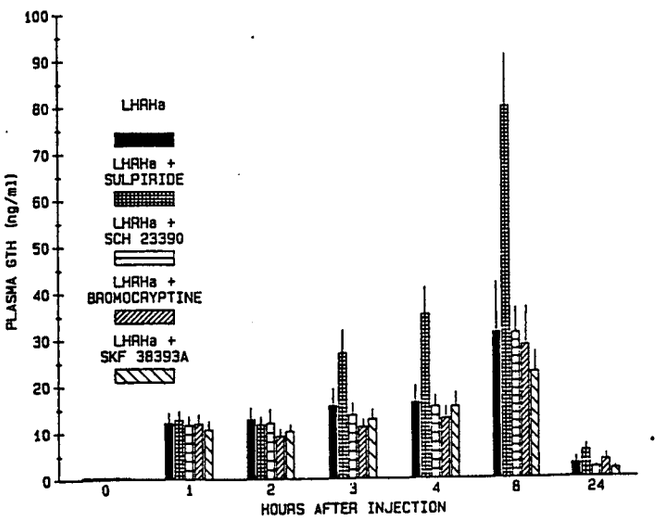

Fig. 1. The effect of injections of LHRHa alone, or in combination with specific dopamine D1 and D2 agonists and antagonists, on plasma GTH levels in mature female catfish.

Results

LHRHa caused a rise in plasma GTH levels, starting at 1 hr postinjection and reaching a maximum at 8 hr. At 24 hr the level was almost back at control value. The D1 agonist SKF 38393A and the D1 antagonist SCH 23390 did not have an effect on the LHRHa induced GTH levels. Sulpiride, the D2 antagonist, very strongly enhanced the LHRHa induced GTH release and caused ovulation in all animals. On the other hand, bromocryptine, the D2 agonist, did not influence the LHRHa stimulated GTH release (Fig. 1).

Conclusions

The results of experiments using LHRHa in combination with DA and the aspecific agonist apomorphine (de Leeuw et al., 1986) and pimozide (de Leeuw et al., 1985) demonstrated the involvement of DA receptors in the interaction with GnRH. The present results indicate that a D1 receptor does not play a role in the dopaminergic regulation of the GTH release. The effect of sulpiride points towards the involvement of D2 receptors. The effect of bromocryptine, however, is somewhat puzzling. As a D_2-agonist it could be expected to have an effect similar to that of DA, i.e. a D2 agonist an inhibition of the LHRHa stimulated GTH release. Investigations using other D2 agonists are in progress to clarify this problem.

References

de Leeuw, R., Goos, H.J.Th., and van Oordt, P.G.W.J., 1986. The dopaminergic inhibition of gonadotropin release: an in vitro study with fragments and cell suspensions from pituitaries of the African catfish, Clarias gariepinus (Burchell). Gen. Comp. Endocrinol. 63:171-177.

de Leeuw, R., Resink, J.W., Rooyakkers, E.J.M. and Goos, H.J.Th., 1985. Pimozide modulates the luteinizing hormone-releasing hormone effect on gonadotropin release in the African catfish, Clarias lazera. Gen. Comp. Endocrinol. 58:120-127.

THE REPRODUCTIVE PHYSIOLOGY OF TRIPLOID PACIFIC SALMONIDS

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Summary

We have examined the effects of induced triploidy on gonadal and pituitary development in coho salmon (Oncorhynchus kisutch), pink salmon (O. gorbuscha), and rainbow trout (Salmo gairdneri). Only a small fraction (less than one percent) of pre-meiotic cells successfully pass through meiosis in triploid gonads. This degree of gonadal development is not sufficient to trigger physiological maturation in females, but does do so in males. Triploid females remain silver-bright throughout their lives, whereas triploid males develop typical secondary sexual characteristics, produce small amounts of aneuploid spermatozoa, and exhibit the same degree of precocious maturation and post-spawning mortality as do normal males. Plasma levels of sex steroids and plasma and pituitary levels of gonadotropin are the same for diploid and triploid males throughout their lives. However, triploid females always have low or non-detectable sex steroid and gonadotropin levels in their plasma. Furthermore, relative to maturing diploid females or diploid and triploid males, triploid females have a very low pituitary gonadotropin content. Triploid females generally have minimal plasma vitellogenin levels, but the administration of 17B-estradiol induces a normal vitellogenic response by the liver. The production of all-female triploids is a useful technique to provide sterile fish for salmonid aquaculture. We have produced all-female triploids both by the direct feminization of mixed sex triploids and by the induction of triploidy in eggs fertilized with homogametic milt.

DETERMINATION OF VITELLOGENIN LEVELS IN OVULATORY PACIFIC SALMONIDS USING AN HOMOLOGOUS RADIOIMMUNOASSAY FOR COHO SALMON (ONCORHYNCHUS KISUTCH) VITELLOGENIN

T.J. Benfey[1], H.M. Dye[1], E.M. Donaldson[1] and T.G. Owen[2]

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[2]Helix Biotech Ltd., 217-7080 River Road, Vancouver Industrial Park, Richmond, British Columbia

Summary

We report the development of a radioimmunoassay to measure plasma levels of vitellogenin in Pacific salmonids. Coho salmon (Oncorhynchus kisutch) vitellogenin was purified by ion-exchange chromatography and antibodies were raised in rabbits by intramuscular injection of vitellogenin in Freund's complete adjuvant (for details, see Gordon, M.R., et al., 1984, Aquaculture 43: 333-339). Plasma sample dilutions from females at or near ovulation were parallel to the coho standard for all Pacific salmonids tested, i.e., coho, chinook (O. tshawytscha), chum (O. keta), pink (O. gorbuscha) and sockeye salmon (O. nerka) and rainbow trout (Salmo gairdneri), but not for Atlantic salmon (S. salar) or a non-salmonid, the sablefish (Anoplopoma fimbria). Vitellogenin concentrations at ovulation were 9.46 ± 12.84 (SD) mg/ml for coho, 9.10 ± 0.77 mg/ml for chinook, 0.36 ± 0.77 for chum, 0.16 ± 0.16 for pink, and 0.068 ± 0.086 for sockeye salmon. Immature male and female coho salmon had much lower plasma vitellogenin levels, but high levels were rapidly attained after repeated injections of 17B-estradiol.

IN VITRO STEROID PRODUCTION BY OVARIAN FOLLICLES OF ORANGE ROUGHY *HOPLOSTETHUS ATLANTICUS* COLLETT), FROM THE CONTINENTAL SLOPE OFF NEW ZEALAND.

N.W. Pankhurst*

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* Present address: University of Auckland, Leigh Marine Laboratory, R.D. Leigh, New Zealand.

Orange roughy were captured by trawl from depths of 800-1200m and ovarian follicles or segments from fish that had completed vitellogenesis but had not begun final oocycle maturation, were incubated *in vitro*. 11-deoxycortisol (11-DOC), deoxycortisone acetate, testosterone (T) at 0.1 and 1.0μg.ml , and cortisol (F) and 17α-hydroxy progesterone (17αOHP) at 1.0μg.ml significantly increased the proportion of oocytes undergoing final maturation, whereas 17α,20β-dihydroxy-4-pregnen-3-one (17α,20βP), progesterone, pregnenolone, 17β-estradiol (E_2) and low doses of 17αOHP and F did not.

T and 17αOHP were synthesized by isolated follicles in response to treatment with human and piscine gonadotropins, while 11-DOC, 17α,20βP and E_2 were not. This is consistent with measured changes in plasma steroids whereby T is produced during both vitellogenesis and final oocyte maturation, E_2 is present during vitellogenesis, 17α,20βP is not detectable at any stage and plasma 11-DOC changes are apparently unrelated to gonadal condition, with 11-DOC being of interrenal origin.

EFFECT OF (D-ALA^6, TRP^7, LEU^8, PRO^{9}NEt)-LUTEINIZING HORMONE-RELEASING HORMONE (sGnRH-A) AND DOPAMINE ON OOCYTE MATURATION AND STEROIDOGENESIS IN CARP (CYPRINUS CARPIO L.) IN VITRO

M. Sokolowska-Mikolajczyk, D. E. Kime*, T. Mikolajczyk and P. Epler

Department of Ichthyobiology and Fisheries, Agricultural Academy, Krakow, Poland, and *Department of Zoology, The University, Sheffield, United Kingdom.

Summary

The in vitro effects of sGnRH-A, dopamine (DA), carp hypophyseal homogenate (chh), and 17,20β-dihydroxy-4-pregnen-3-one (17,20P) alone and in combinations were examined. In carp, in distinction to other vertebrates, sGnRH-A and/or DA had no effect on oocyte maturation and steroidogenesis, nor did they affect the action of chh and 17,20P.

In some groups of vertebrates GnRH blocks gonadal development via inhibition or modulation of steroidogenesis. In frogs, for example, it increases testicular androgen production (Segal & Adejuwon, 1979) In the case of GnRH action on secretion of pituitary gonadotropins in fish, DA blocks the action of GnRH and decreases blood GtH levels. If GnRH acts at the level of the ovary in fish as in other vertebrates DA could remove the effects of GnRH on steroidogenesis. Carp oocytes were cultured in vitro to examine the effects of GnRH & DA on oocyte maturation and steroidogenesis compared to the maturational effects of chh and 17,20P.

Five 5-year old females were injected with chh (1 mg/kg) 24 hr before the in vitro experiment. From each fish triplicate incubations were carried out in all experimental groups. Ovarian fragments were incubated with sGnRH-A (1µg/ml), DA (1 & 10 µg/ml), chh (100 µg/ml), 17,20P (1 µg/ml) and with combinations of these hormones. After 24 hr incubation oocyte maturation was determined by the germinal vesicle (GV) position and the levels of testosterone (T), 17-hydroxyprogesterone (17P), 17,20P, and testosterone glucuronide (TG) in the incubation media measured.

The results (fig. 1.) show that sGnRH-A and DA did not alter oocyte maturation, or affect the influence of chh or 17,20P on oocyte maturation. Neither sGnRH-A nor DA had an effect on chh stimulated steroid production. All incubations which contained chh had very significantly higher concentrations of T, 17P, 17,20P and TG compared to control incubations.

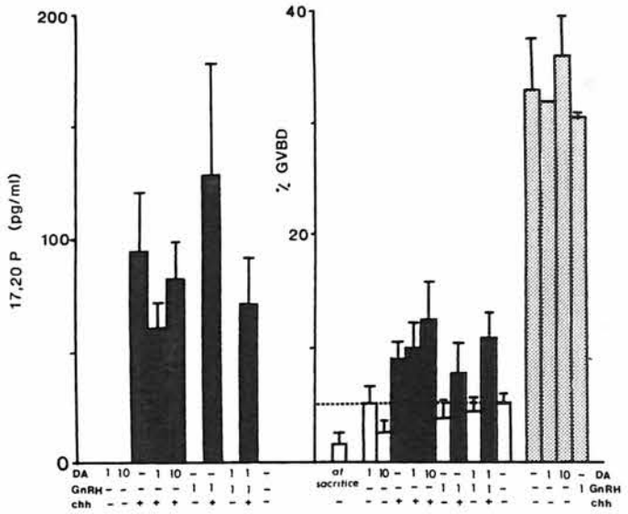

fig. 1. The effect of combinations of DA, GnRH, chh and 17,20P on oocyte maturation and steroidogenesis. Black bars represent incubations containing chh, and shaded bars those containing 17,20P.

The results show that neither DA nor GnRH alone or together affected steroidogenesis including the production of the probable maturation inducing steroid - 17,20P. Chh stimulated steroidogenesis was not affected by addition of either GnRH or DA. As expected, both chh and 17,20P stimulated oocyte maturation in vitro. The results obtained show that in carp sGnRH-A did not affect steroid biosynthesis in the ovary and did not inhibit or stimulate oocyte maturation. These results are confirmed by the fact that during stimulation of artificial spawning with intraperitoneal injections of GnRH-As this hormone has contact with the ovary but does not inhibit oocyte maturation or the ovarian stimulatory effect of pituitary GtH.

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EFFECT OF CO-CULTURE OF PRIMED AND CONTROL OVARIAN TISSUE OF THE COMMON CARP (CYPRINUS CARPIO L.) ON OOCYTE MATURATION AND STEROIDOGENESIS IN VITRO.

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Summary

Possible interactions between ovarian fragments from primed (carp hypophyseal homogenate, chh, treated) and control fish have been examined by in vitro co-culture. Primed tissue enhanced the % of oocytes with peripheral germinal vesicle (GV) in unprimed fragments. 17,20β-dihydroxy-4-pregnen-3-one (17,20P) production was suppressed to unprimed levels in co-culture but other steroids were unaffected.

Maturation of fish oocytes is controlled by pituitary gonadotropins which stimulate the synthesis of ovarian steroids. There is a certain degree of ovarian autonomy in the production of steroids and ovarian secretion may be regulated by intra-ovarian factors. It is not clear whether GtH primed ovarian tissue affects unprimed tissue and/or vice versa. In vitro co-culture of both primed and unprimed ovarian tissue was examined in order to elucidate this problem.

24 four-year old female carp were used. 12 were injected with chh (1 mg/kg body weight) and 12 with saline. 3 injected and 3 control fish were sacrificed at 3, 6, 12, & 24 hr after injection and ovarian fragments taken for in vitro culture and co-culture (fragments from injected & control fish incubated in the same vial). Incubations were carried out with and without addition of chh (100 µg/ml). Oocyte maturation was determined by the GV position and testosterone (T), its glucuronide (TG), 17-hydroxyprogesterone (17P) and 17,20P measured.

The percentage of oocytes with peripheral GV in tissue from unprimed fish was significantly ($P<0.05$) enhanced by the presence of primed tissue. Unprimed tissue caused a significant increase ($P<0.01$) in oocytes with peripheral GV in primed fish due to a decrease in number of oocytes undergoing final maturation (GVBD). T, TG and 17P showed no difference between primed, unprimed and co-cultured tissues. In all groups begun 6 to 24 hr after priming, 17,20P production was elevated in the medium from primed tissue alone but was low in medium from both unprimed tissue and from co-cultures. In no case did the co-culture produce higher amounts of 17,20P than unprimed tissue alone.

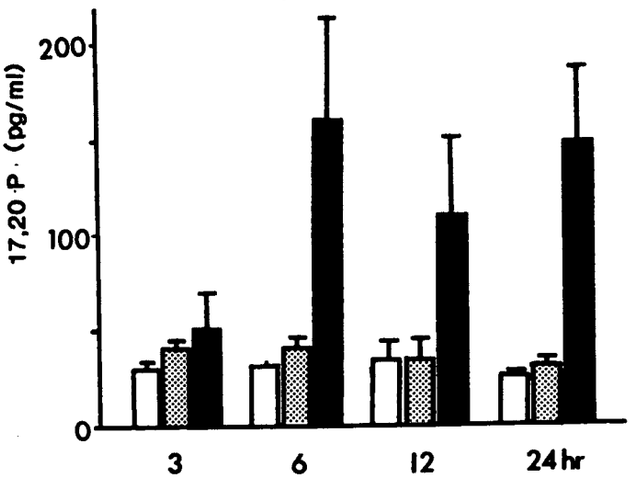

Fig. 1. Effect of co-culture on 17,20P production. Open bars show unprimed tissue dark bars primed tissue and light bars co-culture of primed and unprimed tissue.

The results show that there is an interaction between ovarian tissue from primed and unprimed fish when incubated together. 17,20P production is clearly suppressed by the presence of less mature, unprimed, tissue. Primed tissue accelerates GV maturity in unprimed tissue. Overall the results suggest that the two effects will tend towards a catching up process leading to synchronous maturation of the maximum number of oocytes. This fits well with the spawning pattern of carp where a large number of eggs must be spawned at one time. In our co-cultures the two types of tissue were separated by a mesh and it is probable that in an intact ovary in which different stages of maturity would be closely intermingled the effect would be far more pronounced and result in synchrony of oocyte maturation among all post-vitellogenic oocytes which will then be ovulated at one spawning. This synchronous development is in marked contrast to that in mammals where intra-ovarian factors inhibit development in all but a few oocytes. The inhibition of primed tissue in co-culture could be a similar mechanism to that found in mammals.

SOME PHYSIOLOGICAL ASPECTS OF GnRH RECEPTOR BINDING IN THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*

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In teleosts, the release of gonadotropic hormone (GTH) is stimulated by a gonadotropin-releasing hormone (GnRH). Chum salmon GnRH has been identified as Trp^{7}-Leu^{8}-LHRH (sGnRH). This sGnRH appears to be the predominant GnRH in several teleost species (Sherwood et al., 1984). The functional relationship between GnRH receptors and GTH release has been studied in goldfish (Habibi et al., 1987) and catfish (De Leeuw et al., 1987). The present studies demonstrate specific, saturable GnRH binding sites in the female catfish pituitary. In addition, the binding affinity of several GnRH-like peptides were compared with their in vitro bioactivity.

In order to study the presence of saturable GnRH binding sites, catfish pituitary membranes were incubated with increasing concentrations of the iodinated sGnRH analog, D-Arg^{6}-Pro^{9}GnRH-NEt (^{125}I-sGnRHa) for 2 hr at 4°C. At radioligand concentrations of 50×10^{-10} M and above, specific binding of ^{125}I-sGnRHa was saturable. A Scatchard plot analysis of the saturation data resulted in a straight line suggesting the presence of a single class of high-affinity binding sites ($Ka = 0.901 \pm 0.06 \times 10^{9}$ M^{-1}, Bmax = 1678±150 fmol/mg protein).

The specificity of ^{125}I-sGnRHa binding was demonstrated by displacement experiments, in which pituitary membranes were incubated with 2×10^{-9} M ^{125}I-sGnRHa and increasing concentration of sGnRHa, native sGnRH, the superactive LHRH analogs Buserelin (D-Ser(t-Bu)^{6}-Pro^{9}-LHRH-NEt) and LHRHa (D-Ala^{6}-Pro^{9}-LHRH-NEt) and other peptides. As shown in Fig. 1, the GnRH-like peptides inhibited the binding of ^{125}I-sGnRHa. The competition curves were parallel. The sequence of competition capacity was sGnRH > Buserelin > LHRHa. TRH (thyrotropin-releasing hormone), hCG (human chorionic gonadotropin), somatostatin and vasopressin did not cause inhibition of ^{125}I-sGnRHa-receptor binding.

With respect to the relation between the above-described relative binding affinities of sGnRHa, sGnRH, Buserelin and LHRHa and their bioactivity in the African catfish, the effect of these peptides on the release of GTH from catfish pituitary cell suspension was examined. After a preincubation period of 48 hr, pituitary cells (150.000/-200 µl medium) were incubated with increasing concentrations of sGnRHa, sGnRH, Buserelin and LHRHa. All peptides were equipotent in stimulating GTH release, i.e., they were active in the same dose range and their maximal responses were of equal magnitude. This is in agreement with the results of other in vitro studies (MacKenzie et al., 1984).

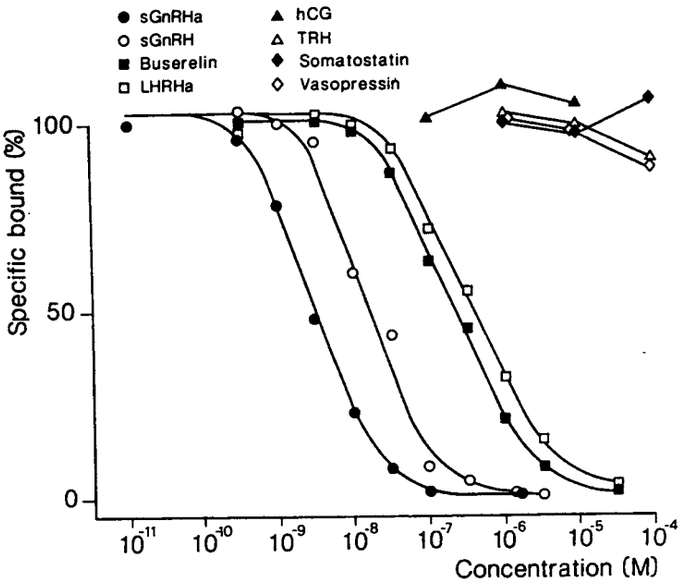

Fig. 1. Displacement of specifically bound ^{125}I-sGnRHa by different peptides.

A Scatchard plot analysis of saturation obtained with membrane fractions of pituitary cells cultured for 48 hr, showed a binding affinity of 0.15×10^{9} M^{-1} and a binding capacity of 62 fmol/500.000 cells. This binding affinity is approximately 5 times lower than the binding affinity of fresh pituitary membranes. This might be caused by the enzymatic treatment of the pituitaries in preparing the cell suspensions, leading to a possible receptor damage. In favour with this idea is the observation that after 7 days in culture the binding affinity is again comparable with the binding affinity of freshly prepared pituitary membranes, suggesting complete recovery of the receptors.

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STUDIES ON THE REPRODUCTIVE BIOLOGY OF GONADAL TUMOUR-BEARING CARP-GOLDFISH HYBRIDS

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Summary

Carp (Cyprinus carpio), goldfish (Carassius auratus) and their intergeneric hybrids were collected from several sites on the lower Great Lakes of North America from 1978 to 1981. The hybrids exhibited epizootics of gonadal neoplasms (a frequency of 68% in 461 hybrids examined) that ranged from small homogeneous nodules to large multinodular masses in various stages of necrosis and liquifaction. Historical evidence has implicated an environmental etiology for these tumours. Hybrids invariably displayed a hyperplasia of proximal pars distalis basophils (the presumptive gonadotrops) of the pituitary gland which seemed progressive as older hybrids had disproportionately larger pituitaries, totally due to the basophil proliferation. Parental species exhibited gonadal tumours at much lower frequencies (3.4% and 8.9% for carp and goldfish, respectively) and these neoplasms were composed of small nodules only.

Electrophoretic examination of tissue enzymes and morphological characters indicated that the hybrids examined for this study were all of the F_1 generation, with one possible exception.

The gonadal histology of male and female carp captured for this study was typical of a successfully reproducing population, whereas the hybrids appeared to be sterile. Meiosis was often completed in male hybrids but spermatids usually degenerated early in spermiogenesis. Females frequently completed vitellogenesis but provided no evidence of final maturation or ovulation. Proliferation of undifferentiated germ cells were observed in male and female hybrids and were considered to represent neoplastic transformations. Proliferations of Sertoli cells were common in males and in the male portion of hybrids exhibiting intersex gonads; the Sertoli cells of these individuals were well differentiated, formed tubules and accumulated large stores of lipid. Extensive degeneration of germinal cells was often apparent in areas of Sertoli cell proliferation as were numerous, large melanin-macrophage centres. Gonadal stromal cell tumours did not form tubules or accumulate lipid. The spindle cells of these neoplasms could be found in homogeneous sheets or intermixed to varying degrees with the early stages of germ cells and/or connective tissue elements.

The pituitary glands of hybrids had greater concentrations of immunologically active gonadotropin (GtH) than sympatric carp. Tumoured hybrids had a higher pituitary GtH concentration than non-tumoured hybrids. The gonadotrops were not considered to have undergone neoplastic transformation.

Heterologous gonad bioassays indicated that hybrid GtH was functional since it induced both phosphorous uptake by the immature chick testis in vivo and steroidogenesis in testis minces from brook charr (Salvelinus fontinalis) in vitro.

Radioimmunoassay determined serum GtH concentrations in hybrids was as high as or higher than levels found in sympatric carp indicating that the hybrid gonadotrops were capable of releasing GtH into the blood.

In carp serum testosterone (T) levels were similar in male and females, 11-ketotestosterone (11-KT) was predominantly a male androgen and 17β-estradiol (E_2) was predominantly associated with females. Non-tumoured male hybrids resembled male carp with respect to T, 11-KT and E_2, while non-tumoured female hybrids resembled female carp with respect to T and 11-KT but not E_2. Female hybrids did not seem to be preferentially converting T to E_2 as were female carp. Hybrids with gonadal stromal tumours exhibited the highest androgen levels which may indicate an androgenic function for the proliferating cell type. Absent or retarded steroidogenesis could not be supported as the cause of the hypophyseal hyperplasia in the hybrids.

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ANTIFREEZE PROTEIN GENE TRANSFER: A POTENTIAL SOLUTION TO OCEAN PEN CULTURE OF SALMON IN ICY WATERS.

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The ocean-pen culture of Atlantic salmon is restricted to areas where the water temperature does not decline below 0°C. This is because Atlantic salmon can freeze to death when the temperature drops to -0.7°C. Many marine fish can inhabit icy waters because they have evolved antifreeze proteins (AFP) which depress the freezing point of their body fluids to safe levels. We are proposing to make the Atlantic salmon more freeze resistant by transferring antifreeze protein genes from a fish which can survive icy waters, the winter flounder, to the genome of the salmon.

Approximately 1800 Atlantic salmon eggs were microinjected with linearized AFP genes immediately after fertilization. Survival to the fry stage was approximately 80%.

Individual fry were analyzed for the presence of the flounder AFP gene by genomic Southern blotting. Two out of the 30 progeny analyzed showed hybridization to the DNA probe. The hybridization bands following cleavage by restriction enzymes Sst I and Bam HI were identical to those of the linearized DNA which was injected into the eggs. These hybridization signals were absent from the control salmon. The intensity of the hybridization signals indicated that at least one copy of the AFP gene was present in each cell.

Research supported by a strategic grant from the Natural Sciences and Engineering Research Council of Canada and by the Medical Research Council of Canada.

THE GOLGI COMPLEX AND FLAGELLUM FORMATION DURING SPERMATOGENESIS IN GOLDFISH

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Summary

The spermatozoa of goldfish, Carassius auratus, have no acrosome and other peculiar features. It has been found that the sperm have Golgi complex in their neck (Guan,1987). OFTEN GROUPS OF TIGHTLY PACKED VESICLES ARE seen within the flagellum. As soon as the sperm are released into water, the flagellum rolls up from the tip towards the head continuously until it is incorporated into the head(Guan,1986).During spermatogenesis, the fine structure of Golgi complex changes as the cells differentiate from spermatogonium to spermatozoon. At the primary spermatocyte stage, the number and activity of Golgi complex increase and the flagellum formation starts. The primitive flagellum usually has 9+0 type axoneme. This fact may suggest that the main role of Golgi complex during spermatogenesis in goldfish lies in the formation of flagellum vesicles and the outside membrane sheath of axoneme. In spermatid, Golgi complex is rather small as Baccetti (1984) has reported.

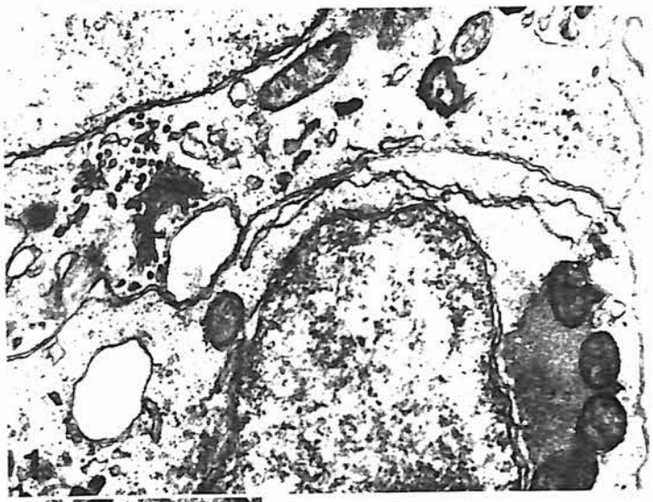

Fig.1. A young primary spermatocyte showing the base body very close to the plasma membrane. 20,000 X.

Fig.2. A primary spermatocyte has a 9+0 type axoneme near to the nucleus. 50,000 X.

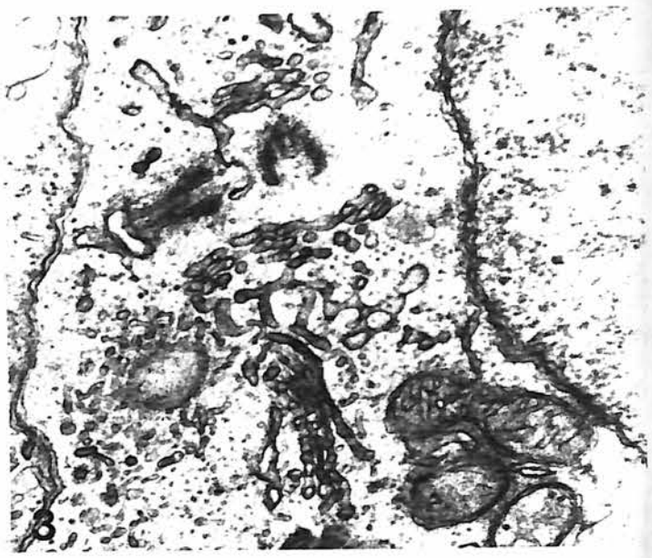

Fig.3. A primary spermatocyte. The base body is located at some distance from plasma membrane and close to Golgi complex.36,000 X.

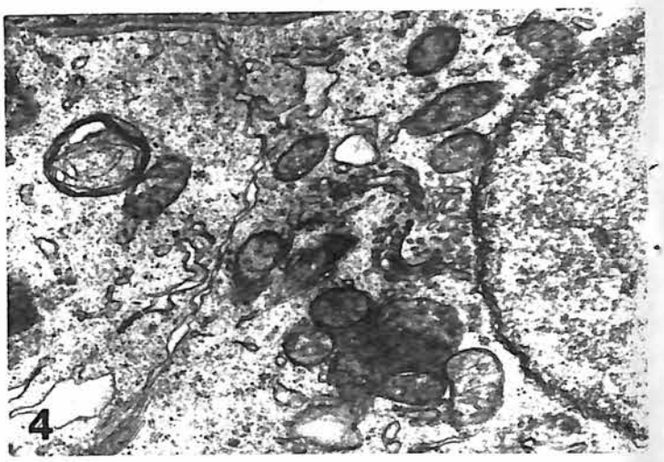

Fig.4. A primary spermatocyte showing the cisternae attached to both side of axoneme to form primitive flagellum which is located inside the cytoplasm. 18,000 X.

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WATERBORNE SELENIUM AND REPRODUCTIVE FAILURE IN CENTRARCHID FISHES

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Abstract

Over the past 10 years wet-basin coal-ash disposal at power plants has been implicated in the disappearance of freshwater fish populations from cooling water reservoirs. Associated with declining fish populations has been an increase in waterborne selenium. Selenium levels in fresh water are about ≤ 1 ug/l but in power-plant reservoirs may be 10-fold or more above background. Selenium is a required element for fishes but can be toxic to fishes depending on concentration and form of the selenium (VI, IV, -II). Selenium accumulates in the food chain of fishes with potential dietary concentrations from 500-1,000 the waterborne concentration. Selenium can also be taken up directly from the water. Fish population studies indicate that centrarchid fishes such as largemouth bass and bluegill are the first species to disappear from selenium contaminated reservoirs. Total body burdens of selenium are elevated compared to fishes from control areas. Carcass concentrations may be as much as 20-fold higher than controls. Selenium levels in the gonads are ≤ than carcass burdens in the testes but exceed carcass levels by about 2-fold in the ovaries. To assess the impact of the elevated levels of selenium in the gonads studies have been conducted by cross breeding bluegill with high and low body burdens of selenium. Neither precent fertilization nor percent hatch differed among parent combinations. All crosses with females with high selenium burdens produced larvae with edema and the larvae did not survive to swim-up. Histopathology studies on longear sunfish from a selenium-enriched reservoir indicate pronounced morphological alterations in the ovaries with atretic follicles, abnormally shaped follicles and reduced yolk mass being observed. Build-up of selenium in the ovaries of centrarchid fishes, which can exceed 1,000 times the water levels, results in damage to the ovary and production of abnormal larvae from spawned eggs. Levels of selenium in excess of 4 mg/kg wet weight in the ovary are associated with reproductive failure. The form of selenium responsible for the reproductive failure has not been identified. Selenium levels in water less than 3 ug/l will probably protect sensitive species from reproductive problems.

STEROIDOGENESIS AND OOCYTE MATURATION DURING INDUCED OVULATION IN CARP

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Summary

A combined in vivo-in vitro approach is described for optimising the conditions of induced ovulation in fish. For carp, two injections of hypophysial homogenate separated by a minimum of 12 h is required to induce the maturational changes necessary for ovulation at the optimum temperature of 20°. The changes in oocyte maturation and steroid production under various conditions are described.

Ovulation in cyprinid fish is artificially induced by means of two injections of carp hypophysial homogenate (chh). We have recently employed a mixed in vivo-in vitro approach to determine the optimum temperature and chh dose during induced ovulation in carp (Epler et al., 1987). This approach has been extended to examine the optimum interval between the two chh injections.

Carp were sacrificed at intervals of 3-24h after injection with chh (primed) or saline (control). A significant increase in number of germinal vesicles (GV) at the periphery of the oocyte occurred between 6 and 12 h after priming (Fig. 1)

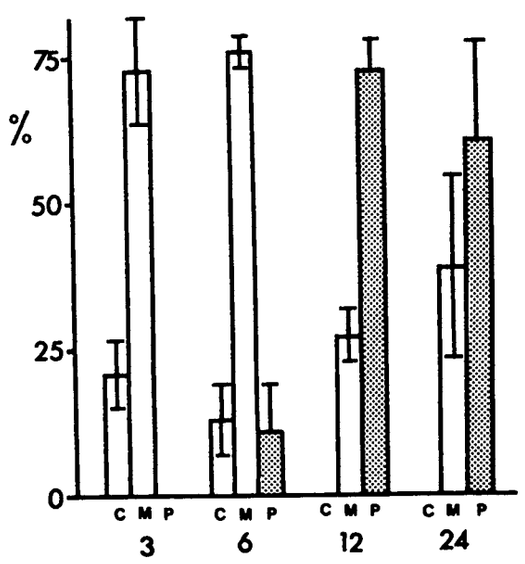

Fig. 1. % oocytes in central (C), migratory (M) and peripheral (P) positions 3-24 h after injection of a priming dose of chh.

The oocytes were incubated with or without chh for 24 h. Chh stimulated in vitro 17,20P production only in oocytes taken 6-24 h after priming. Oocytes taken at 6 and 24 h had similar response. Oocyte maturation increased in all chh incubations and germinal vesicle breakdown increased with time between priming and sacrifice (Fig.2). Testosterone, testosterone glucuronide and 17-hydroxyprogesterone were only produced in incubation with chh but yields were unaffected by priming or time of sacrifice. Chh did not give 17,20P or GVBD in incubations of oocytes from control fish, although the % peripheral GV increased.

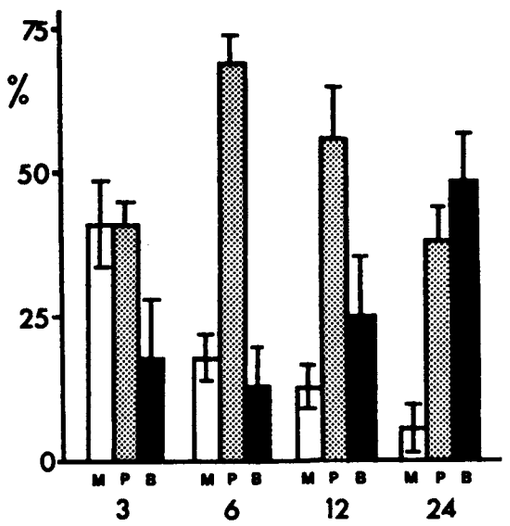

Fig. 2 % of oocytes in migratory (M), and peripheral (P) positions or after GVBD (G) in incubations with chh begun 3-24 h after priming.

The results further confirm the importance of priming on GV migration and induction of potential for 17,20P synthesis. The temporal sequence suggests that 12 h is the minimum time that should be allowed between priming and main injections. The close correlations between the predicted conditions for carp and those already found optimal in aquaculture suggests that the in vivo-in vitro approach might be useful for determining conditions for induced ovulation in other species.

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STEROID GLUCURONIDES IN THE SEMINAL VESICLE OF THE AFRICAN CATFISH

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Summary

In teleosts pheromones of gonadal origin may act as sex attractants and may induce reproductive behaviour. As sex pheromones steroid glucuronides have been suggested. In male African catfish, Clarias gariepinus, such sex pheromones may originate in the seminal vesicles. Indeed, an aqueous extract of seminal vesicles could be used to attract females after ovulation. In order to study the capacity of seminal vesicles to synthesize steroids and steroid glucuronides, tissue fragments were incubated with tritiated pregnenolone and androstenedione respectively. Moreover, steroid glucuronides were identified and quantified in seminal vesicle fluid of feral spawning, and feral and cultivated non-spawning catfish. After deglucuronidation with ß-glucuronidase the steroids were determined by gas chromatography-mass spectrometry.

The seminal vesicles were able to synthesize androgens, 11-oxygenated androgens and 5ß-reduced C_{21} and C_{19}-steroids. Moreover, a distinct synthesis of steroid glucuronides, i.e. 5ß-pregnane-3α,17α-diol-20-one, testosterone, 5ß-dihydrotestosterone, 5ß-androstane-3α,17ß-diol and etiocholanolone was observed. These steroid glucuronides, as well as the glucuronides of 5ß-androstane-3ß,17ß-diol, 5ß-androstane-3α,11ß-diol-17-one and 5ß-androstane-3α,17ß-diol-11-one, were traced in the seminal vesicle fluid. The latter were also identified as free steroids after in vitro incubation.

A short outline of the methods:

1) Steroids and steroid glucuronides were extracted from the seminal vesicle fluid by reversed-phase chromatography with Seppak C_{18} columns, followed by elution with ethanol (100%) and aqueous ethanol (50%).
2) The isolated steroids and steroid glucuronides were separated by an extraction with dichloromethane against water.
3) The water fraction was treated with ß-glucuronidase and the deglucuronidated steroids were extracted with dichloromethane.
4) The deglucuronidated steroids were derivatized into (oxime-) trimethylsilyl-ethers.
5) The derivatized steroids of standards and seminal vesicle fluid were applicated on a Hewlett-Packard 5992B gaschromatography-mass spectrometer with a fused silica capillary column.
6) Retention times and mass spectra of steroids from the seminal vesicle fluid were compared with those of standard steroids.
7) Selected ion monitoring (SIM) was carried out on characteristic ions to detect steroids at lower concentrations.
8) For quantification, calibration curves were prepared between standard steroids and an internal standard 5α-androstan-3ß-ol. To correct for losses during extraction ^{3}H-estrone-glucuronide was added at step 1, while to correct for losses during derivatization and GC-MS analysis 5α-androstan-3ß-ol was added at step 4.

After GC-MS application, the deglucuronidated steroid fraction of the seminal vesicle fluid showed mass spectra at the retention time of the standards 5ß-pregnane-3α,17α-diol-20-one, etiocholanolone and 5ß-androstane-3α,17ß-diol-11-one, highly similar to those of the corresponding standard steroids. SIM analysis showed the presence of characteristic ions of 5ß-androstane-3α,17ß-diol and testosterone at the expected retention times of these steroids. An indication of the presence of 5ß-androstane-3ß,17ß-diol, 5ß-dihydrotestosterone and 5ß-androstane-3α,11ß-diol-17-one could not be found.

Quantification of the glucuronides demonstrated that in the feral spawning group the levels of 5ß-pregnane-3α,17α-diol-20-one-, 5ß-androstane-3α,17ß-diol-11-one- and 5ß-androstane-3α,17ß-diol-glucuronide were significantly higher than in the feral and cultivated non-spawning groups. The levels of etiocholanolone- and testosterone-glucuronide showed no significant differences. Comparison of the feral non-spawning and cultivated non-spawning group revealed that only the level of 5ß-pregnane-3α,17α-diol-20-one-glucuronide was significantly higher in the feral non-spawning group.

Reference

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CATECHOLESTROGENS, THEIR SYNTHESIS AND INHIBITORY EFFECT ON THE METABOLISM OF DOPAMINE IN THE BRAIN OF THE AFRICAN CATFISH

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In several teleosts, including African catfish, Clarias gariepinus, gonadal steroids have a negative feed-back action on gonadotropin release, and dopamine can inhibit this GnRH stimulated release.

In order to investigate a possible link between the inhibitory actions of sex steroids and dopamine, the following hypothesis was proposed. In brain centers, sex steroids (androgens) are aromatized to estrogens. These estrogens can be hydroxylated to catecholestrogens, which subsequently are methylated by catechol-O-methyl transferase (COMT). COMT is also responsible for the inactivation of dopamine. Thus the presence of catecholestrogens might inhibit the degradation of dopamine, resulting in a stimulation of the dopaminergic inhibition of the gonadotropin release.

This hypothesis presupposes the presence of the enzymes involved in the conversion of androgens into methoxycatecholestrogens in brain centres, preferably in centres regulating gonadotropin release. Moreover, the affinity of COMT for catecholestrogens should be the same as or higher than its affinity for dopamine.

The first supposition was checked by studying enzyme activity in 0.3 mg brain punches, using a radiometric assay method. The second supposing was evaluated by studying the kinetic characteristics of COMT following incubations of telencephalon homogenates with catecholestrone and dopamine respectively, and by studying substrate competition by incubating similar homogenates with both substrates together.

Aromatase

The highest activity (expressed as pmol estrogen per mg tissue per hour) was detected in the preoptic region (3.7 pmol). The more caudally located area tuberalis, including the nucleus lateralis tuberis and the nucleus recessus lateralis, also showed a relatively high activity (2.5 pmol). A similar activity was found in the most rostral part of the telencepahalon and the dorsal parts of the mesencephalon i.e. tectum opticum and torus semicircularis (2.3 pmol). A moderate aromatase activity was observed in remaining parts of the brain, except cerebellum and hindbrain, in which aromatase activity was hardly detectable (0.1-0.3 pmol).

2-Hydroxylase

This enzyme could be demonstrated throughout the brain. Relatively high activities were observed in the telencephalon, particularly in the area ventralis pars dorsalis (0.5 pmol), in the nucleus recessus posterior (0.5 pmol) and the area tuberalis, including the nucleus lateralis tuberis, and the preoptic region (0.4 pmol) of the hypothalamus, and in the tectum opticum and torus semicircularis of the dorsal mesencephalon (0.4 pmol). The ventral mesencephalon (0.2 pmol) and the hindbrain (0.1 pmol) showed a much lower activity. A moderate activity was found in the remaining parts of the brain (0.3 pmol).

Catechol-O-methyltransferase

This enzyme, although present in most of the brain areas (5-20 pmol), shows a more than moderate activity in areas containing a high aromatase activity.

Substrate competition

Substrate saturation studies indicated a preference of COMT for catecholestrogens. Although the Km values of catecholestrone and dopamine were nearly the same, competition experiments with equal substrate concentrations demonstrated a higher methylation of catecholestrone than of dopamine.

Conclusions

- The enzymes, aromatase, 2-hydroxylase and COMT were detected in regions known to be involved in the regulation of reproduction.
- Since the torus semicircularis and the tectum opticum demonstrated a high activity of the three enzymes, it is suggested that these structures are also involved in reproductive processes.
- The in vitro results of the competition experiments suggest that in vivo the presence of catecholestrogens may lead to a decreased degradation of dopamine, resulting in a prolonged dopaminergic inhibition of gonadotropin release.

I

COMPARISON OF (D-ARG^6,TRP^7,LEU^8,PRO^{9}NET)-LUTEINIZING HORMONE-RELEASING HORMONE (tGnRH-A) AND (D-ALA^6,PRO^{9}NET)-LUTEINIZING HORMONE-RELEASING HORMONE(LHRH-A), IN COMBINATION WITH PIMOZIDE OR DOMPERIDONE IN STIMULATING GONADOTROPIN RELEASE AND OVULATION IN THE CHINESE LOACH(PARAMISGURNUS DABRYANUS)

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The Chinese loach is a relatively new species in freshwater aquaculture in China. In addition to research for development of a reliable technique for induced ovulation for artificial propagation of this species, we have used the loach for comparative studies on the actions of (D-Arg^6,Trp^7,Leu^8,Pro^{9}NEt)-LHRH (tGnRH-A) and (D-Ala^6,Pro^{9}NEt)-LHRH (LHRH-A) on gonadotropin secretion, as well as the activity of two dopamine receptor antagonists, domperidone and pimozide, in potentiating the actions of sGnRH-A and LHRH-A. The results demonstrate that sGnRH-A is more potent than LHRH-A, by a factor of about 10 fold when given alone or in the presence of pimozide or domperidone. Pimozide and domperidone are about equipotent in potentiating the actions of both sGnRH-A and LHRH-A, whereas the difference in potency of sGnRH-A and LHRH-A is similar to that found in goldfish and common carp, the lack of any apparent differences in potency of domperidone and pimozide is different from the situation in these other species in which domperidone is about 10 fold more potent. Since the potentiation of the actions of sGnRH-A and LHRH-A by pimozide or domperidone, on blood levels of gonadotropin, is less in the loach than in goldfish and common carp, the gonadotropin release-inhibitory activity of dopamine in loach is presumably less prominent. On the practical side, a single set of injections of domperidone(5 mg/kg body weight) and sGnRH-A (10 ug/kg body weight) is highly effective in inducing ovulation within 11-14 hours following injections.

HORMONAL REGULATION OF VITELLOGENESIS IN MURREL, *CHANNA PUNCTATUS* (BLOCH)

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Studies with Indian catfish showed that estradiol-17β could induce the synthesis of vitellogenin, and gonadotropin is responsible for the uptake of this lipophosphoprotein into oocytes (Sundararaj & Nath, 1981; Nath & Sundararaj, 1981; Sundararaj *et al.*, 1982). Recently we have examined the influence of estrogen and gonadotropin on the vitellogenin level of liver, serum and ovary in an Indian murrel, *Channa punctatus* and obtained some interesting results. Like majority of Indian teleosts, this fish is a seasonal breeder and breeds during monsoon. All experiments were conducted on fish belonging to preparatory phase I and II when ovary contains nonyolky oocytes (80-90%) and thus suitable for evaluating the effects of hormone administered to them.

Vitellogenin was identified in the sera of vitellogenic and estradiol-17β-treated female murrel by subjecting the serum to gel filtration on Sephadex G-200. Treatment of female murrel with estradiol-17β (E_2) and estrone (E_1) at a dose of 10 μg/fish/day for five days resulted in a significant increase in serum vibellogenin level with concomitant decrease of liver vitellogenin, but there was no incorporation of vitellogenin into oocytes which was reflected by ovarian weight. Estriol, testosterone, progesterone and cortisol treatment showed no effect on vitellogenin profile in liver, serum and ovary. Simultaneous treatment of salmon gonadotropin (SG-G 100) at two dose levels (500 ng or 2.5 μg/fish/day) along with E_2 (10 μg/fish/day) revealed that (i) a synergestic action on the synthesis of vitellogenin was noticed when lower dose of SG-G 100 was used with E_2 for 9 days without promoting the incorporation of vitellogenin into oocytes and (ii) when higher dose was used ovarian vitellogenin content as well as ovarian weight was increased.

The above findings indicate that in murrel, both E_1 and E_2 are effective in the synthesis of vitellogenin but the action of E_2 can be enhanced in the presence of SG-G 100 indicating that SG-G 100 is, perhaps, doing something to liver cells so as to enable the acceleration of the action of E_2. Secondly, for the incorporation of vitellogenin into oocytes a higher dose of gonadotropin is essential.

Work is in progress and effort is being directed to obtain explanation in regard to the mechanism involved in the uptake of vitellogenin from serum to oocytes.

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STEROID GLUCURONIDES AS SEX ATTRACTING PHEROMONES IN THE AFRICAN CATFISH, CLARIAS GARIEPINUS

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Summary

Shortly after ovulation female African catfish are attracted by the odor of male conspecifics. This odor seems to originate from the seminal vesicle, since extirpation of the seminal vesicle made males less attractive, whereas removal of the testes, resulting in a subsequent enlargement of the seminal vesicle, made males more attractive (Resink et al., 1987).

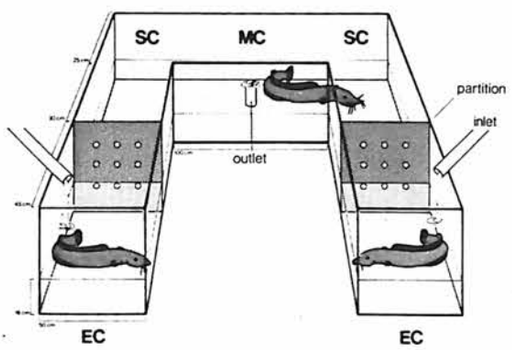

Fig. 1. An U-shaped two-choice tank.

Attraction tests were carried out with ovulated female catfish placed in the testing chamber (TC) of a two-choice tank (Fig. 1). In both end compartments (EC) a male without seminal vesicle was placed behind a perforated partition. When a dosage of 2.0 ppm seminal vesicle fluid was infused into one EC, female catfish spent more time in the adjacent side compartment (SC, Fig. 2A). No attraction was found with doses of 0.1 an 0.5 ppm; a dose of 16.0 ppm resulted in a repulsive effect. The steroids and steroid conjugates of the seminal vesicle fluid were isolated by reversed phase chromatography. The free steroids were then removed with dichloromethane; the remaining water fraction containing the steroid conjugates (glucuronides). Inflow of this water fraction (2.0 ppm) attracted ovulated female catfish, whereas other seminal vesicle fluid fractions did not (Fig. 2B). After treatment with ß-glucuronidase, the water fraction did not cause attraction, indicating the steroid glucuronides as sex attracting pheromones. Bilateral sectioning of the lateral and medial subdivision of the olfactory tracts, respectively, demonstrated that the attraction dependents on the medial tract (Fig. 2C).

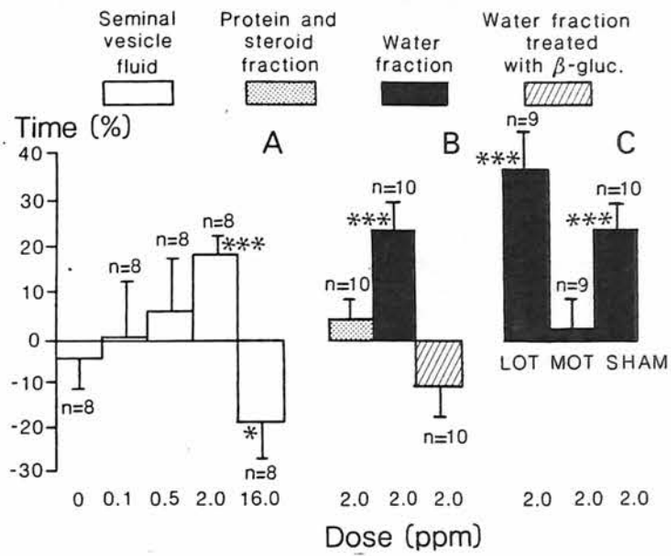

Fig. 2. Change in percentage time spent by normal (A,B), sham operated (SHAM), lateral (LOT) and medial (MOT)olfactory tract sectioned ovulated catfish in a SC due to infusion of different fractions. (* $P < 0.05$, ** $P < 0.025$, *** $P < 0.005$)

In addition, the olfactory sensitivity of female catfish for seminal vesicle fluid fraction was measured by underwater electro-olfactography. 10^{-5} M L-serine was used as a reference stimulus. Seminal vesicle fluid appeared to be a highly stimulatory odorant, mainly because of its steroid glucuronides. Water fractions containing steroid glucuronides evoked a higher response than fractions containing proteins and free steroids, and this response was much lower when the water fractions had been pretreated with ß-glucuronidase.

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Acknowledgement

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STEROIDOGENESIS DURING OOCYTE MATURATION AND OVULATION IN THE OVARY OF THE AFRICAN CATFISH, CLARIAS GARIEPINUS

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Fish farmers are showing an increasing interest in the African catfish, Clarias gariepinus, a.o. because of its high food conversion and its resistance to diseases. Under husbandry conditions, however, this species does not show spontaneous spawning.

Females need to be injected with gonadotropins or relevant steroids in order to induce oocyte maturation and ovulation. Indirectly oocyte maturation and ovulation can be induced by injecting pimozide (PIM) and the gonadotropin releasing hormone analogue des-Gly^{10}, D-Ala^{6} LHRH ethylamide (LHRHa). (De Leeuw et al., 1985). This leads to an increase in plasma gonadotropin (GTH) level, and subsequently to changes in the ovary that result in ovulation.

These changes, especially those concerning steroidogenesis were studied in 40 mature females, 0, ½, 1, 4, 8, 10, 12 and 16 hours after the injection of 10 µg Pim and 0.1 µg LHRHa/g body weight. Upon autopsy part of the ovarian tissue was processed for histological examination, and part was used for in vitro incubation during 3 hours with [^{3}H]-pregnenolone and [^{3}H]-androstenedione as precursors. A homologous radioimmunoassay was used for measuring the plasma GTH levels.

Until 4 hours after injection all ovaries were in the post-vitellogenic stage, and between 8 and 16 hours after PIM-LHRHa administration changed from stages of nuclear migration and germinal vesicle breakdown to ovulation and post-ovulation conditions. The onset of nuclear migration was accompanied by a rise in plasma GTH level from 25 mg ml^{-1} to 150 ng ml^{-1}. This level remained high during subsequent stages.

The maturation and ovulation processes were also accompanied by changes in steroidogenic pathways. In post-vitellogenic ovaries pregnenolone and androstenedione were mainly converted into testosterone, whereas during the stages of nuclear migration and germinal vesicle breakdown steroidogenesis was shifted in the direction of 17α,20ß-dihydroxy-4-pregnen-3-one and three 5ß-reduced C_{21}-steroids, i.e. 5ß-pregnane-3α,17α-diol-20-one, 5ß-pregnane-3α,17α,20ß-triol and 5ß-pregnane-3α,6α,17α,20ß-tetrol.

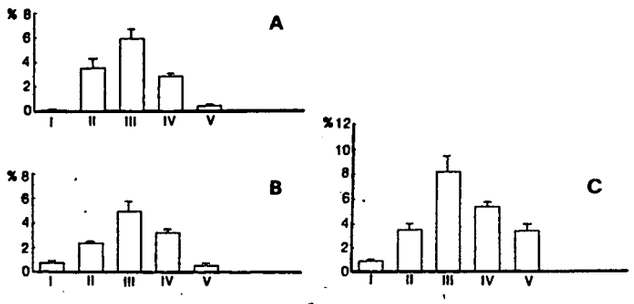

Fig. 1. Yields of 17α,20ß-dihydroxy-4-pregnen-3-one (A), 5ß-pregnane-3α,17α,20ß-triol (B), and 5ß-pregnane-3α,6α,17α,20ß-tetrol (C) during post-vitellogenesis (I), nuclear migration (II), germinal vesicle breakdown (III) ovulation (IV) and post-ovulation (V).

The latter, very polar steroid has not been identified in fish before. During maturation also the production of steroid glucuronides increased, especially that of testosterone-glucuronide, and to a lesser degree that of 5ß-dihydrotestosterone-, 5ß-androstane-3α,17ß-diol- and 5ß-pregnane-3α,17α-diol-20-one-glucuronide. During ovulation and post-ovulation the conversion of the steroid precursors had decreased, leading to a reduced synthesis of the steroids marking oocyte maturation.

These results indicate that not only 17α,20ß-dihydroxy-4-pregnen-3-one, but also some related 5ß-reduced C_{21}-steroids may be involved in inducing oocyte maturation and ovulation. Moreover, the steroid glucuronides and the highly polar 5ß-pregnane-3α,6α,17α,20ß-tetrol might function as sex pheromones and assist in evoking postovulatory spawning behaviour. Experiments are being carried out to evaluate this hypothesis.

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ULTRASTRUCTURAL LOCALIZATION OF FREE CALCIUM IN PITUITARY GONADOTORPS OF THE AFRICAN CATFISH, CLARIAS GARIEPINUS: EFFECTS OF GONADOTROPIN-RELEASING HORMONE

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Summary

The presence and localization of free calcium in catfish gonadotrops was ultrastructurally demonstrated with the combined oxalate-pyroantimonate procedure. It appeared that precipitates of the calcium-pyroantimonate complex were present in the mitochondrial matrix, the cytosol and in particular in cisternae of the endoplasmic reticulum. In vivo treatment of fish with LHRHa strongly increased the deposition of calcium in the cisternae.
Keywords: catfish, gonadotrops, ultrastructure, calcium, LHRH.

Introduction

Gonadotropin release in the African catfish is stimulated by GnRH, which in turn is under inhibitory control of dopamine (De Leeuw et al. 1986). The release of pituitary hormones in general and of gonadotropins in particular is, moreover, Ca^{2+} dependent (Moriarty, 1978). A role for Ca^{2+} as mediator of GnRH-induced gonadotropin release has been suggested by a.o. Hopkins and Walker (1978) and Conn et al. (1979). The present study was undertaken to localize calcium in relation to its functional properties in stimulated pituitary gonadotrops.

Materials and Methods

Twenty mature females of the African catfish, Clarias gariepinus, were used for this study. Fifteen females were treated with the dopamine antagonist Pimozide in combination with LHRHa. The fish were sacrificed in three groups of five specimen each, after respectively 30, 45 and 60 min.; five fish served as controls. After dissection the pituitaries were prefixed in 3% glutaraldehyde in 0.01 M oxalic acid, followed by immersion in potassium oxalate and postfixation in a mixture of 1% OsO_4 and 2% potassium pyroantimonate according to Borgers et al. (1984). After dehydration the tissues were embedded in Epon. Verification of the presence of calcium included treatment of the sections with EGTA.

Results and discussion

Precipitates of the calcium-pyroantimonate complex were present in the mitochondrial matrix, the cytosol and more in particular in the cisternae of the rough endoplasmic reticulum (RER). Occasionally the precipitates were distributed also on the limiting membrane of secretory granules. In fish treated with Pimozide-LHRHa the calcium deposits in the RER cisternae had weakly increased after 30 min, whereas a strong increase and a moderate increase were observed at 45 and 60 min following injection, respectively (Fig. 1). Apparently, the stimulatory effect on the calcium concentration is time-dependent. Stimulation of the gonadotrops was, moreover, accompanied by an increase in number of RER cisternae. After treatment of sections with EGTA the calcium-pyroantimonate precipitates had disappeared. The rise in calcium concentration in the stimulated gonadotrops is probably due to an influx of Ca^{2+} across the cell membrane (Limor et al. 1987). Most probably the RER plays a crucial role in the regulation of the calcium concentration in the cytosol.

fig. 1a plate

Calcium deposits at 45 min after injection with Pimozide-LHRHa; x 14.400.

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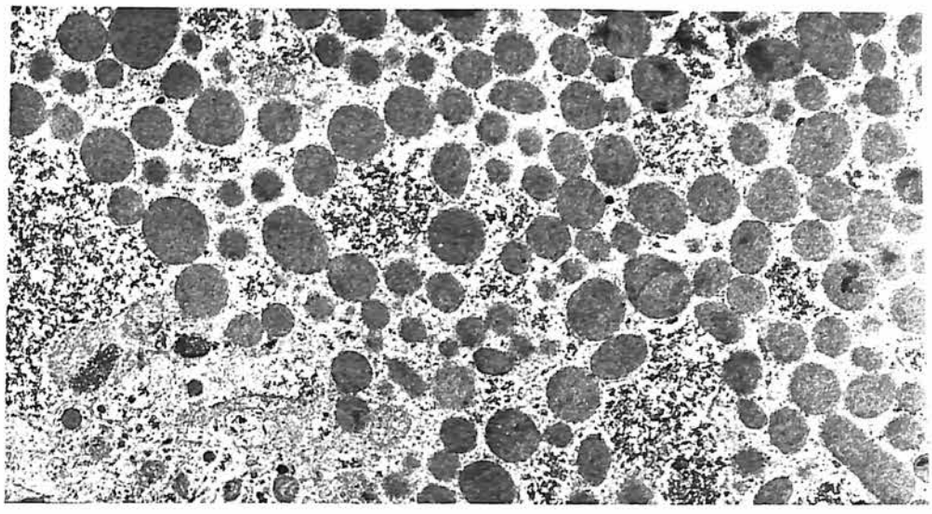

M/S. Peale et al.

A STUDY OF OOCYTE DEVELOPMENT AND INDUCTION OF SPAWNING IN THE WINTER FLOUNDER FOLLOWING LHRH ANALOG IMPLANTATION

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Female winter flounder were collected in February from Newfoundland inshore waters by scuba divers. The fish, in prespawning condition, were held in seawater at controlled laboratory seawater temperatures (5 $\pm$ 2 C) and under ambient photoperiod without feeding for approximately 2 months or until they spawned. On February 18, 1986 the fish were implanted IP with either a blank cholesterol pellet or a cholesterol pellet containing 100μg [D-Ala^6,Pro^9-NHet]LHRH analog. The fish were checked weekly for spawning by attempting to express ovulated eggs from the body cavity. At biweekly intervals, a 14G needle was used to aspirate 300-600 mg egg samples directly from the dorsal ovary. Monitoring of the state of egg development was conducted immediately by submitting egg biopsy samples to microscopic determinations of egg diameter and clearing oocytes for determination of germinal vesicle position. Further oocyte analyses included the determination of % dry weight and estimates of protein, lipid and fatty acid compositions.

The results of our study showed that spawning can be advanced as much as two months by implantation of LHRH analog in female winter flounder held under laboratory conditions of constant temperature and ambient photoperiod. Migration of the germinal vesicle was a good indicator of the approach of the spawning period in the flounder. During this time egg size increased while ovarian dry weight declined, presumably associated with the uptake of water. The level of protein in the ovary remained constant while lipid levels appeared to decline. An analysis of total ovarian fatty acids revealed a profile typical of marine species with the (n-3) fatty acids being the most abundant family. The fatty acid profile did not change in the control fish during the experiment and appeared unaffected by LHRH analog. Since no changes in the total protein or fatty acid profile were observed between prespawning fish and fish induced to spawn with LHRH analog, our data suggests that acceleration of spawning can be accomplished without deleterious effects on these aspects of egg chemistry.

THE INFLUENCE OF [D-Ala^6,Pro^9-NHet]LHRH (LHRH$_a$) TREATMENT ON PLASMA SEX STEROIDS PROFILES OF WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS DURING GONADAL RESCRUDESCENCE

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It is well established that oocyte development in teleosts is regulated by the pituitary with vitellogenesis being mediated by 17β-estradiol and final maturation and ovulation by the action of gonadal progestogens. In the male, testosterone and 11-ketotestosterone are the major androgens that are thought to influence testicular growth including spermatogenesis and spermiation.

In winter flounder, the gonads of both sexes following spawning are completely regressed (July GSI's about 2.7% and 0.9% in female and male respectively). Beginning in August the male gonads develop rapidly through to November (GSI 16%) and in females, onset of vitellogenesis also begins in August continuing progressively through February when the ovary reaches its maximum size (GSI 16%). Thereafter, little vitellogenin uptake occurs and these fish undergo a period of apparent ovarian maintenance retaining the high GSI until May when spawning occurs.

LHRH analogs have been used previously to induce spawning in a variety of fishes. Most of these studies have been performed on mature fish near the time of spawning. The purpose of the present series of studies was to investigate the seasonal variation in pituitary sensitivity to LHRH stimulation in male and female winter flounder as indirectly determined by sex steroid hormone levels. LHRH$_a$ was administered by cholesterol pellet implantation (125 μg hormone) and by intraperitoneal injection (20 μg/kg b.w.). Experiments were conducted for periods of 2-4 weeks. Plasma sex steroids 11-ketotestosterone and 17β-estradiol were measured by RIA.

Males in October (GSI 10%) and November (12%) exhibited an increased level of 11-ketotestosterone following LHRH$_a$ implantation. By contrast sham-operated fish showed a decrease in steroid levels. Injections of LHRH$_a$ twice a week in December (GSI 16%) induced significant increases in 11-ketotestosterone compared to saline injections. All the hormone treated males were spermiating when the December experiment was terminated. Female fish implanted with LHRH$_a$ in October (GSI 7.0%) showed an increase in 17β-estradiol throughout the experiment for two weeks; however, levels of 17β-estradiol decreased in sham-operated fish. When females were injected with LHRH$_a$ in February the levels of 17 -estradiol were also elevated compared to saline injected fish.

In conclusion, the levels of 11-ketotestosterone and 17β-estradiol in males and females, respectively, are increased following LHRH$_a$ administration. This suggests that the pituitary is responsive to exogenous LHRH$_a$ during the gonadal recrudescent stage of reproductive cycle.

A NEUROETHOLOGICAL STUDY OF REPRODUCTIVE BEHAVIOR IN THE SALMON

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During the breeding season animals communicate each other using various species-specific signals, which transmit informations as to their species identity, their sex, their mating readiness, etc. A chain of stimuli and reactions during interactions between the partners are often observed. These signals and the behavioral chain ensure them successful mating and fertilization of the eggs.

Reproductive behavior of the salmon proved to be an exellent subject to analyze the signals and the neural substrates for this behavior. Hime salmon (landlocked form of red salmon, Oncorhynchus nerka; known as 'kokanee' in North America) and chum salmon (Oncorhynchus keta) were used as experimental animals, and various neuroethological methods, which were a combination of ethological, neurophysiological and neuroanatomical techniques, were used. Sexual behavior of these fishes was observed in aquaria and was described quantitatively.

The sexual behavior of hime salmon and chum salmon consists of courting by the male and nestbuilding by the female, as in other salmonid fishes, and culminates in spawning within the nest by both sexes, through stimulus-reaction chain of behaviors by each sex. Three successive stages were distinguished in the sexual behavior: early (stage 1) and late (stage 2) stages before the spawning, and a stage (stage 3) after the spawning. Progress from stage 1 to stage 2 was gradual, while that from stage 2 to stage 3 was rapid. The female performed a set of behavior patterns particular to each stage, while the male behaved in basically similar ways irrespective of the stages, although the level of sexual motivation of the male decreased at stage 3. Thus, the sexual behavior of salmon may be a system well suited for analyzing the signal and the neural bases underlying the hierarchy and temporal patterning of motivated behavior.

To clarify the signals releasing male sexual behavior, model presentation experiments were performed. It was found that key stimuli for eliciting male courtship behavior consisted of three aspects of visual cues, i.e., shape, pattern and movement which simulated features of female engaged in sexual behavior. On the other hand, key stimuli for eliciting male spawning behavior consisted of both vibrational and visual cues. Matching of positions of these cues was found to be important for eliciting spawning behavior. Importance of vibrational cues was also confirmed by experiments, in which electromyographic (EMG) activities of the body muscles and vibration of the body were measured. These stimuli may serve as signals which transmit informations between the partners as to the timing of gamete release and ensure its synchronization between two sexes.

To examine brain areas involved in the sexual behavior, localized electrical brain stimulation and brain lesion experiments were performed. It was found that electrical stimuli applied to specific loci in the telencephalic and preoptic areas elicit various sexual behavior patterns. Within these regions of female there is a hierarchy of neural systems which mediate progressively more complete sexual behavior. On the other hand, heavy deficits in the sexual behavior were brought about after lesioning these areas. It was suggested that the telencephalic and preoptic areas are involved in the control of sexual motivation. Projection areas to or from some of these regions were examined using retrograde/anterograde axonal tracing methods.

Analyses of EMG activities of body muscles during the sexual behavior revealed EMG patterns peculiar to each behavior, suggesting the existence of specific motor-pattern generators (presumably within the spinal cord) for these behaviors. To obtain some inference about controlling mechanisms of them, descending pathways to the spinal cord were examined by retrograde labeling methods using cobaltic lysine complex.

From these ethological/neurophysiological/neuroanatomical results a neural model controlling the sexual behavior was proposed.

The production of homozygous gynogenetic inbred lines of common carp, Cyprinus carpio L.

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Abstract

Standardization of bio-assays for hormones, immune response and vitamins is often hampered by large variation in results, caused by genetic and environmental influences. These problems might partly be solved if highly inbred gynogenetic fish are used.
In gynogenesis, eggs are fertilized with irradiated sperm, which is genetically inactive, and kept diploid by suppression of the second meiotic or first mitotic division. In the first case the degree of homozygosity depends on the rate of crossing over between non-sister chromatids during the first meiotic division. In the second case completely homozygous offspring is produced.
We conducted several experiments to establish the optimum conditions for the production of gynogenetic broodstock fish of common carp, Cyprinus carpio L.
Genetic inactivation of sperm was achieved by irradiating 10 ml of milt, diluted 1:3 with physiological saline, with U.V. (2200 J/m^2/min) for one hour.
Using females, heterozygous for two recessive genes determining a blond fenotype, two modes of gynogenesis were demonstrated:

- Gynogenesis by suppression of the second meiotic division was achieved by cold shocking eggs, fertilized with irradiated sperm, 1-2 or 7-9 minutes after fertilization. Consistent yields of 25- 50 % of viable fry were obtained when eggs were incubated at 24 C and cold shocked at 0 C for 15-45 minutes. Typically, only 3-8 % of the offspring were blond.
- Gynogenesis by inhibition of of the first mitotic division was achieved by incubating eggs at 24 C and heat shocking them 32 min. after fertilization. Highest yields of viable fry were obtained when eggs were shocked at 40 C for 2 min. 23-25 % of the gynogenetic fry thus produced displayed the blond fenotype.

By combining both gynogenetic techniques it will be possible to establish inbred lines of carp with specified characteristics for research purposes.

Effects of repeated human Chorionic Gonadotropin (Chorulon R) -induced spawning on plasma half-disappearance time, absolute fecundity and hatching rate of eggs in the African Catfish (Clarias gariepinus).

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In fish culture practice hCG is often used to induce spawning (Richter et al. 1987). The hCG profiles resulting from a single injection (usually 4 IU/g) are probably long-lived and for this reason it has been suggested not to use the hormone (for instance Anguilla spec. unpublished data). In other fish species (Dicentrarchus spec.) a possible development of hCG-antibodies has been put forward (unpublished data). This immunological reaction could reduce the maturation and ovulation response of oöcytes.

In a first experiment the plasma hCG profiles in African Catfish following intravenous or intramuscular injection were estimated, using a specific enzyme-immuno asssay. The curves of disappearance of hCG, obtained after intravenous administration, were fitted using a two phase model including a distribution and an elimination phase. A half-disappearance time of 4.9 $\pm$ 1.3 h and 55.5 $\pm$ 7.4 h were found for the first and second phase respectively. The curves of disappearance of hCG, obtained after intramuscular administration, were fitted using a three phase model. A half-disappearence time of 60.6 $\pm$ 7 h was found for the third (elimination) phase.

Literature on the half-disappearance time of hCG in fish is, as far as we know, not available. A plasma half-disappearance time of 6 - 10 h was calculated from measurements of plasma gonadotropin levels following intraperitoneal injection of highly purified carp GTH in goldfish (Carassius auratus) (Cook and Peter 1980).

In a second experiment the effects of repeated hCG-induced spawning (three times with six weeks intervals) on the presence of hCG residues, absolute fecundity and hatching rate of eggs was investigated. 4 weeks after the first and second spawning residues of 17.83 and 19.57 mIU hCG/ml were found indicating that an immunological reaction against hCG could not be detected. The absolute fecundity and the hatching rate of eggs of the three subsequent spawnings were 56000 $\pm$ 12000, 80500 $\pm$ 13400, 81500 $\pm$ 21500 and 81.3 $\pm$ 10.8, 86.3 $\pm$ 1.7, 69.7 $\pm$ 7.2 %, respectively. A reduction of egg quantity and -quality by repeated use of hCG could thus not be observed in the African Catfish.

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DISTRIBUTION OF GONADOTROPHIN RELEASING HORMONE (GnRH) IN THE BRAIN OF "CARIBE COLORADO", Pygocentrus notatus. Dayssi Marcano, Franca Gentile*, Hilda Guerrero* and Carmen L.Paiva*. J.M.Vargas Medical School. Universidad Central de Venezuela. Caracas. Venezuela.

Gonadotrophic releasing hormone (GnRH) activity has been found in the hypothalamus and extrahypothalamic regions of the brain of various teleosts. Using radioimmunoassay procedures we have demonstrated the presence of a substance with GnRH-like immunoreactivity (ir-GnRH) in different brain regions of the Venezuelan freshwater fish "caribe colorado", P. notatus. Immunoassayable GnRH was present in extracts of the optic tectum, cerebellum and the brain stem in both male and female fish. Fluctuactions in the ir-GnRH levels, that depend on the reproductive state and enviromental conditions (rainfall), occurred in the optic tectum and cerebellum extracts obtained from female fish. The ir-GnRH content in the optic tectum and the cerebellum was highest in May, paralleling the gonosomatic index. In the optic tectum, the increase was more than five-fold greater than that of the pre- and post-spawning levels. In the cerebellum, ir-GnRH levels increased four-fold in May compared to March levels. No such changes were observed in male tissues. Both male and female brain stem extracts showed the lowest levels of ir-GnRH, and no changes were observed during the reproductive cycle.

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LOCALIZATION OF TESTOSTERONE IMMUNOREACTIVITY IN RAINBOW TROUT TESTES

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Keywords: rainbow trout, testis, spermatogenesis, testosterone, immunohistology

Introduction

Sparse information is available about the testicular site(s) of androgen action. Since conventionally raised steroid antisera are suitable for immunohistological procedures, we used this technique to localize testosterone (T) immunoreactivity (ir) in rainbow trout testes during different phases of the reproductive cycle.

Material and Methods

The immunohistological procedure (peroxidase anti-peroxidase technique) was carried out as described previously (Schulz, 1986).

Results and discussion

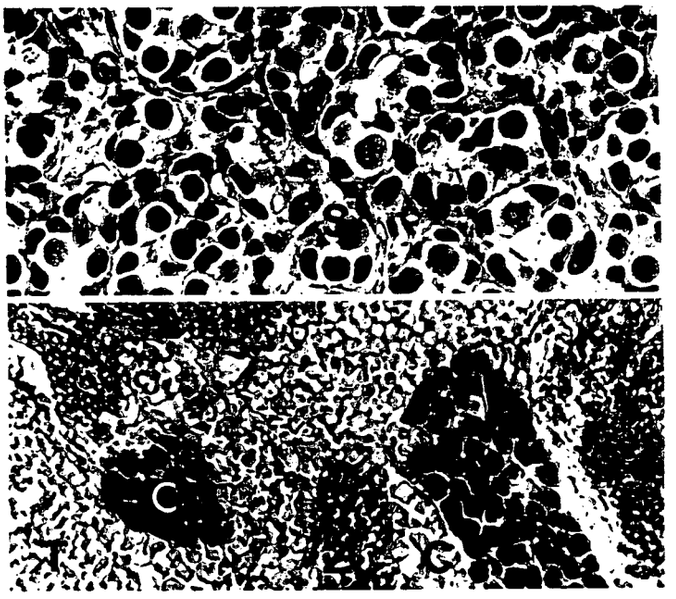
Most spermatogonia and certain groups of spermatocytes were intensely labeled. Spermatides were always unlabeled while spermatozoa often showed an intermediate staining. In spermatogonia and spermatocytes, the granular label was concentrated on the nuclear area.

Sertoli cells were always stained. At the initiation and after completion of spermatogenesis, the label was restricted to the nucleus while during active spermatogenesis the perinuclear cytoplasm appeared to be labeled too. In advanced males, the extralobular space carried an intermediately strong label and mostly did not contain distinctly stained structures.

Control experiments demonstrated that the label corresponds to T-ir but also indicated that other C-19 steroids contribute to the staining, so that the label should be considered as representing androgens. A cross-reaction of C-18 or C-21 steroids, however, can be excluded.

The nuclear staining suggests that the androgen localization observed here may be of physiological relevance. On this presumption, androgens could be involved in the maintenance of spermatogonia, while the regulation of their proliferation appears to depend on more complex stimuli. In the rat, spermatids are formed only when androgen levels are locally high (Sharpe, 1984). The staining of certain groups of spermatocytes may be related to a similar androgen need, and also seems to reflect the cystic form of spermatogenesis. Since most of the early spermatocytes were stained, the labeled population possibly corresponds to first order spermatocytes, in which a special androgen need could exist e.g. in the context of protamine mRNA synthesis (Iatrou & Dixon, 1978).

The fact that a more intense, cytoplasmic staining of interstitial cells was observed only rarely may be related to the rapid secretion of steroids.



Immature (above) and maturing testes were incubated with T-antiserum diluted 1/1,000 - 5,000. G-spermatogonia, C-spermatocytes, T-spermatides, Z-spermatozoa, S-Sertoli cell

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METABOLIC CLEARANCE RATE OF TESTOSTERONE IN MALE MUMMICHOGS

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Summary

The half-life of testosterone in non-breeding mummichogs was 46 min. The metabolic clearance rate of testosterone from a 10 gram mummichog was calculated to be 1.82 ml/hr, yielding a 24-hr production rate of 16.64 ngT/24 hr.

Introduction

Changes in serum testosterone (T) levels in mummichogs, Fundulus heteroclitus, are not correlated with changes in serum levels of 17B-hydroxy-4-androstene-3,11-dione or 11B,17B-dihydroxy-4-androsten-3-one (Cochran, 1987). This lack of correlation might be due to a difference in the metabolic clearance rates of the androgens.

Materials and Methods

Mummichogs, 5-15 g, were acclimated to 18°C overnight. A 30 gauge needle was used to inject [1,2,6,7-^{3}H]-T (40 uCi/118ng, 2.2×10^{-4}dpm/10 g body weight) into the conus arteriosus. Blood was taken from ten fish at each time point-1,5,10,15,20,30,60, and 120 min- via the caudal artery. Serum was extracted with diethyl ether, and T resolved from a uBondapak C18 column by high performance liquid chromatography (HPLC). ^{14}C-T was used to correct for steroid lost by extraction and HPLC of each sample.

The sperm index and serum concentrations of testosterone were determined for 5 fish using a method described previously (Cochran, 1987).

Results and discussion

The males in the experiment had a sperm index of 1.48±0.97, which is three orders of magnitude less than during the breeding season (Cochran, 1987). Serum T was .38±0.14 ng/ml, compared with 11.3 ng/ml during the breeding season.

The half-life of ^{3}H-T in the serum, 46 min, was significantly shorter than the only other reported half-life for T in teleosts- 11.5 hr (Querat et al., 1982). The metabolic clearance rate was 43.68 ml/24 hr. Thus, in non-breeding mummichogs, the 24-hr production rate of T is 16.64 ngT/24 hr (MCR*serum [T]).

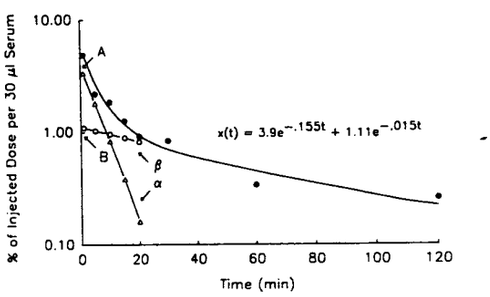

fig. 1. Serum metabolic clearance profile (MCP) of HPLC isolated ^{3}H-T determined in non-breeding mummichogs at 18±1° illustrating the "curve-peeling" method of graphical analysis. The closed circles are the mean (N=10).

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182 ml/h/kg

GONADAL STEROID EFFECTS ON HEPATIC T4 TO T3 CONVERSION IN RAINBOW TROUT (SALMO GAIRDNERI RICHARDSON)

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Determination of appropriate levels of gonadal steroids to administer by slow-release implants

Estradiol-17B (E2) and progesterone (P) were suspended in warm hydrogenated coconut oil and given as intraperitoneal injections to lightly-anaesthetised rainbow trout at levels of 1, 5 or 10 mg/kg body weight. When the fish were returned to water at 10-11 C, the coconut oil solidified in the peritoneal cavity forming a slow-release implant. Blood samples were taken from each fish at the time of injection, and four and eight weeks after the injection.

There was a clear dose-related response in liver weight:body weight ratio and plasma protein levels in the E2-treated fish. In addition, plasma calcium and triiodothyronine levels were significantly elevated and depressed, respectively, in the E2-treated groups, but the changes were not dose-dependent. P had no effect on any of the parameters measured. There was no dose-response relationship in plasma E2 or P levels.

T4 to T3 conversion by liver homogenates

Rainbow trout were given intraperitoneal implants of hydrogenated coconut oil containing either E2 or P at levels equivalent to 1 mg/kg body weight, and samples were taken between 4 and 8 weeks later to measure hepatic T4 to T3 conversion rates *in vitro* using homogenates of the liver as the source of deiodinase. The T3 production rate in the E2-treated fish was twice as great as that of the controls, whereas P appeared to have no significant effect on T4 monodeiodination. The protein content of the homogenates was similar in the three treatment groups.

Table 1. Effect of 17B-estradiol (E2) or progesterone (P) on hepatosomatic index (HSI), and plasma protein, Ca^{++}, thyroxine (T4), and triiodo-L-thyronine (T3) levels in rainbow trout (*Salmo gairdneri* Richardson) at final sampling.

Treatment	N	HSI (%)	Plasma protein (g/dl)	Plasma Ca^{++} (mM)	Plasma T4 (ng/ml)	Plasma T3 (ng/ml)
C	8	0.97±0.04	3.9±0.3	2.5±0.1	4.4±1.0	3.8±0.5
LE2	10	1.19±0.06*	7.8±1.3*	9.6±2.0*	2.6±0.7	2.0±0.4*
ME2	11	1.50±0.12*,a	11.4±2.1*,a	10.1±1.6*	3.5±0.8	1.5±0.2*
HE2	8	1.93±0.15*,a,b	14.4±0.9*,a,b	12.8±2.8*	1.9±1.2	1.4±0.4*
E2C	9	0.88±0.06	3.8±0.2	2.6±0.1	4.3±1.5	2.6±0.3
LP	9	0.96±0.07	4.0±0.5	2.6±0.2	4.9±1.4	2.1±0.5
MP	9	0.97±0.08	4.4±0.4	3.0±0.4	6.2±2.1	3.1±1.0
HP	9	1.01±0.10	3.7±0.4	2.4±0.3	6.6±1.6	2.8±0.5
PC	10	0.98±0.08	3.9±0.2	2.1±0.1	4.8±1.8	3.2±0.6

Note: Data are shown as mean ± S.E.
L = 1.0 mg/kg; M = 5.0 mg/kg; H = 10.0 mg/kg body weight; E2C = control fish held in E2-treated tank; PC = control fish held in P-treated tank.
* significant from control ($p < 0.05$); [a] significant from LE2 ($p < 0.05$); [b] significant from ME2 ($p < 0.05$) by ANOVA and P.S.D.

ULTRASTRUCTURE OF THE TESTIS AND LIVER DURING THE NATURAL BREEDING SEASON OF THE BOWFIN (*AMIA CALVA*)

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Abstract

Ultrastructural changes in the testes and liver of the bowfin (*Amia calva*) were studied at various points in its natural breeding season in specimens caught in Lake Erie, Ontario. This study describes the cytological characteristics of the liver and the epithelial and interstitial components of the testis, examines seasonal changes in hepatocytes and testicular morphology and attempts to evaluate the interactions of liver and various testicular components in the reproductive process in this species. A comparison is made between similar studies on other teleostean species.

THE EXPRESSION OF GERM CELL SPECIFIC DIFFERENTIATION MARKERS, AS DEFINED WITH MONOCLONAL ANTIBODIES, IN CORRELATION WITH THE ONTOGENY OF GONADOTROPIN PRODUCTION IN CARP (CYPRINUS CARPIO L.)

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In carp, developmentally regulated cell surface antigens of germ cells have been identified using monoclonal antibodies raised against spermatozoa (Parmentier et al., 1984; Parmentier and Timmermans, 1985) and isolated spermatogonia. It was shown that the expression of several antigenic determinants started on primordial germ cells (PGCs), concomitantly with the initiation of gonad formation (at the age of 3 weeks) or with the onset of PGC proliferation (at 6 weeks). In contrast, some other antigenic determinants, known to be male specific, arose at the onset of spermatogenesis (at 20 weeks). From studies on many vertebrates, including teleosts, it is generally accepted that gonadal functions are regulated by gonadotropic hormone(s) from the pituitary gland. The present study was designed to gain more insight in the physiology of the germ cell differentiation antigens in carp. Therefore, the correlation between the appearance of specific antigens in differentiating germ cells and the ontogeny of gonadotropin production was studied.

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Hormone Changes during Spawning in Goldfish

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Hormone changes were studied during ovulation in female goldfish Carassius auratus and during spawning in males. Mature female goldfish is known to ovulate spontaneously after the water temperature is raised from 12 to 20°C, and ovulation occurs during scotophase synchronized with photoperiod. Plasma gonadotropin (GtH) levels were gradually elevated in the latter half of photophase (1400-1600hr), and then rapidly rose (2000hr), showing a peak at the time of ovulation (2300-0300hr). Plasma testosterone levels showed a peak before ovulation (2000hr) and then decreased by the time of ovulation. An increase of 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P), an inducer of final oocyte maturation, lagged behind that of testosterone, and elevated levels lasted for a few hours before ovulation (2000-0000hr). Plasma estradiol-17β, a main steroid for vitellogenesis, remained at moderate levels during ovulation.

When females were kept with males in the same tanks, males started to court with ovulatory females between 1600 and 2000hr, and spawning behavior (oviposition in females and ejaculation in males) began immediately after ovulation occurred. The spawning behavior continued for a few hours. Plasma GtH levels in spawning males showed a marked increase which was synchronized with the preovulatory GtH surge in females, and peaked at the onset of spawning. Plasma testosterone and 17,20-P increased almost concurrently with the GtH surge in males, whereas 11-ketotestosterone remained low during the spawning. A large amount of milt was observed during spawning, and the amount was correlated with plasma GtH levels.

This GtH surge in males was induced by pheromones from ovulatory females. Olfactory tract section of the males abolished the GtH surge in the presence of ovulatory females. When males were separated with an opaque partition but with water circulating between male and ovulatory female compartments, the GtH surge occurred although they could not court with females.

It appears that the ovulatory GtH surge in femle goldfish synchronized with photoperiod stimulates the production of sex pheromones, which in turn induce the GtH surge in males. We can propose that the synchronous GtH surge in both sexes causes ovulation and milt production to occur at the same time, favoring a higher rate of fertilization of the eggs.

ROLE OF STEROID HORMONES IN OVARIAN MATURATION IN JAPANESE FLOUNDER

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Summary

Changing levels of steroids in intact and HCG-treated Japanese flounder during ovarian maturation and spawning were examined. T and E_2 were high at last phase of yolk deposit and decreased thereafter. HCG also caused a prompt increase of T before beginning of final maturation. 17α-OHprog and 17α,20β-diOHprog increased sharply at final maturation. The result on in vitro maturation of the flounder oocytes indicated that 17α,20β-diOHprog was the most potent steroid in inducing GVBD. Thus, these results suggest that 17α, 20β-diOHprog is involved in final maturation of Japanese flounder. T may be related to final maturation.

Introduction

There has been an accumulation of information concerning the changes in plasma levels of steroids during sexual maturation in many freshwater teleosts. Current evidence suggests that 17α,20β-dihydroxy-4-pregnen-3-one(17α, 20β-diOHprog) functions as the natural maturation-inducing steroid in salmonid species. Compared with freshwater teleosts, there is scarecely reported the information concerning steroids during ovarian maturation and spawning in seawater teleosts. In recent years, the mass production of useful seawater fish has been widely developed in Japan. In order to asertain the techniques as to seed production, we tried to know hormonal roles of steroids during ovarian maturation, ovulation and spawning in Japanese flounder (Limanda yokohamae).

Results and discussion

Exp.1 Changes in steroids during maturation, ovulation and spawning (Fig.1)

Testosterone (T) and estradiol-17β (E_2) were high at last phase of yolk deposit, but decreased sharply at ovulation. Both 17α-hydroxyprogesterone (17α-OHprog) and 17α, 20β-diOHprog increased at ovulation and decreased thereafter.

Exp.2 Changes in steroids of Japanese flounder following HCG treatment

HCG caused a prompt increase of T 6 hr. after injection. But the high level of T returned to low within 24 hr. E_2 decreased to a low level before final maturation. The elevation of 17α-OHprog which is a precursor for 17α,20β-diOHprog occurred earlier and persisted longer than the level in 17α,20β-diOHprog.

Exp.3 In vitro effects of steroids on GVBD in flounder oocytes

Effectiveness of steroids on GVBD was investigated in vitro using the folliculated oocytes. Both progesterone and 17α-OHprog were effective in inducing GVBD at relative high concentrations. But a rate of 48% GVBD was recorded with the low concentration (1 ng/ml) of 17α,20β-diOHprog. This steroid was the most potent in inducing oocyte final maturation. Therefore, the results on in vitro and in vivo suggest that 17α,20β-diOHprog is involved in final maturation of Japanese flounder. T may has a role in serving as an intraovarian modulation of gonadotropin action at the level of follicular tissues just before oocyte final maturation.

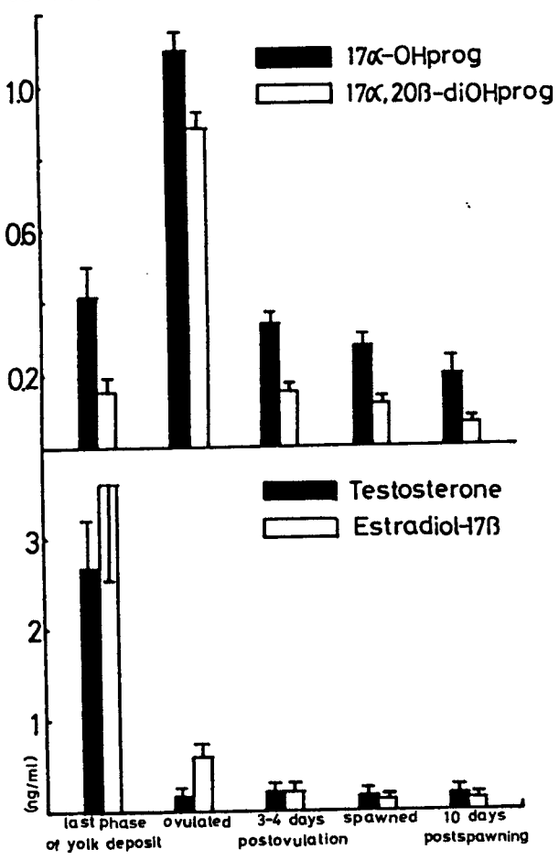

Fig.1 Changes in steroid hormones during maturation, ovulation and spawning in Japanese flounder.

GONADOTROPIC AREA DEVELOPMENT CORRELATED WITH TESTIS AND GONOPODIAL DEVELOPMENT IN THE CUATRO OJOS, *ANABLEPS DOWI* (PISCES: ANABLEPIDAE).

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Specimens of *A. dowi* were collected from an estuary in El Salvador, Central America. Histological preparations were made of pituitaries and testes and were stained with a modified Masson's trichrome. Based upon the well-established localization of the gonadotropic (GTH) region in the closely related poeciliid fishes, the cells of the GTH region of *A. dowi* also were basophilic and located in an identical position. In order to estimate the degree of development of the GTH zone, the % area of the entire pituitary occupied by the GTH zone was calculated from tracings of mid-sagittal sections using a compensating planimeter. Specimens whose testes contained only isolated spermatogonia or small cysts (<10 cells/cyst/section) had an average GTH area of $0.75\pm0.48\%$ and ranged in size from 5.4-8.8cm. Those whose testes had larger cysts of spermatogonia (>10 cells/cyst) had GTH areas of $2.78\pm1.16\%$ and measured 7.9-10.8cm. Specimens whose testes had cysts up to the spermatid stage ranged from 11.5-12.0 cm with GTH areas of $4.04\pm1.28\%$. Free spermatozoa within the main ducts were seen only in specimens 12.3cm or larger, where GTH areas ranged from 6.7-19.3%. The first evidence of gonopodial development was seen in two specimens (7.2 and 7.3cm) whose GTH areas were only 0.6% in each case. If larger % areas indicate higher levels of GTH, then these observations suggest that increasing levels of GTH may stimulate spermatogonial proliferation, while higher levels are necessary for the meiotic and later stages.

T

RELATIONSHIP BETWEEN GnRH RECEPTOR BINDING AND BIOLOGICAL ACTIVITY IN THE GOLDFISH PITUITARY

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Studies of goldfish pituitary GnRH receptors using an analog of teleost GnRH (DArg6, Trp7, Leu8, Pro9-NEt)-GnRH as labeled ligand demonstrated the presence of two classes of binding sites; a high affinity low capacity site, and a low affinity high capacity binding site (Habibi et al., 1987; Biol. Reprod., in press).

In the present study, we investigated the seasonal variations in GnRH receptor properties, and the functional relationships between GnRH receptor binding and biological activity in the goldfish pituitary. Positive correlations were obtained between pituitary GnRH receptor content of both high and low affinity sites and pituitary responsiveness to GnRH in terms of gonadotropin release. However, structure-activity studies based on teleost and mammalian GnRH molecules with amino acid substitutions at positions 6 and 10 suggest that high affinity GnRH binding sites are involved in the control of pituitary gonadotropin release in the goldfish pituitary. In addition, the findings indicate that the presence of Trp7 and Leu8 in the GnRH molecule (i.e. teleost GnRH format) results in better recognition of the peptide by the receptors in the goldfish pituitary.

ACTIONS OF A GnRH-AGONIST AND A DOPAMINE-ANTAGONIST ON PITUITARY GnRH AND DOPAMINE RECEPTORS IN THE GOLDFISH

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In teleosts, GnRH and dopamine act at the pituitary level to regulate the release of gonadotropin (GtH) (Peter et al., 1986). The purpose of this research was to examine the interaction of domperidone, a dopamine antagonist, and an agonist-analog of teleost GnRH (tGnRH-A) on pituitary GnRH and dopamine receptors and GtH release in goldfish. In goldfish, injection of tGnRH-A or domperidone, alone, increased serum concentrations of GtH in a dose-related manner; domperidone and tGnRH-A potentiated each other's action to increase serum concentrations of GtH. GnRH-receptor properties were investigated following treatment with domperidone or tGnRH-A in vivo. Injection of domperidone or tGnRH-A increased the number of high-affinity GnRH binding sites in the pituitary compared to controls. Similarly, tGnRH-A significantly increased the number of specific [3H]-spiperone binding sites (dopamine receptors) in the pars distalis without affecting affinity; an increase in the number of binding sites in the neurointermediate lobe was not significant.

In summary, in vivo studies indicate that tGnRH-A and domperidone potentiate each other's activities on GtH release. Receptor studies suggest that this in part be due to an increase in the number of pituitary GnRH receptors. In addition, the findings suggest that GnRH may influence GtH release by affecting dopamine receptors at the pituitary level.

EFFECTS OF A GONADOTROPIN-RELEASING HORMONE (GnRH) ANALOG ON GOLDFISH OOCYTE MEIOSIS AND STEROIOGENESIS IN VITRO.

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In mammals, GnRH has been demonstrated to inhibit steroidogenesis in isolated granulosa and luteal cells, and to impare gonadotropin-induced follicle development and ovulation in vivo. However, little is known about the actions of GnRH on oocyte meiosis. The present study provides the first demonstration of a direct action of GnRH on progestogen-induced meiosis, using goldfish oocytes in vitro as model system. Incubation of prophase-I arrested follicle-enclosed goldfish oocytes with carp gonadotropin (GTH), or one of the two commonly found progestogens in teleost blood, 17a-hydroxyprogesterone (HP) and 17a,20B-dihydroxyprogesterone (DHP), causes reinitiation of oocyte meiosis, as indicated by dissolution of the germinal vesicle (GVD). Addition of an agonist analog of teleost GnRH [(DArg6, Trp7, Leu8, Pro9-NEt)-GnRH; tGnRH-A] significantly reduced the magnitude of the GVD response to GTH, HP and DHP in a dose-dependant fashion. Production of testosterone, stimulated by GTH treatment, was also inhibited by tGnRH-A. The GTH-stimulated DHP production which could be demonstrated in the presence of a phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine, was not affected by tGnRH-A. These observations support the hypothesis that GnRH or a GnRH-like peptide might influence the control of oocyte meiosis at the ovarian level in goldfish.

Differential distribution of two molecular forms of immunoreactive gonadotropin-releasing hormone in discrete brain areas of goldfish, (Carassius auratus)

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Summary

Two molecular forms of immunoreactive gonadotropin-releasing hormone (ir-GnRH) were identified in the extracts of various brain areas of goldfish by reverse phase high pressure liquid chromatography (RP-HPLC) and radioimmunoassay (RIA). The two ir-GnRH peaks coeluted with [His^5,Trp^7,Tyr^8]-GnRH (cGnRH-II) and [Trp^7,Leu^8]-GnRH (sGnRH) and had immunological properties comparable to those of the synthetic peptides respectively. The concentration ratio of the early eluting cGnRH-II-like immunoactivity (cGnRH-II-LI) to the late eluting sGnRH-like immunoactivity (sGnRH-LI) was higher in the caudal parts (cerebellum, medulla and spinal cord) compared to the rostral parts (olfactory bulbs, telencephalon, optic tectum-thalamus, hypothalamus and pituitary) of the brain. The differential distribution of two ir-GnRH forms suggests that different irGnRH forms may have different physiological functions.

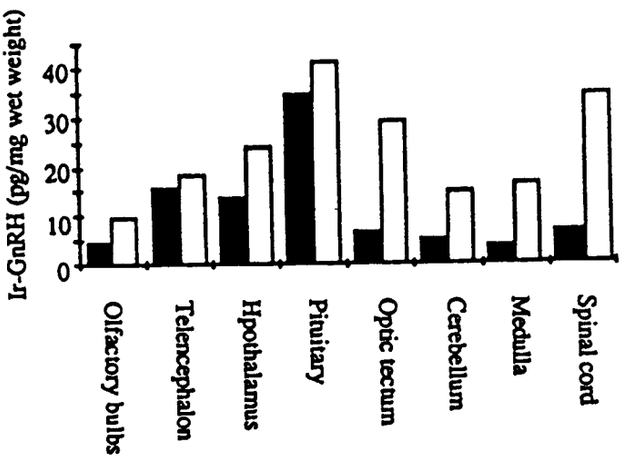

Fig. 1. Comparison of the concentrations of two molecular forms of GnRH immunoactivity in discrete brain areas of female goldfish (■ late eluting sGnRH-LI; □ early eluting cGnRH-II-LI).

Introduction

The existence of multiple molecular GnRH forms in brain of a single species has been well demonstrated in different vertebrates including teleosts (for review, see Sherwood, 1986). However, the functional roles of different GnRH forms in brain are not clear. Here, we characterized the identity and distribution of the ir-GnRH forms in discrete brain areas of goldfish using RP-HPLC and RIA.

Results and discussion

The two immunoreactive GnRH peaks coeluted with synthetic cGnRH-II and sGnRH, and had immunological properties comparable to synthetic peptides in a RIA (Yu et al, 1987). RIA using antisera with different specificity towards known vertebrate GnRH structures did not reveal the presence of mammalian GnRH (mGnRH), [Gln^8]-GnRH (cGnRH-I) and [Tyr^3,Leu^5,Glu^6,Trp^7,Lys^8]-GnRH (lamprey GnRH) in the goldfish brain.

As shown in Fig.1, the concentrations of the late eluting sGnRH-LI, but not the early eluting cGnRH-II-LI, were lower in the caudal parts of the brain. The early eluting cGnRH-II form showed a clear predominance over the late eluting sGnRH form in the caudal parts, but were about equally prevalent in the rostral part of the brain.

Although differential localization of neurons containing the two ir-GnRH forms awaits future immunocytochemical studies,the differential distribution of the two ir-GnRH immunoactivities suggests possible differentiation of functions among the two ir-GnRH forms. One hypothesis is that while both GnRH forms may serve a neuroendocrine function at the pituitary, the early eluting cGnRH-II form may preferably serve as a neurotransmitter in the other parts of the goldfish brain. Multiple sites of GnRH actions in the rat brain have been indicated by the wide distribution of the ir-GnRH and the GnRH binding sites (see Millan et al, 1986). To further the study, this laboratory is currently investigating the differential in vitro release of two ir-GnRH forms from discrete brain areas in goldfish.

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CURRENT STATUS OF LH-FSH-LIKE FISH GONADOTROPIN

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The situation in Tetrapods

Two gonadotropins (GTH), lutropin (LH) and follitropin (FSH), exist in each mammalian species. They are made of two glycoproteic 100-130 amino acids long subunits (SU) called α and β; LH, FSH and also TSH have α in common. All SU's exhibit zoological specificity. The α's from the various species on the one hand, all the β's (LHβ's, FSHβ's and also TSHβ's) on the other hand are homologous. A similar situation was found in representatives from the various Tetrapod classes even though only one GTH seems to be present in a few species. Biological, biochemical and immunological data suggest a general homology of Tetrapod α's on the one hand and β's on the other hand. Therefore the three lineages within the β family (LH, FSH, TSH) should have derived from a common molecule by two genic duplications occuring in Tetrapod ancestors.

The situation in fish

In all groups of fish (except Myxinoids), various data indicate the presence of pituitary GTH exhibiting some similarities with the LH-FSH family. The search for factor(s) belonging to this family was essentially carried out in one Selachian, two Holosteans and several Teleosts. In each case, it led to the purification of only one GTH, a glycoprotein exhibiting zoological specificity. Subunit structure was demonstrated in most of the cases. Sequence studies were carried out in carp and in salmon ; they demonstrated the homology of the two SU's with mammalian α and β respectively, Teleost GTH β being slightly closer to mammalian LH β than FSHβ. These data suggest that only one GTH lineage (LH) exists in fish (together with the TSH lineage) and that the FSH lineage only appeared in primitive Amphibians. Studies at the gene level are necessary to test these hypotheses.

Biosynthesis, secretion and control of Teleost GTH

As in Mammals, hormonal biosynthesis involves the association of α and β SU's. This reaction was shown to be much more rapid and less thermodependent in a Teleost than in Mammals, a difference which allows the fish hormone production even at low temperatures. A pulsatility of GTH secretion, with a period and an amplitude which vary in the course of the sexual cycle was demonstrated in the trout. Pituitary GTH synthesis and secretion are stimulated by gonadoliberin (GnRH) which is released in the pituitary, and this GnRH effect in inhibited by dopamine. Pituitary GTH cells are also controlled by sexual steroids ; besides the classical negative retrocontrol which exists in adults, a positive retrocontrol was demonstrated in immature salmonids and eels ; in these animals sexual steroids strongly stimulate the synthesis (but not the release) of GTH and they increase the mRNA's for α and β SU's.

Mechanism of action of Teleost GTH

When data obtained on various species are put together, GTH appears able to stimulate the various aspects of the pituitary dependent gonadal development eg, in female, follicle growth, vitellogenesis, steroidogenesis, maturation and ovulation. Receptors (R) for GTH (with a K_D close to 10^{-10}M) were demonstrated in gonads. In these organs, GTH was shown to stimulate adenylate cyclase (AC) activity as well as cAMP accumulation. Dibutyryl cAMP was shown to increase steroidogenesis in tilapia. However the situation is much more complex than it could appear. Several data suggest important differences according to species, sex and stage of sexual development. These differences can be concerned with the location and properties of receptors - the heterogeneity of which was suggested -, their coupling to AC, the activity of protein kinases and of steroidogenetics enzymes, etc... Moreover the existence of second messengers other than cAMP cannot be excluded.

Conclusion

One should question how the successive and different stages of complex sexual cycles are precisely controlled even though a single glycoprotein GTH is present. The type of action exerted by GTH probably depends on the pulsatility of the GTH secretion (as suggested by data on the stimulation of vitellogenin uptake) and on the conditions of the gonadal cells which are themselves under the control of various hormonal or paracrine influences, as it is more and more obvious in Mammals. Indeed pituitary factors different from GTH participate in the stimulation of vitellogenin uptake (the "carbohydrate-poor GTH") and in the control of the estradiol dependent hepatic synthesis of vitellogenin (the growth hormone being a likely candidate for this effect). To understand the meaning and the mechanisms of these various interactions is clearly a main goal for future research.

IMMUNOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF TWO GLYCOPROTEIN GONADOTROPINS FROM THE CARP PITUITARY

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Summary

The question of the number of gonadotropins (GtHs) in teleosts and their homology to other vertebrate GtHs is currently unresolved. Tetrapod species, with the possible exception of squamate reptiles, have two GtHs, namely LH and FSH (Licht, 1986). These GtHs share a common α-subunit but have different β-subunits which dictate hormonal specificity. Work from Idler's laboratory suggests that teleosts have two GtHs (Idler and Ng, 1983), although the homology of one form with low carbohydrate content to other vertebrate GtHs is not clear. Other workers have demonstrated only a single form of GtH in teleosts (Burzawa-Gerard, 1982; Licht, 1986).

We have recently isolated two separate glycoprotein GtHs from an alcoholic extract of common carp pituitaries by DEAE-cellulose chromatography and gel filtration on Sephadex G-75. Both forms of GtH, designated GtH-1 and GtH-2, consist of two subunits. Two GtHs with similar physicochemical characteristics have also been purified from chum salmon (Kawauchi et al., 1986).

The immunochemical relatedness of carp GtH-1 and GtH-2 was determined using antisera generated to isolated α and β subunits of existing carp GtH preparations (provided by B. Breton and E. Burzawa-Gerard). Both ^{125}I-labelled GtH-1 and GtH-2 bind to α-subunit antisera whereas only ^{125}I-GtH-2 shows significant binding to β-subunit antisera. In the β-subunit system, GtH-1 had only about 5% the activity found with GtH-2; whereas GtH-2 and F-11 carp GtH from B. Breton were equipotent. There were no differences in the potencies of GtH-1, GtH-2 and F-11 GtH when tested with the α-subunit antisera. These results demonstrate that the two carp glycoprotein GtHs follow the tetrapod pattern in that they share a similar α-subunit but have different β-subunits.

Carp GtH-1 and GtH-2 exhibit similar biological activity in goldfish, causing stimulation of gonadal steroidogenesis and the induction of oocyte final maturation. The relative activities of GtH-1 and GtH-2, based on their effects on steroidogenesis, change with stage of ovarian development; GtH-2 having enhanced steroidogenic activity in preovulatory as compared to vitellogenic follicles. GtH-1 and GtH-2 are equipotent in inducing oocyte final maturation. These data suggest the existence of two classes of GtH receptors in goldfish preovulatory follicles.

In summary, we have isolated and characterized two distinct GtHs from the common carp pituitary. GtH-2 is comparable to previously isolated cyprinid maturational GtH whereas GtH-1 is a newly identified GtH. Based on their chemical nature (glycoprotein) and biological activity, both GtH-1 and GtH-2 appear to be distinct from the low carbohydrate content GtH (Idler and Ng, 1983).

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GtH 1 ≠ GtH BB sur anti β pas de liaison 5%
GtH 2 ≅ GtH BB sur anti β
GtH1 ≅ GtH2 ≅ GtH BB sur anti α

CONCANAVALIN A CHROMATOGRAPHY SEPARATES TWO FORMS OF MATURATIONAL GONADOTROPIN IN GOLDFISH

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Summary

It is well known that gonadotropins (GtHs) in tetrapod species as well as teleosts fishes exist in several molecular forms (Wakabayashi, 1980). Variation in carbohydrate content accounts for the heterogeneity of mammalian GtHs and also for differences in the biopotency of specific isohormones, through alterations in receptor binding affinity and in vivo clearance properties (Chappel et al., 1983). In the present studies, we have attempted to separate different molecular forms of maturational GtH in the pituitary and serum from goldfish by adsorption to the plant lectin Concanavalin A (Con A). Con A binds glycoproteins with exposed glucose or mannose residues, and has previously been used to separate different molecular forms of mammalian LH and FSH (Chappel, 1981; Chappel et al., 1982).

The goldfish pituitary and serum contains two forms of maturational GtH which are separated by chromatography across a column containing Con A linked to Sepharose beads. One species of GtH does not bind to Con A and passes through the column unretarded (Con A-unbound); the other species binds to Con A (Con A-bound) and is eluted from the column by the addition of 1-O-methyl-α-d-glucopyranoside. The displacement curves for both Con A-unbound and Con A-bound forms of GtH were parallel to that of the carp GtH standard when tested by RIA using a β-directed antisera. Furthermore, both species of GtH stimulate testosterone production by goldfish testis pieces incubated in vitro. However, when equivalent amounts of immunoreactive material were tested, the Con A-unbound form showed reduced bioactivity compared to Con A-bound GtH.

In order to determine whether the biological and immunological characteristics of pituitary GtHs change with physiological state, pituitary glands and serum samples were obtained from goldfish subjected to experimental manipulations which induce high rates of GtH secretion. Injection of the dopamine receptor antagonist domperidone caused a significant increase in the immunoreactive Con A-unbound: Con A-bound ratio and a concomitant decrease in pituitary GtH bioactivity. In separate studies, injection of the dopamine receptor antagonist pimozide and an LHRH agonist analog caused a time related increase in the proportion of Con A-unbound GtH in goldfish serum and a marked reduction in the biological: immunological activity ratio of serum GtH. These studies have shown that under high secretion rate conditions, there are marked changes in of glycosylation of GtH and reduced bioactivity of the secreted forms.

In summary, we have found two forms of maturational GtH in goldfish pituitaries and serum which differ sufficiently in carbohydrate content or arrangement to permit their separation by Con A adsorption. Additionally, these results are consistent with the work in mammals showing that alterations in carbohydrate content influence the bioactivity of GtHs.

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Effects of 5-HT on gonadotropin levels in male and female goldfish, Carassius auratus.

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Summary

Plasma gonadotropin (GtH) levels were measured in sexually recrudescing male and female goldfish after the intraperitoneal (i.p.) or brain intraventricular adminstration of serotonin (5-hydroxytrptamine, 5-HT). In both sexes, intraperitoneal administration of the drug evoked a significant increase in GtH levels. The brain administration of 5-HT did not elicit any change in GtH levels.

Goldfish-gonadotropin-serotonin-anterior pituitary.

Introduction

To study the involvement of 5-HT on regulation of GtH secretion in goldfish, 5-HT was administered "in vivo" intraperitoneally or by brain intraventricular injection.

Experiments were done in sexually recrudescing male and female goldfish acclimated to a natural simulated Edmonton photoperiod and 18°C. Serotonin was dissolved in 0.7% NaCl with 0.1% sodium metabisulphite for intraperitoneal injection. In the case of the brain administration, the vehicle used was 0.7% NaCl with 0.004% ascorbic acid. Ketanserin tartrate was dissolved in a vehicle of 1:2 (v/v) dimethyl sulfoxide and propyleneglycol, and injected in a volume of 1 ul/g fish. Serum GtH was measured using a radioimmunoassay for carp gonadotropin.

Results and discussion

In both male and female goldfish, a significant increase in serum GtH levels was seen 0.5 hours after the administration of 5-HT (10 ug/g) as shown in Fig. 1. The serum GtH levels decreased to control levels by 1 hour postinjection. Taking the above results into consideration, dose-dependency of 5-HT was demonstrated in both male and female goldfish at 0.5 hours post-injection. There was no response to different doses of 5-HT into the brain ventricle of sexually recrudescing females. The specificity of the response to 5-HT administered i.p. was demonstrated by pretreating the animals with the specific S_2 receptor antagonist, ketanserin (Leysen et al. 1982).

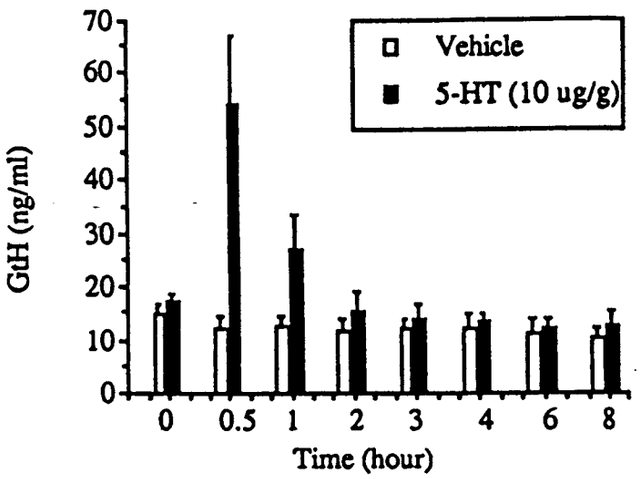

Fig.1. Time course effects of 5-HT (i.p.) on serum GtH levels in female goldfish.

The evidence from these experiments suggests that 5-HT stimulates GtH release in a dose-dependent manner. The specificity of this action of 5-HT is demonstrated by blocking the response by pretreatment with ketanserin. The levels of this stimulatory action are not known. However, it is important to note that 5-HT fibers were reported in the goldfish proximal pars distalis (Kah and Chambolle, 1983).

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CRYOPRESERVATION OF CLARIAS GARIEPINUS SPERM AND FERTILIZATION SUCCESS

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Summary

Although catfish are successfully cultured on a commercial scale in many parts of the world, information on attemps to cryopreserve their gametes are very rare. Until recently no attempts were made to cryopreserve the sperm of the sharptooth catfish, Clarias gariepinus (Steyn & Van Vuren, 1987). C. gariepinus has been successfully spawned artificially and is now being evaluated for its aquacultural potential (Schoonbee et al. 1982). The significance of milt preservation in artificial spawning programmes is indisputable.

Assessment of sperm motility before freezing and after thawing is necessary in the development of a cryopreservation technique. Fertilization ability of preserved sperm should be determined once such a teqhnique is perfected. The efficiency of the cryopreserved sperm can only be estimated when the milt-ova insemination ratio is known. The use of large volumes of preserved milt could conceal low sperm survival and fertilizing capacities.

The most effective extenders in previous experiments were employed (Van Vuren & Steyn 1985). The most effective freezing rate (Fig. 1) was used. Sperm could be stored in liquid nitrogen for 14 days to 28 months with acceptable fertilization rates (Table 1). The volume of milt could be lowered to 0,1 ml with fertilizing successes well over 50% and a storage time of between 12 and 24 months. Sperm preserved for 14 days were just as efficient as sperm preserved for 16 months. Glycerol rendered the best membrane protection opposed to DMSO and methanol.

A remarkable similarity existed between the cryodiluent success sequences obtained previously and the present investigation. These findings confirm the value and necessity of motility evaluations before attempts are made to perform fertilization experiments. The low hatching rates accomplished with fresh milt can be attributed to the quality of eggs used in the experiments. Due to the lack of proper hatching facilities it is possible that overripe ova were used. The last experiments indicated that the volume of longterm preserved sperm should be more than 1 ml in order to obtain higher hatching rates.

CRYOPRESERVATION OF CLARIAS GARIEPINUS MILT AND FERTILIZATION SUCCESSES

Dilution ratio 1:1; Equilibration time 20 min.; Thawing temperature 25°C; Ova Volume/number - 20ml / ± 7600

Milt Sample No	Volume	Extender	Cryoprotectant	Freezing rate	Storage time	Ova Hatched %	Ova Hatched Estimated number
A	0,3ml		Milt not preserved	(Control)		51,0	3876
B	0,3ml	4	11% Glyc	Fig. 1	14 days	51,2	3891
B	0,3ml	4	11% DMSO	Fig. 1	14 days	47,0	3572
C	0,3ml	4	1% Glyc	Fig. 1	16 months	41,0	3116
D	0,1ml		Milt not preserved	(Control)		42,0	3192
B	0,1ml	4	11% Glyc	Fig. 1	12 months	59,0	4484
B	0,1ml	4	11% DMSO	Fig. 1	12 months	55,0	4180
C	0,1ml	4	1% Glyc	Fig. 1	28 months	69,5	5282
E	0,3ml		Milt not preserved	(Control)		50,3	3822
C	0,3ml	4	1% Glyc	Fig. 1	28 months	68,0	5168
C	1,25ml	4	1% Glyc	Fig. 1	28 months	82,8	6292

Glyc = Glycerol, Meth = Methanol, DMSO = Dimethyl sulphoxide

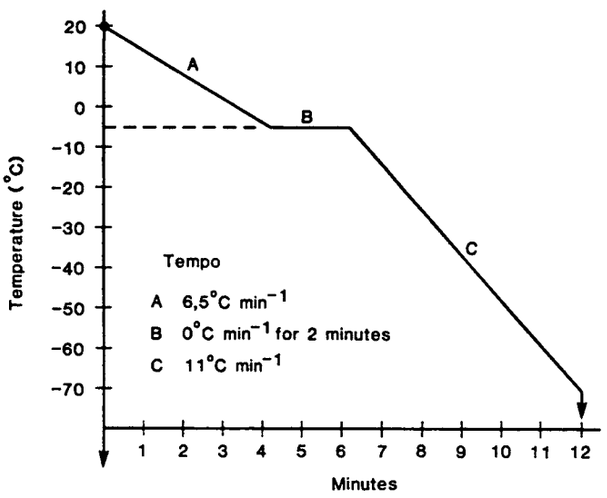

fig. 1: Freezing rate employed.

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EFFECT OF SPERM CELL ABNORMALITIES ON THE CRYOPRESERVATION OF SALMO GAIRDNERI MILT

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Summary

The techniques of Erdahl & Graham (1980) were strictly adhered to during attempts to cryopreserve the milt of S. gairdneri. Experiments were first conducted on a local strain which was originally imported to southern Africa during 1897. The experiments were also duplicated on another strain which was recently imported. Cryopreservation experiments were unsuccessful during both investigations. Experiments were conducted during onset and middle of the spawning season, extending over a two year period. The results were always negative. When fresh milt was employed during hatching trials, the milters of both strains showed to be fertile and perfectly able to fertilize 80% and more ova. An electron microscopic investigation lead to the discovery of sperm cell abnormalities. The tails of sperm cells in fresh S. gairdneri milt, were affected with nodular like deformations. After freezing and thawing, the tails were severely damaged and occasionally snapped at the deformation, resulting in immotile sperm. The cause of the abnormality is unknown. However, we believe it is possibly due to the environmental conditions or the composition of the diet. In order to identify possible irregularities in the chemical composition of such milt, we determined the concentrations of specific chemical components of the milt. Milt for the chemical determinations was frozen in liquid nitrogen, directly after sampling and thawed prior to centrifugation. Due to the lack of comparable information on the chemical composition of semen from trout in other parts of the world, the latter could not be interpreted. The chemical composition of the milt and the environmental conditions under which above mentioned trout strains were raised, are presented in Tables 1 & 2.

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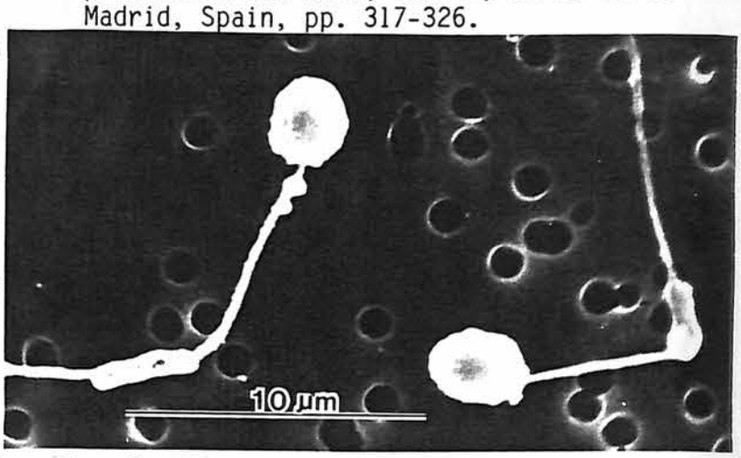

fig. 1. Flagellar deformations on S. gairdneri sperm

Table 1 Chemical properties of the seminal plasma

Parameter	$\bar{X}$	(n=10)	SD	Unit
Ph	7.24	±	0.14	$-\log_{10}(H^+)$
Osmolality	254	±	56.84	m osm kg^{-1}
Na^+	88.70	±	23.34	m mol ℓ^{-1}
K^+	36.25	±	11.43	m mol ℓ^{-1}
Ca^{2+}	0.98	±	0.14	m mol ℓ^{-1}
Mg^{2+}	1.35	±	0.21	m mol ℓ^{-1}
Cl^-	103.60	±	27.11	m mol ℓ^{-1}
Glucose	0.86	±	0.40	m mol ℓ^{-1}
Lactate	0.12	±	0.05	m mol ℓ^{-1}
Total protein	0.46	±	0.16	g/100 ml
Albumin	0.26	±	0.12	g/100 ml
Cholinesterase	78.45	±	27.33	u/ℓ
ATP	0	±	0	m mol ℓ^{-1}
Phospholipids	0.35	±	0.16	m mol ℓ^{-1}
Cholesterol	0.21	±	0.16	m mol ℓ^{-1}

TABLE 2 Water quality of De Kuilen trout hatchery

		Summer		Autum			Winter			Spring			Summer	
Parameter		Jan.	Feb.	Mar.	Apr.	May.	Jun.	spawn Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Unit
Temperature	Min.	18.5	16.0	16.0	13.0	9.0	8.0	7.0	8.5	11.5	14.0	13.0	15.0	°C
	Max.	24.5	24.0	22.5	20.5	17.5	15.0	15.5	17.0	22.5	24.0	25.0	25.0	
Ph	Min.	8.1	7.8	7.1	6.8	6.9	-	6.5	6.5	-	6.8	-	6.9	-log 10(H^+)
	Max.	8.6	8.5	7.8	7.9	7.5	-	7.0	6.9	-	7.1	-	7.3	
Disolved O_2	Min.	10.4	10.0	10.0	10.8	10.6	-	10.7	10.3	-	10.0	-	10.0	mg ℓ^{-1}
	Max.	10.5	10.9	10.7	12.0	11.4	-	11.0	11.0	-	10.8	-	10.2	
Conductivity		30	60	60	50	45	-	40	40	-	45		45	µS cm^{-1}

CRITICAL DAYLENGTH AND TEMPERATURE LEVEL FOR PHOTOPERIODISM IN GONADAL MATURATION OF GOLDFISH

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Our previous study revealed that the ovarian development of goldfish, Carassius auratus, in autumn and early winter was markedly accelerated at 24°C/16L but suppressed at 24°C/12L. This photoperiodic response became indistinct at 16°C. The present investigation was undertaken to determine: a, the critical daylength at which the photoperiodic effect on ovarian activity changes from acceleration to suppression; b, the temperature level below which the photoperiodic effect becomes obscure.

When yearling goldfish of comet variety were exposed to 12, 13, 14, 15 or 16L at 24°C from Oct. 26 to Dec. 27, females were suppressed at 12 or 13L but matured to spawn at other daylengths. The critical daylength was thus located between 13 and 14L. Males were not suppressed at any daylengths and spermiated to pass quickly into post-spawning stage. They commenced next maturational cycle after completion of regressive stage.

When exposed to 18 or 21°C combined with 12 or 16L from Dec. 5 to Mar. 5, a few females matured and spawned at 21°C/12L, while most females did so on other regimes. The temperature level below which the photoperiodic response became indistinct was between 21 and 18°C. Males continued spermatogenesis at 18°C/16L, but they spermiated and passed into post-spawning stage quickly on other regimes.

APPLICATION OF HONEY IN CRYOPRESERVATION OF SPERM OF MILKFISH (CHANOS CHANOS) AND BLACK PORGY (ACANTHOPAGRUS SCHLEGELI)

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Abstract

For cryopreserving sperm of marine finfish, extenders are needed to dilute viscid milt and to provide nutrients for long-term storage. The aim is to find a more functional, convenient and inexpensive extender for milkfish and black porgy.

Pure honey is not only a natural bacteria inhibitor but also reasonably priced and readily available. The result of this study indicates that 1% honey (449 mOsm/kg) was an optimum substitute for milkfish serum in the preserving of milkfish sperm in terms of prefreezing and post-thawing motility. A high motility score of 5 was obtained in both serum-DMSO and 1% honey-DMSO groups. Nevertheless, fertility needs to be studied in the near future.

On the other hand, 0.5% honey (210 mOsm/kg) was used to substitute 5.6% glucose in preserving black porgy sperm and rather satisfactory prefreezing, frozen-thawed, and three-day frozen-thawed motility as well as fertility were obtained. The motility score of 5 was found with all 5.6% glucose-DMSO, 5.6% glucose-glycerol, 0.5% honey-DMSO, and 0.5% honey-glycerol groups after 3-day cryopreservation. The mean fertility rates were 68.09% and 60.63% in 5.6% glucose-DMSO and 0.5% honey-DMSO respectively as well as 74.87% and 78.45% in 5.6% glucose-glycerol and 0.5% honey-glycerol respectively.

CONTROL OF K^+ SECRETION IN THE BLOOD-TESTIS BARRIER OF BROOK TROUT (*Salvelinus fontinalis*)

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Introduction

The sperm duct epithelium is part of the blood-testis "barrier", but its role in reproduction is much more than as a simple barrier to large solutes. The sperm duct is the site of active Na^+ uptake (Marshall 1986) that is stimulated by dibutyryl cyclic adenosine monophosphate (cAMP). It is involved in steroidogenesis of androgens (Schultz 1986) and in active resorption of spermatozoa in the post-spawn period (Billard & Takashima 1983). The unique ionic composition (high $[K^+]$ and low $[Na^+]$) of seminal plasma maintains quiescence of maturing spermatozoa (Morisawa *et al.* 1983). This study examines the K^+ secretory transport mechanism, its possible hormonal control in the trout sperm duct and evaluates this tissue as a general model for epididymal function in vertebrates.

Results & Discussion

Sperm duct epithelia from adult, mature brook trout (400-600 g; GSI 1.1-4.3% 2.1 ± 0.14% mean ± SEM n=33) were mounted *in vitro* and bathed on both sides with Ringer's solutions for electrophysiological and radioisotopic flux studies as described previously (Marshall 1986). Short-circuit current (Isc), transepithelial potential (Vt) and tissue conductance (Gt) were measured using dual current-voltage clamps (WP-Instruments DVC-1000). $^{22}Na^+$ and $^{86}Rb^+$ (a tracer for K^+ transport) were used to measure unidirectional influx (J_{ms}), efflux (J_{sm}) and net flux ($J_{net} = J_{ms} - J_{sm}$).

The net secretion of Rb^+ in cAMP-stimulated voltage-clamped tissues is indicative of active transport of K^+ (Table 1). Initially, unstimulated tissues have a high Isc (10-40 uamp.cm^{-2}), but this falls to zero in about 20 minutes; cAMP restores the Isc and net Na^+ transport (Marshall 1986). The estimated rate of cAMP-stimulated K^+ transport, based on the relative permeability of K^+ and Rb^+ in rainbow trout ($P_K:P_{Rb}$ = 1.27; Sanders & Kirschner 1983), would be about 150 neq.cm^{-2}.h^{-1}. Ouabain did not inhibit Rb^+ net flux in the first hour of exposure, but the net flux dropped to zero by hour 3, indicating that Na,K-ATPase is involved in K^+ secretion, but not directly. The slow depletion of intracellular K^+ that is typical after blockade of Na,K-ATPase would account for the delayed drop in Rb^+ net flux.

Table 1 Ouabain inhibition of Rb^+ secretion

Period	J_{sm}	J_{ms}	J_{net}
	(neq.cm^{-2}.h^{-1})		
Control†	64.2 ± 9.8	5.8 ± 1.9	58.4 ± 10
Ouabain (10^{-5} M, serosal)			
hour 1	75.0 ± 7.4	14 ± 2.6	61.2 ± 7.7
hour 2	*40.3 ± 3.0	*25 ± 5.8	*15.7 ± 5.2
hour 3	*32.7 ± 3.5	*28 ± 8.9	*4.8 ± 8.1

* $P<0.01$, compared to controls, n=7.
† with cAMP and IMX

The secretion of Rb^+ was unaffected by mucosally-added Ba^{2+} (1.0 mM), indicating that Ba^{2+}-sensitive K^+ channels are not involved. Ba^{2+} on the serosal side stimulated Rb^+ net secretion from 30.8 ± 9.4 to 51.6 ± 13.2 neq.cm^{-2}.h^{-1} ($P<0.01$, paired t-test; n=7), suggesting that basal K^+ channels blocked by Ba^{2+} increase intracellular $[K^+]$, leading to augmentation of Rb^+ exit across the apical membrane.

The antiandrogen cyproterone acetate (CA, 0.2 mg.kg^{-1} per IP injection in castor oil; 3 injections over 7 days) was used as a pretreatment to test for androgen maintenance of the epithelia function. CA-treated animals had significantly reduced cAMP-stimulatable Isc and Rb^+ net flux, suggesting that androgens help maintain ion transport rates in the sperm duct. We do not yet know if a gonadotropic hormone, via cAMP, might also stimulate the transport.

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INTERACTION OF PROGESTOGEN WITH OVARIAN FOLLICLES OF THE GOLDFISH CARASSIUS AURATUS DURING MEIOTIC MATURATION

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Summary

During follicle incubation, media, into which 17alpha hydroxyprogesterone (HP) was introduced, showed exponential reduction of HP with a concommitant increase in the number of HP metabolites. Follicle extracts revealed that small amounts of HP were taken up by the follicles early in the incubation (i.e., 3 hr) and converted to several metabolites; the major one of these, which accumulated in the follicle, comigrated with 17alpha, 20beta, dihydroxy progesterone.
Keywords: meiosis, oocyte, steroid metabolism.

Introduction

A peak of HP occurs in the plasma of female goldfish before ovulation (Kagawa et al., 1983). HP is one of several steroids which are effective in eliciting meiotic maturation in goldfish follicle-enclosed oocytes in vitro (Jalabert, 1976; Nagahama et al., 1983; Lessman, 1985). In order to understand the interaction between HP and the follicle, RIA, TLC-autoradiography and HPLC were used to assess changes in added HP during meiotic maturation.

Results and discussion

Initially, RIA was used to assess changes in exogenous HP over time (Table 1). HP in the media decreased exponentially with time to less than 10% of the initial amount by 48 hr, while HP associated with follicles was less than 5% of the initial amount throughout the 48 hr incubation. Meiotic maturation as assessed by germinal vesicle dissolution (GVD) showed a clear dose response to both added HP and disappearance of HP from the media; control follicles showed low endogenous levels of HP which was associated with a low level of spontaneous GVD (Table 1).

In order to qualitatively assess the changes, tritiated HP was incubated with follicles for 0, 6, 24 and 48 hr. Media and follicle extracts were run on TLC plates and autoradiographed. The results indicated an increasing number of metabolites (9 major spots for media, 6 major spots for follicles by 24 hr) with time. To provide quantitative data on HP changes, tritiated HP was incubated for 0,3,6,16, and 24 hr; the media and follicle extracts were run on reverse phase HPLC. The elution profile of radioactivity indicated 6 major peaks in the follicle extracts and 9 major peaks in the media extracts by 24 hr.

Table 1. Changes in HP assessed by RIA of incubates of goldfish ovarian follicles.

Incubation	steroid added (ng)			
time (hr)	0	20	200	2000
0	1	22	193	1992
	(nd)	(nd)	(nd)	(1)
2	nd	17	128	2012
	(nd)	(nd)	(2)	(88)
4	nd	14	111	1480
	(nd)	(1)	(2)	(50)
6	nd	7	98	1403
	(nd)	(nd)	(3)	(76)
24	nd	3	32	348
	(nd)	(nd)	(1)	(35)
48	2	1	11	156
	(nd)	(nd)	(1)	(29)
%GVD	17	42	83	97

Data presented as mean ng HP measured in media and follicle washes (x3); data in parentheses are ng HP associated with washed follicles, nd = none detected; N=3 females. Germinal vesicle dissolution assay (%GVD) determined at 48 hr on 3 replicate batches of 20 follicles/female/dose.

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EVIDENCE FOR A GONADOTROPIN-RELEASING HORMONE BINDING PROTEIN IN GOLDFISH SERUM

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Summary

Binding of salmon gonadotroping-releasing hormone (sGnRH) and its superactive analogue, [D-Arg^6, Pro^9-NEt]-sGnRH to a macromolecular component in goldfish serum was studied, using ^{125}I-[D-Arg^6, Pro^9-NEt]-sGnRH as labeled ligand. Bound was separated from free labeled ligand by gel filtration with Sephadex G-50 mini- columns. The binding of labeled ligand to goldfish serum was dose-dependent. The results indicate a single class of specific binding site having low affinity and high capacity. The existence of a GnRH binder in serum may, in part, contribute to the long lasting pharmacological action of GnRHs in goldfish.

Control of Sexually Related Dimorphic Growth by Gonadal Steroids in Yellow Perch (Perca flavescens)

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Mechanisms that control sexual differences in growth and ultimate size have been well studied in mammals but are poorly understood in fish. In yellow perch (Perca flavescens), females begin to outgrow males at a size coincident with the onset of vitellogenesis and spermatogenesis (90-120 mm total length). Also, exogenous estrogens can promote and androgens can retard perch growth. To determine whether endogenous gonadal steroids influence sexually related dimorphic growth, perch were gonadectomized at 55-65 mm total length, a size at which neither estradiol-17β (in females) nor testosterone (in males) could be detected in the plasma. As these fish reached 90-120 mm total length, intact (laparotomized) females began to grow faster than gonadectomized females. Gonadectomized males grew faster than intact males and at the same rate as gonadectomized females. These results suggest that gonadal estrogens promote growth in females and gonadal androgens impair growth in males.

♀ > ♂

♀ ≥ 80g

♂ ≥ 50g

THE EFFECT OF CASTRATION ON PLASMA ANDROGENS IN MATURE MALE RAINBOW TROUT

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Keywords: rainbow trout, castration, plasma androgens, radio-immunoassay

Introduction

GtH/androgen ratios in blood plasma change during maturation and maturity in a way not explicable within the concept of a negative feed-back of androgens on GTH-secretion. Furthermore, GTH-levels are relatively low before spawning and when spawning grounds are not available. Thus, the testes could be unsufficiently stimulated to solely account for the high androgen concentrations in plasma which may be attained and maintained in part by extragonadal mechanisms. In order to test the significance of these mechanisms, we quantified plasma androgens before and at different times after surgical gonadectomy.

Material and methods

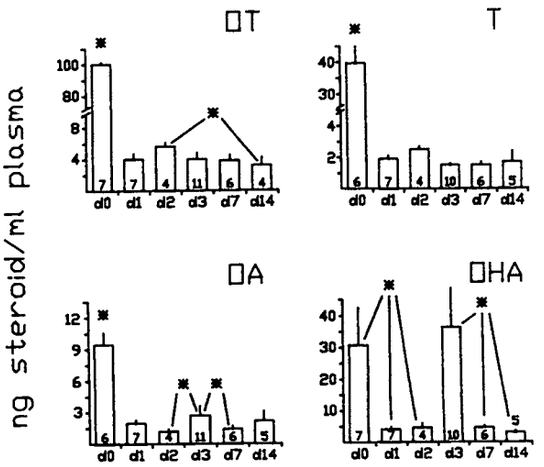
Milt producing males served as experimental animals. Plasma was gained from an initial control group, and 1, 2, 3, 7, and 14 days after castration. The concentrations of 4 androgens (testosterone-T, 11-oxotestosterone-OT, 11ß-hydroxyandrostenedione-OHA, androstenetrion-OA) were measured by radio-immunoassay after thin-layer chromatography of ether extracts. Samples from males with gonadal tissue remnants in the body cavity were discarded.

Results and discussion

The concentrations of all androgens declined precipitously one day after castration (Fig.). In comparison with the initial control group and with the exception of OHA, they remained at low levels thereafter but always were clearly above the limits of detection. While T levels did not change after castration, 11-oxygenated androgens oscillated significantly which was most pronounced in the case of OHA (Fig.).

Supposing a linear decrease of OT and T after castration, -most likely overestimated- plasma halflife-times below 90 min are calculated with the data from the initial control group and from one day after castration.

The results indicate that (i) halflife-times in plasma appear too short to assist in the maintenance of high plasma androgen concentrations, (ii) the testes produce the bulk of sex steroids and their precursors, (iii) extragonadal steroid sources contribute to the organism's steroid balance.



Plasma concentrations (mean +/- SEM; n=4 to 11) before (d0) and until 2 weeks (d14) after castration of milt producing male trout. * - $p < 0.05$, Student-t test.

A non-specific stimulation of the pituitary-interrenal axis following surgery cannot be excluded. The interrenals appear to synthetize OHA and OA and the concentration changes of the two androgens could be understood as reactions to "stress"-like stimuli. An increased output of cortisol which could fuel a hepatic production of androgen precursors (Kime, 1978) could as well be relevant. The remarkably high OT and T concentrations, however, remain to be explained. Possibly, a 17ß-hydroxysteroid dehydrogenase detected in trout blood cells (Schulz, 1986) is of importance in this context.

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This study was supported by DFG-grant Bl 62/15-2 to V.B.

GNRH- AND GTH-IMMUNOREACTIVE STRUCTURES IN THE PITUITARY OF SALMO GAIRDNERI RICHARDSON

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Summary

Immunoreactive (ir-) GtH- and ir-GnRH structures were localized in alternating serial sections of rainbow trout pituitary glands with the peroxidase-antiperoxidase (PAP-) technique. Immunoreactive (ir-) GtH cells are present within all three parts of the adenohypophysis. GnRH-ir axons enter the neurohypophysis and GnRH-ir material occurs in the entire neurointermediate lobe near by capillaries and sinusoids as well as in the neural protrusions penetrating the proximal pars distalis (PPD).
Keywords: GnRH, GtH, PAP, hypophysis, teleostei, Salmo

Introduction

In most vertebrate groups the rapid transport of Gonadotropin-Releasing Hormone (GnRH) from the sites of storage into the adenohypophysis is guaranteed by a capillary plexus within the median eminence which leads to a portal capillary network within the adenohypophysis. However, in cyclostomes and teleosts such a portal system is lacking so that the GnRH transport to the target cells is not yet clear in detail.

Results and discussion

Pituitaries of Salmo gairdneri were investigated by immunohistochemistry in order to observe possible structural interdependences between GnRH- and GtH-containig sites. After fixation of the heads in Bouin's fluid the brains were excised and conventionally embedded in paraplast. The PAP-technique was applied to 7 um serial sections using antisera to synthetic mammalian GnRH and salmon-GtH with known specifity. Ir-GtH cells are identified within the entire adenohypophysis. The granular reaction product is located in the cytoplasm and the nuclei remain unstained. In the rostral pars distalis (RPD) these cells are spindle-shaped and are integrated into the follicular prolactin cell structures. In the PPD the ir-GtH cells are elongated and are found within epithelial layers together with nonreactive cells. The pars intermedia (PI) contains only a few ir-GtH cells which have an ovoid shape and form small groups. GnRH-ir axons enter the neurohypophysis as a distinct tractus. In the pituitary stalk as well as in the neurointermediate lobe large amounts of ir material is present near capillaries and sinusoids. The neural protrusions penetrating the PPD contain GnRH-ir material, too, which is also often found in the neighbourhood of GtH-ir cells in the PPD and the PI (fig.1). These findings indicate two possibilities of GnRH transport into the adenohypophysis:
a: via bloodstream from the nervous structures interdigitating with the PI and
b: direct contacts between GnRH containing axon endings and GtH cells.

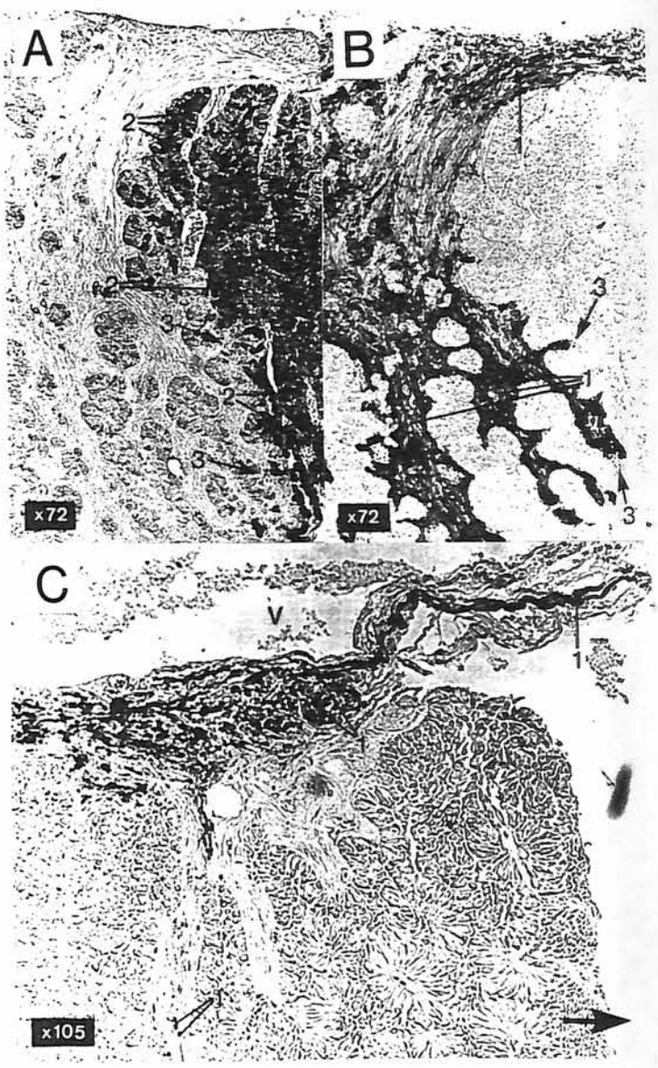

Fig. 1. A-B: PAP-staining of adjacent sagittal sections of S. gairdneri pituitary with rabbit anti-GtH (A) and anti-GnRH (B). C: GnRH-ir fibers entering the PPD.
1=GnRH-ir fibers, 2=PPD-GtH cells, 3=capillary, V=ventricle. Arrow points rostrad.

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PROTEIN KINASE C AS A MEDIATOR IN GOLDFISH OVULATION.

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The possible involvement of protein kinase C in goldfish (Carassius auratus) ovulation was investigated. In vitro ovulation of oocytes that had undergone germinal vesicle breakdown and follicular detatchment in vivo was observed with both a phorbol ester, phorbol-12-myristate-13-acetate and a calcium ionophore, A23187. A combination of these two factors exhibited classic synergism and induced ovulation at much lower concentrations. Since protein kinase C is physiologically activated by diacylglycerols, three sn-1,2-diacylglycerols were also tested in combination with A23187. Of these sn-1-oleoyl-2-acetylglycerol (OAG) was ineffective, while sn-1,2-dioctanoylglycerol (diC_8) and sn-1,2-dihexanoyl--glycerol (diC_6) were found to synergise with the calcium ionophore even at the lowest levels tested. DiC_8 was more effective than diC_6 at comparable levels. Lithium chloride, in the absence of any other factor was found to be stimulatory to ovulation at concentrations of 25mM or higher, however, a consistent dose response was not observed. In vitro ovulation stimulated with phorbol ester and ionophore could be blocked with nordihydroguaiaretic acid (NDGA) at 10µg/ml while indomethacin (IM) at similar or higher (20µg/ml) levels was ineffective. This suggests the involvement of lipoxygenase products in conjunction with protein kinase C activation during ovulation in this species. Similar results with NDGA and IM were also obtained with arachidonic acid and PGF2α induced ovulation. While a direct participation of protein kinase C or its mechanism of action remains to be demonstrated these results indicate its involvement in the control of ovulation in fish. (Supported by NSF Grant No. 8517718).

PROTEOLYTIC ENZYMES IN THE FOLLICULAR WALL OF BROOK TROUT AND GOLDFISH DURING MEIOTIC MATURATION AND OVULATION. Amy K. Berndtson and Frederick Goetz, Department of Biological Science, University of Notre Dame, Notre Dame, IN.

In teleost fish, proteolytic enzymes are believed to be involved in the mechanism of ovulation. To determine if follicular protease activity was present in brook trout (Salvelinus fontinalis) and goldfish (Carassius auratus), follicle walls were dissected from oocytes at different stages throughout meiotic maturation and ovulation and assayed using substrate-sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) techniques. In this system, either gelatin or casein was incorporated into gels to serve as a substrate and, following electrophoresis, a Tris-$CaCl_2$ buffer (pH 7.8) was used to incubate the gels. In both species, six major proteolytic enzymes digested gels containing gelatin. The first two (78 and 70 kD) were inhibited with 5.0 mM 1,10 phenanthroline, 2.0 mM dithiothreitol (DTT), Ca^{++}-free medium, 10.0 mM benzamidine and 5 mM diisopropyl fluorophosphate (DFP). The second two (67 and 59 kD) were inhibited with 5.0 mM 1,10 phenanthroline, 2.0 mM DTT and Ca^{++}-free medium. The last two (22 and 20 kD) were only slightly inhibited with 5.0 mM DFP. These two enzymes also digested gels containing casein. Thiol, carboxyl and other serine protease inhibitors had no effect on follicular proteolytic activity. Preliminay in vitro studies have shown that ovulatory hormones, such as prostaglandin $F_{2\alpha}$, altered the proteolytic activity of several enzymes. All six enzymes were marginally active during meiotic maturation and maximally active just prior to and after ovulation. This study demonstrates that both metallo and serine proteolytic enzymes are present in the follicle wall of brook trout and goldfish and suggests that these enzymes, may play an integral role in the mechanism of ovulation. Funded by NSF grant number DBC-8517718 to FWG.

THE MECHANISM AND HORMONAL REGULATION OF OVULATION: THE ROLE OF PROSTAGLANDINS IN TELEOST OVULATION. Frederick W. Goetz, Mukul Ranjan, Amy K. Berndtson and Priscilla Duman. Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana, USA 46556.

Prostaglandins (PGs) have been implicated as stimulators of oocyte expulsion in various fish species and PG levels have been shown to change relative to the time of ovulation in several species. The purpose of this paper is to present old and new data concerning PG stimulation and synthesis in a salmonid, the brook trout (Salvelinus fontinalis), and a percid, the yellow perch (Perca flavescens). In both species PGs can stimulate ovulation of oocytes in vitro, though the most effective PG and the relative sensitivity to PG stimulation differ markedly for each. F prostaglandins are the most effective PGs in the stimulation of brook trout ovulation and a rather high exogenous PG level is necessary to obtain a response. In contrast, PGE2 is the most stimulatory PG in yellow perch and the follicles are more sensitive by orders of magnitude. In brook trout, PGF does increase in plasma and ovarian tissue during ovulation as measured by RIA. In addition, ovarian PGE levels while appearing low at ovulation, are extremely high in gravid fish prior to meiotic maturation. From recent precursor incorporation studies it appears that the follicle wall of brook trout can produce PGF at ovulation from arachidonic acid. Incorporation studies have shown, however, that there are actually several ovarian sites for PG synthesis and that a greater conversion of precursor to PGE and PGF actually occurs in extrafollicular (i.e. of vitellogenic oocytes) tissue. PGE synthesis is exceptionally high in connective tissue elements of the ovary while PGF can be produced by small nonvitellogenic follicles. In fact, a large portion of the PGs measured by RIA probably comes from these tissues. Whether this extrafollicular PG synthesis is directly involved in ovulation or plays a role in some other aspect of ovarian control (e.g. control of blood flow) is unknown but poses some interesting new perspectives from which to experiment.

In perch, 17a, 20b-dihydroxy-4-pregnen-3-one, which induces maturation and ovulation in vitro, also alters PGF synthesis relative to ovulation as measured in incubation fluid by RIA. From preliminary time course experiments, it appears that there is an increase in the production of PGF at the time of ovulation. Estrogenic, androgenic and 11-oxygenated corticosteroids are unable to alter PGF synthesis though other progestational steroids do have an effect on PGF levels that appears to be related to their ability to stimulate ovulation in vitro. Whether the PGF is involved in the in vitro stimulation of perch ovulation or is a consequence of ovulation is still unclear and being investigated. As in trout, the primary site of PGF synthesis, demonstrated by incorporation experiments with arachidonic acid, appears to be extrafollicular.

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ENDOGENOUS OPIOID MODULATION OF GONADOTROPIN SECRETION IN MALE GOLDFISH

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In mammals, the secretion of gonadotropin-releasing hormone (GnRH) and dopamine (DA) from the hypothalamus is modulated by endogenous opioid peptides (EOP's). Opioid agonists inhibit, and antagonists stimulate the secretion of both GnRH and DA, which in turn modulate the secretion of anterior pituitary hormones. In teleost fish, it is now well established that GnRH stimulates, and DA inhibits, the secretion of gonadotropic hormone (GtH) from the pituitary. The present studies were performed to determine whether the EOP system participates in the regulation of GtH secretion in male goldfish, Carassius auratus, via actions on GnRH and/or DA secretion.

In vivo treatment with the opioid antagonist naloxone (NAL) resulted in a transient decrease in circulating GtH levels. When animals received NAL 2 hrs prior to an injection of a GnRH agonist (GnRH-A), the stimulatory effects of GnRH-A on GtH levels were blocked. Similarly, prior treatment with NAL blocked the stimulatory effects of the dopamine antagonist domperidone. Together, these results suggest that NAL stimulates DA secretion, resulting in decreased secretion of GtH.

When NAL was administered simultaneously or 1 hr following GnRH-A, the response to GnRH-A was potentiated. This suggests that NAL can stimulate endogenous GnRH secretion in vivo, but that this effect is only evident when exogenous GnRH is present. In support of this, in in vitro hemipituitary incubations, NAL elicited increased GnRH release into the medium.

In summary, we have presented evidence for the involvement of the EOP system in the neuroendocrine regulation of GtH secretion in male goldfish. Our data suggests that EOP's alter GtH secretion by modulating the release of both GnRH and DA.

THYROID-REPRODUCTIVE RELATIONSHIPS IN THE CHANNEL CATFISH, Ictalurus punctatus: EVIDENCE FOR ESTRADIOL-INDUCED CHANGES IN PLASMA THYROID HORMONE BINDING.

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Blood samples taken at monthly intervals from female channel catfish maintained in pond culture were analyzed for levels of testosterone (T), estradiol (E_2), thyroxine (T_4), and triiodothyronine (T_3) by radioimmunoassay. Levels of all four hormones were low during the months of September to January, corresponding to times of lowest temperature and shortest photoperiod. A significant increase in the levels of all four hormones was found in February in the absence of any change in pond temperature. T returned to basal levels in March, and remained at basal levels throughout the remainder of the year. In contrast, E_2 levels remained significantly elevated throughout the period of maximal gonadal growth until spawning (April-May), at which time they also returned to lowest levels. The levels of both thyroid hormones declined prior to the initiation of spawning, but then increased during the spawning period in both prespawning and postspawning females to reach highest levels in postspawning fish during the summer. A time of year thus exists (February) during which the levels of all four hormones increase simultaneously; this also corresponds to the time at which maximum gonadal growth is initiated. Additionally, during the summer highest thyroid hormone levels are found during the time when steroid hormone levels are lowest, temperatures are highest, and feeding activity (as judged by stomach contents) is greatest.

To determine whether observed changes in thyroid hormone levels were due in part to endocrine interactions with changing E_2 levels, both male and female catfish were injected with estradiol-17β at a dose of 1 mg/kg body weight every four days, and blood was sampled on day 16. Estradiol treatment stimulated a significant increase in total plasma protein and in plasma protein phosphorus, indicating that vitellogenin production was induced. When plasma samples from E_2-injected animals were analyzed at serial dilutions in unextracted thyroid hormone radioimmunoassays, however, E_2 treatment was found to induce significant nonparallelism between unknown plasmas and the standard curve. This nonparallelism was due to abnormally high precipitation of protein-bound radioiodinated thyroid hormones at high plasma dilutions, indicating that proteins induced by E_2 treatment were binding thyroid hormones. To examine changes in thyroid hormone binding to plasma proteins induced by E_2, plasma samples from control and E_2-injected fish were examined for free thyroid hormone levels using a Sephadex column chromatography technique (J. G. Eales and S. Shostak, Gen. Comp. Endocrinol. 58: 291-302; 1985). E_2 treatment stimulated a significant decrease in free thyroid hormone levels, indicating increased protein binding in treated animals. Additionally, plasma samples were labeled in vitro with radioiodinated T_4 and subjected to gel filtration chromatography. Increased total binding of T_4 to plasma proteins was found to be due to substantial binding of T_4 to higher molecular weight proteins in E_2-treated animals, indicating that vitellogenin may function as a thyroid hormone binding protein in this species.

Analysis of plasma binding of thyroid hormones
→ ~~protein~~ lie ai proteine di PM non-albumine
farsi affinate

→ après traitement per E2 binding
converti dans precipitazione di PM ad alto
in la vtg - Plus forte affinité

PHOTOPERIODIC HISTORY AND THE ENTRAINMENT OF THE ANNUAL CYCLE OF REPRODUCTION IN THE FEMALE RAINBOW TROUT (SALMO GAIRDNERI)

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Abstract

Recently, it has been proposed that the annual cycle of reproduction in the female rainbow trout is coordinated by an endogenous circannual clock which is timed by the seasonal changes in daylength (Duston & Bromage,1986,1987). Earlier hypotheses relating to the timing of maturation have been based on apparent requirements for long and short photoperiods during the early and later portions of the ovarian cycle respectively, with daylengths over 12 hours generally being considered as 'long' days.

A requirement for a specific daylength differs fundamentally from our understanding of the behaviour of the endogenous clock, for we consider that it is the direction of change of photoperiod which entrains the rhythm of maturation and that the absolute length of the photoperiod is not important. Further support for this proposal is provided in the current work.

Three groups of 2 year old virgin female rainbow trout, with a natural spawning period of December - January, were transferred in mid - January from outside ponds (ambient photoperiod) to lightproof 1200 litre tanks and provided with artificial lighting controlled by electronic time clocks. Groups A and B were exposed to 18L:6D and 10L:6D respectively until May 8, followed by 6L:18D until spawning. Group C was exposed to 6L:18D until May 8, followed by 2L:22D until spawning. The water temperature was constant at 8.5 - 9.0 `C and the light intensity at the water surface was 25 lux. Serum levels of oestradiol-17B, testosterone and vitellogenin (as calcium) were measured at monthly intervals to provide further information about the effects of photoperiod on the rate of ovarian development.

As a result of the photoperiod treatments fish in Group A commenced spawning on August 23, Group B on October 2 and Group C on December 2 with the periods of spawning lasting 9, 8 and 11 weeks respectively.

It is proposed that the 2-3 month advancements of spawning exhibited by the fish in Groups A and B were due to two separate phase advances of the endogenous clock which controls maturation. The first occurred at the start of the experiment in January when the fish were subjected to an increase in photoperiod, from ambient (8.5L:15.5D) to either 18L:6D (Group A) or 10L:14D (Group B), and the second when the photoperiod was reduced for both groups in May. In contrast the fish in Group C, where spawning was modestly delayed, were subjected to a phase delay in January, when the experimental 6L:18D photoperiod was shorter than that of ambient,and a phase advance in May when the photoperiod was reduced.

Collectively, these data indicate that it is the direction of change of daylength which is responsible for the entrainment of reproduction. Daylength per se is of little importance to this response. Furthermore, as any photoperiod may be percieved by the fish as 'long' or 'short' providing it is longer or shorter than the daylength to which the fish have been previously exposed, then photoperiodic history is of considerable significance to our interpretation of light effects on maturation in fish.

This work was supported by NERC awards to N.R.B.

EGG PROTEINS AND VITELLOGENIN IN THREE MARINE FISH SPECIES

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Introduction

During recent years, the aquaculture of marine organisms has attained a growing interest. Cold-water living species, like the cod (Gadus morhua) and the Atlantic halibut (Hippoglossus hippoglossus) are particulary suited for breeding in the northern, countries, like Norway.

For a successful reproduction and development of the larvae, the quality of the eggs is of major importance. An adequate supply of nutrients during the early development is vital for the embryo. The source of the nutrients are the yolk proteins, which are derived from vitellogenin (VTG) and stored in the egg.

VTG is synthesized in the liver of maturing females in response to estrogen stimulation. It is rapidly secreted and transported to the ovaries, where it is incorporated into the oocytes. After the incorporation, the VTG molecule is transformed into the yolk proteins.

In some marine fish species, a further hydrolysis of the yolk proteins has been observed in mature, hydrated eggs. This has been suggested as part of the mechanism of the osmotic gradient that makes water uptake into the cell possible.

The object of the present study was to characterize the yolk proteins in eggs at different stages of maturity, and plasma vitellogenin in three different species of marine fish: the plaice (Pleuronectes platessa), the cod and the Atlantic halibut.

Results and discussion

SDS-gel electrophoresis on 7-15% polyacrylamide gradient gels revealed the presence of a female-specific protein of high molecular weight (approximately 180 000 daltons) in the plasma of fish of all three species. When plasma of mature females was chromatographed on DEAE-sephacel, this protein eluted last from the column.

Eggs from cod, plaice and halibut were divided into three or four different stages, according to size and degree of hydration. The eggs were crushed and a "yolk" fraction obtained by centrifugation.

When analyzed electrophoretically, hydrated eggs differed in their protein composition from non-hydrated eggs. Changes in the yolk protein pattern has been observed in mature, hydrated eggs from marine fish species. These observations are in accordance with the results of this study, where hydrated eggs contained proteins of lower molecular weight than non-hydrated eggs.

METABOLISM OF STEROIDS IN THE OVARIES OF THE UROHAZE-GOBY (GLOSSOGOBIUS OLIVACEUS)

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Summary

In vitro steroidogenesis was examined using ovaries of the urohaze-goby (Glossogobius olivaceus) in the breeding season. Cell-free homogenates of fully matured ovaries (just before ovulation) were incubated with ^{14}C-labeled pregnenolone, dehydroepiandrosterone, progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone in the presence of NAD^+ or NADPH. Main products from these substrates were progesterone, androstenedione, 5β-pregnane-3,20-dione, 17α-hydroxy-5β-pregnane-3,20-dione, 5β-androstane-3,17-dione, and 5β-dihydrotestosterone, respectively.

Introduction

The steroidogenesis in the testes of the urohaze-goby is characterized by the predominant activities of 5α-reductase and Δ^5-3β-hydroxysteroid dehydrogenase + Δ^5-Δ^4 isomerase. Furthermore, 11-oxotestosterone, which is an androgen peculiar to teleosts (Idler et al., 1960) is not synthesized in vitro in the testes of this species (Asahina et al., 1985). In this study, we investigated the steroid metabolism in the ovaries of the urohaze.

Results and discussion

When pregnenolone or dehydroepiandrosterone was incubated with the cell-free homogenates of the ovaries in the presence of NAD^+, the main metabolite was progesterone or androstenedione, though each yield was low (Table 1). These results indicate that the ovaries of the urohaze have Δ^5-3β-hydroxysteroid dehydrogenase and Δ^5-Δ^4 isomerase activity.

Ovarian tissue of the urohaze converted progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone into 5β-pregnane-3,20-dione, 17α-hydroxy-5β-pregnane-3,20-dione, 5β-androstane-3,17-dione, and 5β-dihydrotestosterone, respectively (Table 1), showing a relatively high activity of 5β-reductase. In contrast, 5α-reductase activity, which is dominant in the testes of the urohaze (Asahina et al., 1985) was only weakly detected when progesterone was used as a substrate (Table 1).

Since the ovaries of the urohaze did not convert 17α-hydroxyprogesterone into 17α,20β-dihydroxy-4-pregnen-3-one, the maturation inducing steroid (MIS) in salmonids (Nagahama et al., 1985), MIS might be another steroid(s) in this species.

Table 1. Metabolism of various substrate steroids in the ovaries of G. olivaceus.

Substrate	Metabolite	Yeild
Pregnenolone	Pregesterone	0.12*
Dehydroepi-androsterone	Androstenedione	0.27
Progesterone	5β-Pregnane-3,20-dione	1.19
	5α-Pregnane-3,20-dione	0.20
	X-1	0.04
	X-2	0.04
17α-Hydroxy-progesterone	17α-Hydroxy-5β-pregnane-3,20-dione	5.27
	X-3	0.13
Androstenedione	5β-Androstane-3,17-dione	1.22
	Testosterone	0.10
Testosterone	5β-Dihydro-testosterone	0.63
	Androstenedione	0.04
	5β-Androstane-3,17-dione	0.01

* nmol of steroid / 5 mg protein / 60 min.

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NATURAL SEX REVERSAL

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Abstract

The normal occurrence of spontaneous sex inversion among teleosts is a fairly new item within current concepts on vertebrate reproductive physiology. The subject has almost totally been seized by ethologists and by ecologists who cogitate upon selective advantages of particular reproductive strategies. Investigations on causative events that are mandatory for a functional change of sex remain largely neglected. Our knowledge on the role of hormones being presumedly involved in the process of sex inversion is close to zero. This is true even with regard to sex steroids. In terms of developmental physiology we must admit that we don't have the slightest idea about differential steps leading either to spermatogenic or oogenic meiosis. The nature of "social factors" being claimed as causative for sex inversion in certain species remains in the dark. Many reports prove the technical feasibility of manipulations that can induce a phenotypical change of sex. Yet the mechanisms underlying such a process are not understood. It is unknown whether spontaneous sex change in an adult can be compared reasonably with sexual alterations in juveniles. Current research on teleost ambisexuality needs some reorientation in order to save it from the mark of a mere biological oddness.

STEROID METABOLISM IN GONADAL TISSUES OF THE SIMULTANEOUS HERMAPHRODITE SERRANUS CABRILLA

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Abstract

Male and female tissues of the gonads of the simultaneous hermaphrodite Serranus cabrilla were separated from each other and incubated with 4-(14C)-testosterone for two hours without cofactors at room temperature. Fishes were captured in June, the spawning season, and in September. Gonads of juvenile and adult fishes were used. About 90 % of the recovered radioactivity (19 metabolites) could be identified with a separation procedure including paper and thin-layer chromatography, microreactions and cristallization to constant specific activity (details in: Reinboth and Becker, 1984).

The main results are summarized in the tables.

Table 1. Enzymatic activity of the male tissues.

Date	Sept.20	June 7	June 4	June 6
weight mg	190	60	150	300
status	juv.	juv.	adult	adult
17β-HSD	12,3	6,1	12,3	4,3
11β-hydroxylase	28,5	4,9	0,9	3,3
5β-reductase	8,2	9,4	2,2	15,3
5a-reductase	0,4	1,7	4,1	0,5

Table 2. Enzymatic activity of the female tissues.

Date	Sept.20	June 7	June 4	June 6
weight mg	250	170	2x300	3x360
status	juv.	juv.	adult	adult
17β-HSD	11,0	8,5	16,0	10,7
11β-hydroxylase	7,4	-	0,2	0,7
5β-reductase	10,6	31,3	23,1	44,9
5a-reductase	0,6	1,1	0,8	1,5

The most surprising finding is the high activity of 11β-hydroxylase in the gonads of juveniles outside the breeding season. This applies even to ovarian tissue. It can be ruled out that the obvious similarities between the shares of different metabolic groups in the two heterosexual tissues from juveniles in September should be explained by incomplete mechanical separation.

Among the results from the samples taken in June it can be noticed that in ovarian tissue 11β-hydroxylase-activity was low or absent. But 5β-reductase activity was considerably higher in female tissue. In comparison to the data from two other ambisexual species the metabolization rate of testicular tissue was relatively low. The bulk of conjugates were sulfates. Estrogens were not detectable.

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IN VIVO METABOLISM OF EXOGENOUS 11-KETOTESTOSTERONE IN JUVENILE GUPPIES

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Abstract

Juvenile guppies at ages of 5 or 10 days resp. were incubated in 50 or 10 ml of water supplemented with 11-KT at a dose of 20 µg/l, together with 1,5 - 4 µCi of tritiated 11-ketotestosterone. After 7 resp. 24 h the animals were anaesthetized, weighed and homogenized in Ac/EtOH 1:1. Metabolites of the substrate were separated according to a scheme previously described by our laboratory (Reinboth and Becker, 1984) with slight modifications.

No major differences were observed between the metabolism of the animals under the different incubation conditions. At the end of the incubation at least 70 % of the activity in the water was still the substrate.

By far the largest fraction were glucuronides with a yield of 80 % after 7 h and 90 % after 24 h of incubation. Only a few percents were sulfates.

No Δ4-androgens could be identified. The 3 major constituants were an unidentified compound, which was more polar than androstane-3,11,17-triols,5β-androstane-3a,17β-diol,11-one (identified by cristallization to constant specific activity) and 5a-androstane-3β,17β-diol,11-one. The latter was identified by TLC, microchemical reactions and cristallization to constant specific activity after oxidation. A minor fraction run together with 11-KT in $CHCl_3$/Me 9:1 but behaved differently after microchemical reactions.

The lack of Δ4-androgens in extracts from whole juvenile guppies and the strong androgenic effect of 11-KT suspended in the rearing water under the conditions used lead us to speculate on a possible androgenic effect of reduced 11-oxygenated C19-steroids.

Reference

Reinboth, R. and B. Becker, 1984. In vitro studies on steroid metabolism by gonadal tissues from ambisexual teleosts. I. Conversion of (14C)-testosterone by males and females of the protogynous wrasse Coris julis L. Gen. Comp. Endocrinol. 55: 245-250

EFFECTS OF STEROID TREATMENT AND TEMPERATURE ON GONADAL DIFFERENTIATION AND PITUITARY GONADOTROPES IN THE GUPPY, POECILIA RETICULATA, PETERS

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Abstract

Neonate guppies were treated with different doses of 11-ketotestosterone for up to 21 days at temperatures of 25 and 28°C respectively. The animals were fixed in Bouin-Hollande and embedded in Paraplast. Serial sections at 6 um thickness were used for gonad and pituitary histology. Pituitary gonadotropes were stained by immunohistochemistry, using an antiserum raised in rabbits against a commercially available salmon GTH (Syndel SGA-GTH).

At 28°C by far most of the genetic females developed testes containing a few degenerating oocytes. At 25°C only less than half of the genetic females showed signs of spermatogenesis. In these animals the treatment inhibited the formation of the ovarian cavity and the differentiation of oocytes. However, some oocytes hypertrophied and underwent degeneration.

In males, the treatment accelerated spermatogenesis, regardless of the rearing temperature.

The gonadotropic zone in the pituitary was further developed in females than in males. Androgen-treated males showed a somewhat larger GTH-positive area in their pituitaries. In females, animals responding by spermatogenesis to the treatment had a larger GTH-positive area in their pituitary than controls, whereas females with degenerated ovaries had less GTH-immunoreactive material.

A direct comparison of genetic females of one litter treated at 25°C and 28°C for the same period of time (expressed in day-degrees) was carried out. The GTH-positive area in the pituitary of females reared at 28°C was about twice as large as that in females raised at 25°C.

The study underlines the action of the sex inverting treatment on the differentiation of the pituitary gland and indicates a role of gonadotropes in the process of a successful androgen induced sex inversion.

TESTIS GROWTH AND SPERMATOGENESIS IN TELEOST FISH

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Summary

The morphological diversity of spermatogenesis in fish is shown either in the structure of the testis or the morphology of the spermatozoa. Two main types of testis structure are identified. In the tubular type, spermatogenesis starts at the blind end of a tube where the stem cells are located, and cysts move down the tube where the sperm, sometimes grouped in bundles, are released into the efferent ducts. In the lobular type of testis, the stem cells are scattered at the periphery of a tube-like structure (lobule) having a central lumen into which the spermatozoa are freed from the cysts after spermatogenesis has been completed; these sperm then move down the lobule to be released into the deferent duct.

The quantitative aspects of testis growth are often described by the gonadosomatic index (GSI) which changes during the reproductive cycle. The maximal value of this index varies widely between groups of fish: from 0.2% in some Tilapia and Siluriforms to 10% in cyprinids and salmonids. The GSI value is maximal in seasonal spawners just after meiosis at the spermatocyte stage and declines during spermiogenesis due to the elimination of cytoplasm from the spermatids. Loss due to germ cell degenerescence during spermatogenesis has been reported in some species; their spermatogenetic production is quite variable: 7.10^9 spermatozoa/g body weight/year in rainbow trout (Parasalmo mykiss), 4.10^9 in common carp (Cyprinus carpio), $2.7.10^9$ in guppy (Poecilia reticulata) and 6.10^8 in pike (Esox lucius). In seasonal spawners testis weight decreases during spermiation period due to the release of sperm during spawning. In fish farm conditions the amount of spermatozoa which can be collected for artificial insemination is highly variable: between 20 and 90% of the total spermatogenetic yield in trout and 4.5% in pike. In carp and goldfish the number of spermatozoa released after stripping is low unless some stimulation is given (presence of ovulating females or gonadotropin injection). After the reproductive season the spermatozoa remaining in the testis are resorbed in some species such as trout and pike, but remain until the next cycle in carp.

The quality of the germ cells is estimated on the spermatozoa by various parameters such as chemical composition, motility, fertilizing capacity and *in vitro* survival. Sperm quality declines in some species such as trout and sea bass (Diecentrarchus labrax) as shown by the lower fertilizing capacity, motility and capacity for cryopreservation of the sperm. In rainbow trout ATP appears to be necessary for sperm survival *in vivo* in the genital tract as well as *in vitro*. In trout sperm this demand seems to be easily covered by mitochondrial ATP synthesis. Such a great amount of energy is needed to initiate motility (the beat frequency of 60 Hertz is one of the highest found in sperm) that the mitochondria cannot cope: the endogenous store of ATP is exhausted within 20 to 25 seconds and the sperm become immotile. Rainbow trout sperm physiology is highly dependent on the ionic environment in the seminal plasma, and especially on potassium and calcium. During spermiation external potassium inhibits motility, but before spermiation is initiated sperm immotility is not due to potassium but to some other unknown factors. External calcium is needed to initiate motility.

Spermatogenetic activity may be either associated with or dissociated from spawning time; it does not necessarily coincide with female oogenetic activity. The timing of spermatogenesis during the year depends on internal or external factors such as temperature, photoperiod or other climatic events which represent long-term changes. The initiation or stimulation of spermiation depends on more rapid environmental changes (temperature change or presence of spawning females). The high diversity of spermatogenetic patterns are in parallel with the variety of modes and strategies of reproduction, spawning behavior, diversity of habitats and many other environmental factors.

OVARIAN CYCLE OF WHITE STURGEON (ACIPENSER TRANSMONTANUS)

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Summary

Sturgeons differ from most true teleosts by longer-than-annual ovarian cycle. Chronology and environmental control of this unusual cycle are unclear. Understanding of these factors is important for management of wild and cultured stocks.

We examined ovarian histology and plasma concentrations of estrogen (17 β -estradiol) and alkali-labile phosphoprotein (ALPP) of adult females (100-205cm fork length) collected during the winter in San Francisco Bay, California. Ovarian stages were quantified by ranking discrete histologic structures (such as three layers of zona radiata, platelet yolk, melanine granules, etc.). Three distinct stages were present: 1) previtellogenic oocytes in early growth phase (0.1-0.3mm) with basophillic cytoplasm and peripheral carbohydrate vesicles; 2) early vitellogenic follicles (0.6-1mm) with platelet yolk and undifferentiated zona radiata; 3) ripe follicles (3-4mm) with micro- and macroplatelet yolk (polarized), differentiated zona radiata and cortical melanin granules.

Winter female stock (n = 398) was composed of seventy-three percent fish in stage one, eleven percent in stage two, and sixteen percent in stage three. Stage 3 was recruited in current year spawning (spring), whereas stage 2 in next year spawning. Stage one (quiescent) was two or more years away from spawning recruitment.

In stage 1, plasma estrogen was below detectable level (RIA, 0.25 ng/ml) and ALPP was also low (4.2 ug/ml). Concentrations of both metabolites significantly rose in stage 2 (4.76 ng/ml and 29.2 ug/ml, respectively) and remained elevated throughout the stage 3. Fish in this latter stage were induced to spawn two months after capture. At ovulation, concentration of estrogen fell to 0.29 ng/ml and ALPP to 20 ug/ml (Figure 1).

Although concentrations of both metabolites exhibit similar trends during the ovarian cycle, we found no correlation between the estrogen and ALPP in group of individual fish undergoing vitellogenesis. Even though the estrogen stimulates hepatic protein synthesis, plasma levels of vitellogenin may depend on rates of synthesis, degradation, and uptake. Additionally, plasma levels of ALPP may not be strictly under estrogen control.

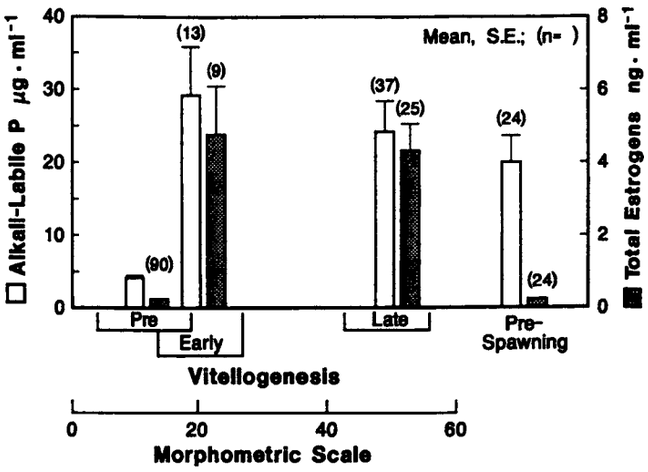

Figure 1. Plasma concentrations of estrogens and alkali-labile phosphoprotein in white sturgeon females at different stages of ovarian development. Morphometric scale is the histologic ranking of oogenesis.

In conclusion, our data indicates that the ovarian cycle in sturgeon is, indeed, an unusually long process but its active part, vitellogenesis, is quite similar with other non-teleost and teleost fish (Dodd & Sumpter, 1984). While vitellogenesis in sturgeon takes approximately one year, duration of previtellogenic stage is, most probably, longer. Sturgeon females lose more body calories at spawning than any other fish (Krivobok & Tarkovskaya, 1970). A timetable of the ovarian cycle in iteroparous sturgeon allows recovery and growth of fish needed for each new wave of vitellogenesis.

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A NOVEL ACTION OF GONADOTROPIN-RELEASING HORMONE IN THE GOLDFISH (Carassius auratus): THE STIMULATION OF GROWTH HORMONE SECRETION

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SUMMARY

The stimulation of gonadotropin (GTH) secretion by the hypothalamic peptide gonadotropin-releasing hormone (GnRH) is well documented in teleost fishes [Peter et al., 1986]. We report here results of experiments demonstrating that GnRH also acts on the goldfish pituitary to stimulate the secretion of growth hormone.

Intraperitoneal injection of GnRH elevates serum levels of growth hormone in both male and female goldfish. In one experiment using female fish, mammalian GnRH (mGnRH), salmon GnRH (sGnRH), [D-Ala^6,Pro^9 NEt]-mGnRH (mGnRH-A), or [D-Arg^6,Pro^9 NEt]-sGnRH (sGnRH-A) caused elevated serum growth hormone levels, although the response to mGnRH, mGnRH-A and sGnRH-A was longer lasting than the response to sGnRH. In a second experiment, injection of mGnRH-A resulted in a dose-dependent increase in serum growth hormone levels, with a dose as low as 0.001 μg mGnRH-A/g body weight causing elevated serum growth hormone levels.

The influence of GnRH on GTH and growth hormone secretion was also studied using goldfish pituitary fragments maintained in a perifusion system. In these experiments, exposure of fragments to two minute pulses of various concentrations of sGnRH rapidly stimulated the secretion of both GTH and growth hormone. Analysis of the in vitro dose-response curves indicates a half-maximal effective dose of sGnRH in the nM range for both growth hormone and GTH secretion. These results indicate that GnRH acts directly at the level of the pituitary to alter both growth hormone and GTH secretion.

A major difference in the neuroendocrine regulation of GTH and growth hormone secretion is suggested by experiments examining the influence of dopamine, a teleost GTH release-inhibitory factor [Peter et al., 1986], and somatostatin, a teleost growth hormone release-inhibitory factor [Marchant et al., 1987], on GnRH-induced hormone release. Injection of the dopamine antagonist pimozide greatly potentiates the effects of GnRH on serum GTH levels. However, pimozide did not potentiate the GnRH-induced elevation in serum growth hormone levels. Furthermore, apomorphine, a dopamine agonist, completely blocked sGnRH-induced GTH secretion in vitro, but was without effect on sGnRH-induced growth hormone release. Conversely, somatostatin completely abolished sGnRH-induced growth hormone secretion, but was without effect on GTH secretion.

Based on these results, we hypothesize that growth hormone and GTH release in the goldfish are regulated, at least in part, through a common releasing factor, GnRH. However, the release-inhibitory factors regulating growth hormone and GTH secretion from the goldfish pituitary are separate and distinct. (Supported by NSERC and AHFMR)

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CURRENT STATUS OF INDUCED SEX MANIPULATION

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Summary

There has been significant recent progress concerning the refinement of existing techniques and the development of new techniques for the regulation of sex in cultured fish. The groups of species that have received particular attention have been the salmonids, tilapias and carps. The greatest diversity of approaches has been utilized in the salmonids and this review will focus on this group.

There are two main approaches to the production of monosex female and male fish; the use of estrogens and androgens to directly influence sex differentiation and the use of chromosome set manipulation to regulate the contribution of genetic information from the male and female gametes to the zygote.

To produce sterile fish the hormonal and a variety of genetic techniques can be used alone or in combination. Thus sterile fish can be produced by the direct administration of androgens during and after sex differentiation, by the production of female triploids and in some situations by the use of interspecific hybrids. In addition, fish can be sterilized by exposure to ionizing radiation at a time when the differentiating gonad is particularly susceptible.

The utilization of techniques for the manipulation of sex will be discussed in the context of their integration into aquaculture production systems.

INDUCED OVULATION IN SABLEFISH (ANOPLOPOMA FIMBRIA) USING GONADOTROPIN RELEASING HORMONE ANALOGUES

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Summary

The sablefish (or Alaska Blackcod, Anoplopoma fimbria, Pallas 1811) is a commercially important species which inhabits the continental shelf of the North Pacific Ocean and is the subject of an increasing fishing effort off the west coast of Vancouver Island and the Queen Charlotte Islands (McFarlane & Beamish, 1983). Sablefish are known to spawn at depths greater than 300 m with spawning activity peaking in February (Mason et al., 1983). The species has been identified as having great potential for commercial mariculture because of its hardiness, ease of adaptation to captivity, ability to feed on a variety of marine protein sources and fast growth rate.

Gonadal hydration and ovulation were induced in captive adult sablefish during their normal breeding season by intraperitoneal injections of 0.1 mg/kg gonadotropin releasing hormone analogue (D-ala^{6},des gly^{10}-LHRH-ethylamide).

The fish (4.94 + 0.6 kg average weight of females) were caught in the wild in October 1986 and held in covered outdoor tanks at the Pacific Biological Station, Nanaimo, B.C. for three months prior to the experiment conducted during the period Jan. 22 to Mar. 5, 1987. The sexes were identified by measurement of plasma levels of estradiol-17β, later confirmed by gonadal catheterization and collection of gametes. Female fish only were injected, males spermiated independently.

A single injection of the analogue alone or combined with 5 mg/kg domperidone (a dopamine receptor antagonist) induced gonadal hydration and ovulation within 4 to 20 days in all the treated fish. Control groups injected with saline or domperidone alone (5 mg/kg) did not ovulate within the same period.

Ovarian hydration was monitored through periodic collection of eggs by ovarian catheterization and observation of the changes in total weight and condition factor of the fish. Blood samples were also collected to study the changes in plasma concentration of estrogen during final maturation.

A second experiment using single hormone analogues (GnRHa): 0.47-0.54 mg/kg D-trp^{6}-LHRH, 0.1 mg/kg D-ala^{6}-LHRH ethylamide and 0.1 mg/kg D-arg^{6}-salmon GnRH ethylamide resulted in a combined total of 7 of 10 injected fish ovulating within 5 to 19 days.

Fertilization rates were variable ranging from 0.0 to 76.4% (D. Alderdice et al., personal communication, 1987). Incubation of the fertilized eggs lasting 12 days at 5.2 + 0.2 C resulted in successful hatching in 4 of 12 test fertilizations. Larval rearing and feeding is underway (G.A. McFarlane et al., personal communication, 1987).

This is to our knowledge the first report of successful induced ovulation, fertilization and larval production in sablefish. We have shown that the application of several high potency LHRH and GnRH analogues are effective in inducing ovulation in the sablefish, that the use of a dopamine inhibitor does not seem to potentiate the ovulation inducing effect of GnRHa at the dosage tested, and that weight changes and the increase in the diameter of cannulated eggs are a reliable indication of ovulatory response.

Further work is required on injection timing and dosages, on the nutritional, hormonal and environmental manipulation of long-term captive broodstock whose gonads do not develop to a stage where they may be induced to ovulate, and on incubation parameters and techniques. The development of these techniques, resulting in reliable sources of sablefish seed, are critical for the successful development of a sablefish mariculture industry in B.C.

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INFLUENCE OF ESTROGEN, AROMATIZABLE AND NON-AROMATIZABLE ANDROGEN DURING ONTOGENESIS ON SEX DIFFERENTIATION IN COHO SALMON (ONCORHYNCHUS KISUTCH)

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Summary

The sex steroids are capable of influencing sex differentiation in the majority of gonochoristic teleost species. Therefore, they are a valuable tool for both exploration of the process of sex differentation and the manipulation of gonadal sex for purposes of fish culture. If the steroids are the natural sex inducers or play a decisive role in sex differentiation and the moment during ontogenesis when they act occurs prior to the first observable histological sex differentiation, as postulated by Yamamoto, then a peak of sensitivity to exogenous sex steroids in terms of final phenotypic sex should be found.

To test this hypothesis estradiol-17β and 17α-methyltestosterone (MT) were administered, by immersion for 2 hr at a concentration of 400 µg/l, to separate groups of developing coho salmon at weekly intervals from the late eyed egg stage until the 3rd week of feeding. Histological samples of the gonadal region were prepared from a control group at each treatment interval using a plastic embedding process. Treated groups were grown for 18 weeks at 10°C and then examined histologically to determine phenotypic sex.

It was found that a single exogenous estrogen treatment significantly increased the proportion of phenotypic females when administered between 8 days pre-hatch and 13 days post-hatch, while single androgen treatments significantly increased the proportion of phenotypic males when administered between 6 and 13 days post-hatch. The maximum response occurred one week earlier for estrogen (84% females) than for androgen (73% males) thus creating two well-differentiated peaks of steroid sensitivity. The natural estrogen 17β-estradiol was more effective at the dosage tested than the potent androgen MT. Histological analysis indicated that steroid sensitivity occurred prior to the time when gonadal sex could be distinguished.

A second study was conducted to investigate the phenomenon of paradoxical feminization during androgen treatment. Aromatization from C-19 to C-18 steroids has been proposed as an explanation for this phenomenon which is encountered when administering androgens at high concentrations and/or during a long period of time.

Groups of developing coho salmon were treated either wih the aromatizable androgen MT or with the non-aromatizable androgen 17α-methyldihydrotestosterone (MDT) one week after hatching at concentrations ranging from 6.25 to 6,400 µg/l for 2 hr in an immersion bath.

Histological examination after 18 weeks at 10°C showed that the proportion of phenotypic males increased with the dose of either androgen, and that at the highest concentration the proportion of females increased relative to that of the males for the aromatizable androgen (MT) but not for the non-aromatizable androgen (MDT).

The results of both studies are presented as a three dimensional model illustrating the influence of sex steroids on sex differentiation.

GONADOTROPIN-RELEASING HORMONE CONTROL OF OOCYTE MATURATION, OVULATION, AND SPAWNING IN THE GILTHEAD SEABREAM, SPARUS AURATA

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Abstract

The gilthead seabream, Sparus aurata, is one of the major candidates for marine fish farming in Europe. Ovarian development in this species is asynchronous, resulting in daily cycles of maturation, ovulation, and spawning over a 2-3 month period. However, in most cases, females do not spawn spontaneously in captivity. In such females, gonadotropin (GtH) content in the pituitary increases towards the breeding season (winter) but the hormone is not released into the blood stream. The pituitary is responsive to GnRH year round, with maximal GnRH induced GtH release in March-April. The mammalian GnRH analog [D-Ala6, Pro9-NET] - LHRH (mGnRHa) is 30 times more potent than native mammalian or salmon GnRH in inducing in vivo GtH release in the female Sparus. A unique injection of the mGnRHa (at 7.5µg/kg BW) administered to females with oocytes at final stages of vitellogenesis induces a rapid but short-lived (48 hours) increase in plasma GtH levels followed by oocyte maturation, ovulation, and spawning. Administration of the hormone in sustained release delivery systems significantly improved the spawning response. In spite of continuously elevated GtH levels, treated females spawned at precise 24 hour intervals. mGnRHa treatment resulted in a rapid increase in blood $E_2$17β levels that paralleled the pattern of the GtH elevation. The $E_2$17β is probably required for the continuation of vitellogenesis in the younger oocytes. However, the mGnRHa treatment resulted in only a very slight rise in the plasma levels of 17α-hydroxy, 20β -dihydroprogesterone (17α ,20β -OHP). This, in addition to very low and constant levels of 17α ,20β -OHP in females undergoing daily cycles of oocyte maturation and ovulation, raises the question as to the nature of the maturation inducing steroid in sparus aurata.

GONADOTROPIN BIODYNAMICS FOLLOWING GnRH ADMINISTRATION IN THE GILTHEAD SEABREAM SPARUS AURATA: A COMBINED RADIOIMMUNOASSAY AND IMMUNOCYTOCHEMICAL STUDY

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Abstract

Radioimmunoassay (RIA) and immunocytochemistry (ICC) were used to study the effect of gonadotropin-releasing hormone (GnRH) on pituitary gonadotrope activity in the Gilthead Seabream, Sparus aurata.

Two to three year old females received a single intramuscular injection (7.5 ug/kg body weight) of the mammalian GnRH analog, [D-Ala6, Pro 9- NET]-LHRH (GnRHa). Controls received an injection of saline. At time zero, 0.5, 1.5, and 24 hrs after injection, fish were bled for RIA and decapitated, pituitaries removed and fixed in Bouin's solution for ICC and histological analysis. Blood GTH levels were determined by an homologous Sparus aurata RIA (Zohar and Breton, unpublished). Pituitaries, embedded in paraffin and sectioned at 5 micra, were stained with Masson's trichrome, periodic acid Schiff (PAS) or aldehyde fuchsin (AF). The indirect immunoperoxidase (PAP) method was used to localize gonadotropin (GTH) with antiserum to the beta subunit of carp GTH (anti-cGTHb; E. Burzawa-Gerard) at a dilution of 1:1800. Standard ICC control procedures were used. Comparisons of ir-intensity were made on material processed at the same time.

In S. aurata, gonadotropes form the outer boundary of the caudal pars distalis (CPD). These cells stain with aniline blue, PAS, and AF and they contain immmunoreactive (ir) -GTH. Blood GTH levels of saline treated fish remained low and constant over the experimental period. Thirty minutes after the GnRHa treatment, circulating GTH increased sharply from 2.4 ± 0.22 ng/ml (0 time) to 160±41 (mean ± S.E). The pituitary gonadotropes at this time showed a slight decrease in ir-GTH and their nuclei appeared somewhat enlarged compared to control animals. At 1.5 hrs following the GnRHa treatment GTH levels increased from 2.22 ± 0.16 to 200 ± 33 ng/ml. Gonadotropes were noticeably enlarged and immunoresponsiveness to anti-cGTHb was most intense. At 24 hrs blood GTH levels decreased to 23.33 ± 4.6 ng/ml. The intensity of ir-GTH staining and cell and nuclear size were reduced.

Our RIA, ICC, and cytological observations are well correlated and they suggest that intense release of GTH is already taking place at 0.5 hrs following the administration of GnRHa. At 1.5 hrs the GTH release is accompanied by active synthesis in the gonadotropes. Both synthesis and release are diminished at 24 hrs after GnRHa treatment.

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CIRCANNUAL CYCLES OF SERUM THYROID HORMONES AND OVARIAN SEX STEROIDS IN RAINBOW TROUT

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Summary

Circannual cycles of thyroxine (T_4) and triiodothyronine (T_3) were measured and related to cycles of 17β-estradiol (E_2) and testosterone (T) in female rainbow trout whose spawning time was altered by varying the photoperiod. In all cases thyroid hormone levels were inversely correlated with circulating levels of E_2. These results suggest a strong interrelationship between annual variations of thyroid hormones and sex steroids and a role for thyroid hormones in ovarian development.
Keywords: Estradiol, thyroid, T_4, T_3, ovarian cycles, photoperiod, rainbow trout.

Introduction

Previous studies have reported the occurrence of circannual cycles of thyroid hormones (T_4-thyroxine; T_3-triiodothyronine) in rainbow trout (Osborn et al., 1978; Leatherland & Sonstegard, 1980;1981). While annual variations in circulating levels of thyroid hormones appear to be negatively correlated with estradiol (E_2), a number of uncontrolled environmental variables may have complicated the interpretation of these observations.

The objective of this study was to measure circulating levels of serum thyroid hormones in groups of female rainbow trout held under constant conditions and to correlate these with circulating levels of sex steroids.

Results and discussion

Female rainbow trout maintained under controlled laboratory conditions displayed marked annual variations of thyroid hormones. Circulating levels of T_3, which in most cases exceeded levels of T_4, were highest during previtellogenesis and progressively decreased during exogenous vitellogenesis as serum levels of E_2 increased. While the maintenance of groups of female trout under different photoperiod regimes successfully altered the spawning time of the trout, the inverse relationship between circulating levels of thyroid hormones and E_2 was not altered.

These results strongly suggest that an interrelationship exists between thyroid and ovarian function. Elevated levels of thyroid hormones during previtellogenesis may indicate a role for thyroid hormones during early ovarian development. Recently, Cyr & Eales (1986) have reported that thyroid hormones, particularly T_3, act upon the ovarian follicle of rainbow trout *in vitro* to amplify gonadotrpin action with regards to E_2 secretion. Decreasing levels of thyroid hormones during vitellogenesis may be the result of E_2 action upon thyroid hormone metabolism. The administration of E_2 to rainbow trout has been shown to decrease circulating levels of T_3 (Leatherland, 1985; MacLatchy et al., 1986). This effect appears to be, at least in part, the result of a decrease in hepatic 5'-monodeiodinase (MacLatchy et al., 1986).

While the precise interaction between the thyroid and the ovary remains to be clarified, it is conceivable that these two endocrine systems interact with one another to regulate the partitioning of energy between somatic growth and sexual development.

Acknowledgement

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THE DISASSOCIATION OF MATURATION FROM WATER UPTAKE IN *FUNDULUS HETEROCLITUS* OOCYTES

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The effects of a non-aqueous medium on the maturation of follicle-enclosed oocytes of the mummichog *Fundulus heteroclitus* *in vitro* were examined. Hydration of oocytes can be successfully uncoupled from meiotic maturation in oocytes stimulated with 17α hydroxy-20β-dihydroprogesterone by incubating follicles in paraffin oil. It was found that germinal vesicle breakdown (GVBD) occurs just as readily *in vitro* in paraffin oil as in 75% L-15 incubation media. Furthermore, follicles stimulated by steroid that matured in paraffin oil appear similar to those maturing in an aqueous medium, only smaller. Therefore, it seems apparent that incubation of follicles in a nonaqueous environment does not repress normal oocyte maturation. When stimulated follicles incubated first in paraffin oil were transferred to an aqueous medium, they swelled immediately. Oocytes treated in this manner were also observed to swell to the same extent as controls, regardless of time of transfer. Polyacrylamide gel electrophoresis indicated that proteolysis of yolk proteins of stimulated follicles incubated *in vitro* in aqueous medium occurred prior to GVBD. In contrast, proteolysis of yolk proteins was not observed until some time after GVBD in stimulated follicles incubated in paraffin oil. Therefore, proteolysis is delayed but not inhibited by the absence of hydration in *F. heteroclitus* oocytes.

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TESTOSTERONE AND ESTRADIOL-17β PROFILES OF FEMALE MILKFISH, *CHANOS CHANOS*, UNDERGOING CHRONIC LHRH-A AND 17α-METHYLTESTOSTERONE THERAPY

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Abstract

A chronic LHRH-a and 17α-methyltestosterone therapy was used to enhance the number of mature female milkfish. Our two specific goals were: to characterize the maturation process of the milkfish's ovary at both a macroscopic level (i.e., egg diameters) and at the physiological level (i.e., serum testosterone and estradiol-17β titers); and, to gain insight into the mechanism by which the chronic hormonal therapy brings about a larger percentage of mature females compared to untreated females. These goals were achieved by coupling the *in vivo* monitoring of the ovary with the determination of serum levels of testosterone and estradiol-17β using RIA procedures.

The milkfish's ovary can be characterized as the group synchronous type in which a female may mature anywhere from one to five times during the breeding season. The rate of growth of the oocytes from treated females appears to be linear between 250-750 um and ranges between 21.4-27.4 um/day.

The serum testosterone levels slowly rise (1-15 ng/ml) as the ova increase from 300 to 500 um in size. A dramatic rise is then observed as the eggs of a clutch grow to 800 um and complete vitellogenesis. This rise is concurrent with a decrease in serum estradiol levels. The maximum serum testosterone value observed was 74 ng/ml.

Estradiol levels increase as the eggs grow from between 300 to 600 um (maximum value observed is 19 ng/ml). After ova have attained an egg diameter of 600 um, estradiol-17β levels drop dramatically and return to initial levels, although egg growth continues. The egg diameters provide a good index of the timing of these changes.

There are no significant differences between the steroid levels of treated and untreated females. This observation is discussed in relation to the mechanism by which the chronic hormonal therapy brings about a larger percentage of mature females.

THE COST AND PHYSIOLOGICAL EFFECTIVENESS OF CPH, HCG AND LHRH-A IN THE INDUCED SPAWNING OF GREY MULLET *MUGIL CEPHALUS*

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Abstract

Two hormone injections, a primer and a resolver, are required to induce spawning in grey mullet. Combinations of CPH, HCG and LHRH-a, each used as a primer or resolver, were tested in 60 spawning attempts. Both the response, in terms of fecundity, fertilization rate and hatching rate, and the cost effectiveness of each combination were evaluated. The cost effectiveness was also examined in a hypothetical "perfect" spawn, where the complicating factors affecting the fertilization and hatching rate (i.e., initial egg state, male state, etc.) were eliminated.

In the hypothetical spawn, the least expensive treatment was either CPH/LHRH-a or LHRH-a/CPH, followed in order of increasing cost by LHRH-a/LHRH-a, CPH/CPH and LHRH-a/HCG.

In actual tests, CPH/LHRH-a was the most cost-effective, as well as the most physiologically-effective treatment. The implications of combining gonadotropin preparations with gonadotropin-releasing preparations will be discussed.

ACCELERATION OF MATURATION AND DOUBLE SPAWNING IN ONE SEASON IN THE GREY MULLET (*MUGIL CEPHALUS*) FOLLOWING CHRONIC HORMONE TREATMENT

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Abstract

The maturation rate of female grey mullet (*Mugil cephalus*) was compared among two hormone treatment groups and one control group. Hormone treatment was administered when the mean oocyte diameter was between 200-300 um (i.e., early vitellogenesis). One treatment group received an LHRH-a pellet plus a 17 α-methyltestosterone (17-MT) capsule. The other treatment group received an LHRH-a pellet plus a testosterone (T) capsule. The fish in both treatment groups and the control group were cannulated four times between November 18, 1986, when treatments were given, and January 13, 1987. The mean oocyte diameter was obtained for each fish following each check.

There was no significant difference between the control group and the LHRH-a/17-MT treated group one month after treatment. The mean oocyte diameters for each group were 288 and 246.1 um, respectively. There was, however, a significant difference in the LHRH-a/T treated group whose mean oocyte diameter was 538.1 um. The majority of this group were spawned by mid-January and three fish subsequently rematured and were spawned a second time in February.

The mullet is considered an annual spawner and there are no previous reports of this species spawning multiple times in one season. The potential differences between the action of 17-MT and T will be discussed.

ACTIVITY PATTERNS OF SEVERAL ENZYMES IN THE OOCYTES OF THE GREY MULLET, *MUGIL CEPHALUS*, DURING INDUCED SPAWNING.

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Abstract

In this preliminary study, the activity of five enzymes in the oocytes of female grey mullet was examined during induced spawning. From each female, a sample of 200 oocytes (follicles attached) was obtained every 4-8 hours. The first sample was taken just prior to the first hormone injection and sampling continued through the completion of hydration and GVBD. Each set of samples was homogenized, electrophoresed on vertical starch gels and stained. The gels were examined qualitatively for relative sample to sample changes in band density.

Three of the enzymes, creatine kinase (CK), malate dehydrogenase (MDH) and leu-gly-gly peptidase showed synchronous patterns of activity which varied between first injection and GVBD. The other two enzymes, leu-tyr peptidase and esterase (EST) showed less variability. With the exception of esterase, hydrated oocytes showed little or no activity in the enzymes tested.

Correlation of activity patterns to hormone injections, potential problems associated with this type of analysis and the advantages of using mullet for studies on the reproductive physiology of fishes will be discussed.

THE PURIFICATION AND RADIOIMMUNOASSAY OF GONADOTROPIN IN THE ATLANTIC CROAKER (*MICROPOGONIAS UNDULATUS*), A MARINE TELEOST.

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Introduction

The measurement of plasma gonadotropin levels in fish has, with few exceptions, been limited to salmonid and cyprinid species. We report here the purification of a maturational gonadotropin and the development of a homologous radioimmunoassay (RIA) for its measurement in the Atlantic croaker, a marine teleost, (family Sciaenidae, order Perciformes) found on the Atlantic and Gulf coasts of the U.S.A.

Methods

Pituitary glands were collected from fish caught during their annual offshore spawning migration in October and November by sports fishermen. Four thousand five hundred pituitaries were collected (wet weight 8.97g). Ethanol fractionation yielded 259 mg of glycoprotein-enriched material and this was further purified by gel-filtration on Sephacryl S200, chromatofocusing, filtration on Sephadex G75, affinity chromatography on CON-A Sepharose and refiltration on G75. Throughout the purification, protein was monitored by O.D. 280 and gonadotropic activity was assessed by an *in vitro* germinal vesicle breakdown (GVBD) bioassay. The maturational GTH activity eluted from the chromatofocusing column at low pH (3-4) and eluted from CON-A Sepharose in the bound (CON-A 2) fraction. Antiserum was raised against the biologically active fractions from the final G-75 column (.072 mg), and the same material was used as standard and for preparation of assay tracer. The RIA protocol is similar to those previously published, involving delayed addition of tracer and separation of bound from free counts with 2nd antibody and polyethylene glycol.

Results and Discussion

The assay was able to measure GtH in the range 25 ng/ml-50 pg/ml with an interassay variance of 11%. The assay was highly specific in that a variety of mammalian glycoprotein hormones (LH, FSH, TSH, HCG) showed no cross-reactivity. Pituitaries from two other species of the family Sciaenidae; the redfish (*Sciaenops ocellatus*) and the spotted seatrout (*Cynoscion nebulosus*) showed a partial cross-reaction in the RIA (i.e. they diluted non-parallel to the croaker GTH standards). It is assumed, therefore, that heterologous RIAs for the measurement of gonadotropin in these species can easily be developed using the available reagents. Biological validation of the assay consisted of: 1) The demonstration of a rise in plasma levels of immunoreactive GTH following administration of LHRHa *in vivo*, 2) A dose-response to LHRHa by pituitaries in short-term culture *in vitro* and 3) A rise in immunoreactive plasma GTH following castration of male croakers. Immunoreactive GTH has been found only in the pituitary and blood and not in any other tissue.

EFFECTS OF CAPTURE AND HANDLING ON CIRCULATING LEVELS OF GONADAL STEROIDS AND CORTISOL IN THE SPOTTED SEATROUT, CYNOSCION NEBULOSUS

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A wide variety of adverse environmental stimuli have been shown to impair reproductive endocrine function in mammals. Stressors can exert direct effects on reproductive physiological function by inhibiting gonadotropin secretion. Stressors can also indirectly influence reproduction by causing hypersecretion of hormones which interfere with reproductive processes, such as corticosteroids and catecholamines. In contrast, there is a lack of information on the effects of stressors on the reproductive physiology of teleosts. An understanding of the effects of stressors on teleost reproductive endocrine function would be valuable, not only in the design of reproductive physiology experiments, but also in the development of practical methods for breeding fish in captivity.

In the present study the effects of angling stress and confinement on plasma cortisol, testosterone and 11-ketotestosterone titers in spermiating male spotted seatrout (Cynoscion nebulosus) were examined. The fish were caught by hook and line and either bled immediately, or confined to a 41 x 56 cm flow-through tank for 5, 15, 30, or 60 minutes before blood samples were taken. Plasma steroid levels were measured by radioimmunoassay.

Mean plasma cortisol concentrations significantly increased between 15 and 30 minutes of confinement. However, there were marked individual differences in the magnitude of the corticosteroid stress responses; some fish showed only minor increases in plasma cortisol (20-40 ng/ml). Testosterone levels did not change significantly during the time-course of the experiment. 11-ketotestosterone levels decreased significantly between 30 and 60 minutes of confinement from a mean of 0.8 ng/ml to 0.4 ng/ml. These results indicate that circulating levels of sex steroids are not altered during the first 30 minutes of exposure to moderate stressors. The effects of different handling techniques and chronic stressors on circulating levels of steroid hormones will also be discussed.

SEASONAL VARIATION OF ESTROGEN-RECEPTOR CONCENTRATIONS IN THE LIVER OF SPOTTED SEATROUT CYNOSCION NEBULOSUS

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Introduction

Hepatic estrogen receptors have been demonstrated in several teleost species. However, there have been no descriptions of the changes in intracellular distribution and concentration of receptors during the annual reproductive cycle. Furthermore, no seasonal studies of vitellogenesis have yet defined the relationship between the synthesis of vitellogenin and the concentration of estrogen receptors in the liver. The purpose of this project was to characterize the hepatic estrogen-receptor of spotted seatrout and to investigate receptor function with regard to the control of vitellogenesis.

Methods

Fish were captured by gill net between July 1984 and October 1985. Livers were frozen on dry ice and stored at -80°C until assayed. Cytosol fractions (100K x G) were prepared using 50 mM Tris, 1.5 mM EDTA, 1.0 mM DTT and 30% v/v glycerol, pH 7.4 at 4°C (TEDG). After washing the nuclear pellet, the nuclear receptors were solubilized in TEDG with 0.6 m KCl (Mak and Callard, 1985). Saturation analysis was performed on these extracts using 0.2 to 20 nM tritiated estradiol. Nonspecific binding was assayed with parallel incubations of a 100 fold molar excess of diethylstilbestrol. Affinity constants were determined by the method of Scatchard (1949). Single point assays were used to estimate receptor concentrations for the seasonal study.

Results

A single class of high affinity binding sites (K_d = 1.17 ± 0.32 nM) was found in the cytosolic fraction. Binding was very temperature sensitive. At 4°C, full exchange was accomplished after 12 hours with a half-time of dissociation of 60 minutes. Binding was highly specific for estrogens: estrone and estriol were thirty times less competitive than either estradiol or diethylstilbestrol. Androgens (dihydrotestosterone, testosterone, 11-ketotestosterone, 11β-hydroxytestosterone and androstenedione), corticosteroids (cortisol, corticosterone, cortisone, 21-deoxycortisol, 11-deoxycorticosterone and 11-deoxycortisol) and pregnene derivatives (progesterone, pregnenelone, 20β-dihydroprogesterone, 17α-hydroxyprogesterone, 20β-dihydroprogesterone and 17α-hydroxy,20β-dihydroprogesterone) did not displace estradiol from the receptor site.

In January, females (all with regressed ovaries) had low levels of cytosolic and nuclear receptors, <0.4 and <0.03 pmole per gram liver, respectively. Vitellogenesis began in mid-February as water temperatures rose (>20°C). By mid-May, when the majority of the females had mature ovaries, concentrations of receptors increased to 5.25 and 1.10 pmole per gram liver (cytosolic and nuclear, respectively.). Reproductive activity declined by September and cytosolic and nuclear receptor levels also decreased to 1.78 and 0.71 pmoles per gram liver, respectively. In November, after the reproductive season, receptor levels fell to levels comparable to those in regressed fish although 25% of the total binding remained within the nuclear fraction.

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PLASMA LEVELS OF GONADAL STEROIDS DURING THE REPRODUCTIVE CYCLE OF FEMALE SPOTTED SEATROUT (*CYNOSCION NEBULOSUS*)

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Most investigations of the seasonal changes in plasma levels of gonadal steroids have been conducted on teleost species which have synchronous oocyte development and spawn once a year. The purpose of the present study was to determine the natural cycle of gonadal steroids in a multiple spawning marine species, *Cynoscion nebulosus*, in which oocyte development is asynchronous throughout the reproductive season (April-October).

Spotted seatrout were captured with a trammel net at their spawning sites on shallow grass flats in a Texas bay. Blood was obtained by cardiac puncture within minutes of capture, centrifuged, and the resulting plasma was stored at -80°C until analyzed for steroid hormones by specific RIA procedures.

In January the ovaries were completely regressed and ovarian recrudescence began at the end of February. Plasma estradiol concentrations increased during the period of oocyte growth in March and by April had reached a peak of 0.8 ng/ml in fish with fully grown oocytes. Thereafter, estradiol concentrations declined to approximately half April values and remained at this level until the end of the reproductive season in September. Histological examination of the ovaries indicated a continuous recruitment of vitellogenic oocytes throughout the spawning season. The results suggest that once exogenous vitellogenesis is underway, vitellogenin production can be maintained with lower plasma levels of estradiol. Large numbers of vitellogenic oocytes were still present in the ovaries of fish that had ovulated, which may explain why plasma estradiol levels were not conspicuously lower in these individuals. Testosterone, which is the immediate precursor of estradiol in spotted seatrout, had a seasonal cycle of plasma fluctuations similar to that of estradiol. There were also diurnal changes in the plasma concentrations of estradiol and testosterone, with minimum levels occurring at dusk and maximum levels at dawn.

Final maturation of fully grown oocytes is highly synchronized in this species. Lipid coalescence and germinal vesicle migration did not begin until around dawn on the day of spawning. By early afternoon later stages of oocyte maturation, germinal vesicle breakdown and hydration were observed. Ovulation began late in the afternoon and by dusk the eggs were free flowing. Interestingly, plasma estradiol and testosterone levels decreased around the time of ovulation. Plasma levels of 17α,20β-dihydroxy-4-pregnen-3-one and 11-deoxycorticosterone, steroids which have been implicated in the control of final oocyte maturation in other teleost species, were low and did not change significantly during final oocyte maturation. Another steroid, 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) was synthesized during final oocyte maturation by spotted seatrout oocytes *in vitro*. Currently a radioimmunoassay is being developed to measure plasma levels of 20β-S in spotted seatrout during final oocyte maturation and ovulation.

THE PRODUCTION OF A NOVEL MATURATION INDUCING STEROID *IN VITRO* IN THE ATLANTIC CROAKER (*MICROPOGONIAS UNDULATUS*)

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This study describes the identification of 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-dihydro-11-deoxycortisol, 20β-S) as a major steroid product of the ovary of Atlantic croaker (*Micropogonias undulatus*) incubated *in vitro*. The potency of 20β-S to induce final oocyte maturation (FOM) of Atlantic croaker oocytes *in vitro* is unsurpassed by any other steroid tested to date.

Experiment 1

The purpose of this experiment was to isolate and identify the most potent steroid inducers of final oocyte maturation (FOM). Ovarian tissue (20 grams) in the process of FOM was removed from Atlantic croaker and was incubated in tissue culture media supplemented with hCG and pregnenolone for 8 hours. Steroids were extracted from the media and fractionated by HPLC and TLC. Fractions were bioassayed for potency to induce FOM *in vitro*.

Several steroids produced by oocytes undergoing FOM were isolated. However, only one steroid (20β-S) was both produced in large quantities and was a very potent inducer of FOM. The ovarian steroid was identified as 20β-S by a variety of methods, including HPLC, TLC, GC-MS, UV absorbance, and reactions with specific enzymes. Another steroid with equal potency to induce FOM was produced in small amounts and was tentatively identified as 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P4). However, over 10 times more 20β-S than 17α,20β-P4 accumulated in the incubation media. Other studies suggest that under more physiological conditions, the accumulation of 17α, 20β-P4 may be an artifact of the high concentration of the steroid precursor. A small amount of testosterone (about 1/20 of the amount of 20β-S) and no estradiol-17β accumulated in the incubation media.

Experiment 2

The time-course of the change in steroidogenesis associated with the process of FOM was investigated during both *in vitro* and *in vivo* hCG induction. *In vivo* induction of FOM was accomplished by a single injection of human chorionic gonadotropin (hCG) capable of inducing Atlantic croaker to spawn. The change in the production of 20β-S, 17α, 20β-P4, 11-deoxycorticosterone (DOC), testosterone and estradiol-17β was examined *in vitro* by incubating tritiated pregnenolone with ovarian tissue removed from females before, during, and after spawning.

A parallel study investigated the products of steroidogenesis of full-grown, immature oocytes induced *in vitro* with hCG to undergo FOM. The change in the synthesis of testosterone, estradiol-17β, and 20β-S at 1, 8, 22, 32, and 48 h of culture with or without hCG was detected by incubating ovarian tissue with tritiated pregnenolone.

HCG induced similar changes in steroidogenesis using either the *in vitro* or the *in vivo* method. The percentage of total radioactivity in the form of testosterone was inversely related to the percentage of radioactive 20β-S. Only a small amount of radioactive estradiol-17β was isolated.

The production of maturation inducing steroids previously proposed for other species of teleosts could not be demonstrated in this study. However, enhanced production of 20β-S was associated with the process of FOM in both *in vivo* and *in vitro* experiments.

Summary

The timely synthesis of 20β-S suggests a physiological role in the process of FOM or spawning in the Atlantic croaker. At the time of FOM, 20β-S is the major steroid produced. Synthesis of other proposed maturation inducing steroids (17α,20β-P4 and DOC) under physiological conditions was not detected. The high potency of 20β-S to induce FOM *in vitro* is further evidence that 20β-S as the maturation inducing steroid in the Atlantic croaker.

LOCALIZATION OF CELL TYPES IN THE ADENOHYPOPHYSES OF THREE SCIAENID FISHES: ATLANTIC CROAKER, SPOTTED SEATROUT AND RED DRUM.

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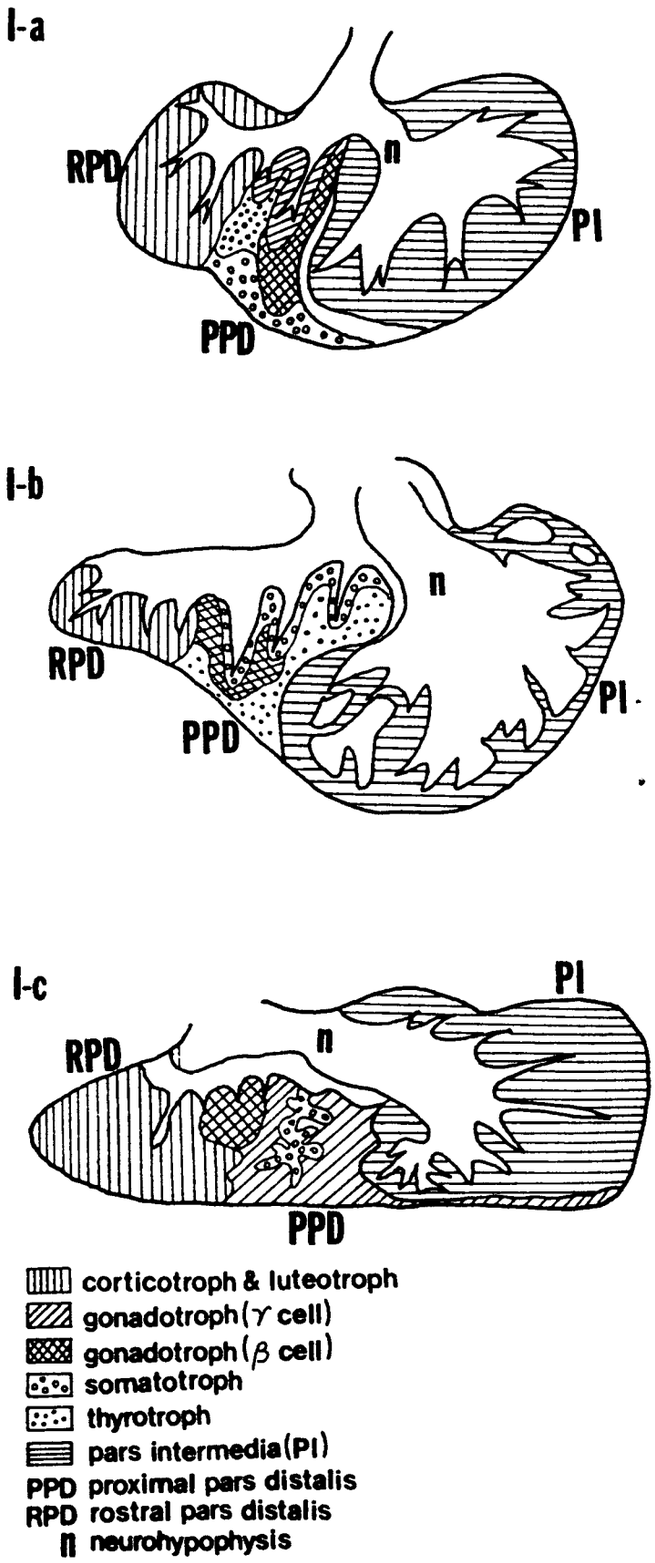
Several aspects of the reproductive and stress physiology of three sciaenid fishes, Atlantic croaker (Micropogonias undulatus), spotted seatrout (Cynoscion nebulosus) and red drum (Sciaenops ocellatus) have been investigated, including the physiology of gonadotropin secretion. However, there is a lack of information on the functional morphology of the pituitary glands in these species and in particular, the identification of cell types in the adenohypophysis. The purpose of this study, therefore, was to determine the locations of the various cell types of adenohypophysis in these three species.

Adult Atlantic croaker, spotted seatrout and red drum were caught in the vicinity of Port Aransas, Texas in January. The fish were decapitated and the pituitary glands were rapidly dissected out and fixed in Bouin's solution. Pituitaries were embedded in paraffin wax. Serial sections of 6 um thickness were stained with Herlant's alcian blue-periodic acid Schiff-orange G (Herlant, 1960), or chromium-hematoxylin-phloxin, or Cleveland and Wolfe's (1932) polychrome staining procedure.

The locations of the various adenohypophyseal cell types in the three species are shown schematically in Figure 1. Corticotroph (ACTH cell) and luteotroph (prolactin cell) were found in rostal pars distalis. Gonadotrophs (alpha and beta cells), somatotroph were found in proximal pars distalis. Two types of gonadotrophs (alpha and beta cells) were identified in croaker (Fig. 1-a) and red drum (Fig. 1-c). In spotted seatrout, however, only one kind of gonadotroph (beta cell) could be located (Fig. 1-b).

Since the categorization of cell types of the adenohypophysis based on histochemical characteristics is not conclusive, immunohistochemical techniques are currently being used to confirm the localization of the pituitary hormones in the adenohypophyseal cells.

Fig. 1. Schematic diagrams of midsagittal section through the pituitary glands of the: (a) Atlantic croaker (b) spotted seatrout and (c) Red drum. Anterior to the left.



Biolassays of Fish Gonadotropin and Presence of a New Type of Gonadotropin Which is Active in Heterologous Species but not in Homologous Species

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Multiplicity of gonadotropin is one of the most interesting problems in fish endocrinology. Although Idler's group separated vitellogenic and maturational gondotropins from several kinds of fish, the problem is still obscure. For example, Ng and Idler (]979) reported that maturational gonadotropin of the flounder had an activity to stimulate vitellogenin uptake by the ovary. Recently, Burzawa Gerard et al. (]985) showed that a nonglycoprotein fraction of the carp pituitary as well as its glycoprotein gonadotropin stimulated vitellogenin uptake by the ovary. Kawauchi et al. (]986) separated two types of chemically distinct gonadotropins from salmon pituitaries. In addition to these, sex specific gonadotropins (Breton et al.,]978) and small and large molecular weight gonadotropins (Ng and Idler,]978) have been reported. Furthermore, hormones with different isoelectric points due to "microheterogeneity" also exists in fish gonadotropin as well as in higher vertebrate ptuitary glycoprotein hormones. Thus, the multiplicity of fish gonadotropin is a complicated problem. The authors attempted to find a key to solve this difficult problem by surveying more than 20 published papers on gonadotropin purification in fish with special emphasis on the assay method. The survey revealed that the specific and quantitative assay method are necessary in this field. The authors employed radioreceptor assay methods for monitoring gonadotropin acitivity in the purification of tuna gonadotropin and obtained unexpected results which will be mentioned later.

As the assay method of gonadotropin, bioassays have been used in most of the 22 papers examined. Among them, homologous and heterologous assays were emplolyed in]] and]3 studies, respectively. Radioimmunoassays were utilized in four of the studies examined and they were all heterologous assays. Radioreceptor assays were employed in three of them and only one of these studies used the homologous assay. The uptake of radiophosphorus by the gondad and steroid release from the gonad were most frequently used as the endpoint of the assays. The oocyte maturation or increase in the gonosomatic index were the second frquently used methods. The uptake of tritiated leucine by the gonad, accumulation of cAMP in the gonad, induction of the ovulation or spermiation were also employed in some studies. The elongation of the ovipositer was used in few studies. However, none of them was as specific as the ovarian ascorbic depletion depletion method and Steelma-Pahley's rat ovarian augmentaion assay for mammalian LH and FSH, respectively. They were also not quantitative enough to assess the rate of contamination of the other type gonadotropin.

One of the present authors and his colaborators have utilized successfully the radioreceptor assay method for purification of gonadotropins of chicken, bullfrog, toad, salmon, silver carp and tuna. These studies revealed the high sensitivity and precision of the method which enabled quantification of the hormone activity of the eluate collected in each fraction collector tube after chromatographies.

For the purification of gonadotropin from pituitary glands of the yellow fin tuna, Thunnus albacares, we employed two radioreceptor assays, one with radioactive silver carp gonadotropin and the testicular receptor prepartion of the goby, Glossogobius olivaceus, and the other with radioactive tuna gonadotropin and the testicular receptor prepartion of the yellow fin tuna. For chromatographies, concanavalin-A Sepharose, Mono Q and Superose]2 were employed: the latter two are for anion exchange and gelfiltration in the FPLC system from Pharmacia, respectively). As final products of the purification, we obtained eight fractions which were separated by the anion exchange and presumably with different electrostatic properties or isoelectric points. Judging from their behaviors in the SDS electrophoresis, they were considered to derive from two principles. These principles were found in the most acidic and least acidic frations without countercontaminations. The least acidic four fractions which were free from the most acidic principle had strong potencies to bind specifically to the yellow fin tuna testis, while they were completely unable to bind to the goby testis specifically. Unexpectedly, the most acidic fraction could not bind to the yellow fin tuna testis but had a strong potency to bind to the goby testis specifically. By using the accumulation of cAMP in the testis of the mackerel (a related species to the tuna) and goby as the index, we bioassayed these gonadotropins. Their biological activities were parallel to the receptor binding activities. In colaboration with Dr. H. Hayashi of Gunma University, we could show that these two types of gonadotropins, i.e. homologous and heterologous type gonadotropins, had different amino acid compositions. The heterologous type gonadotropin may be "vestigial hormone", which is still being used by the goby as the current hormone, or "neutral hormone" such as mesotocin or isotocin.

SEXUAL DIFFERENTIATION IN WHITEFISH (COREGONUS LAVARETUS L.)

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Summary

Development of the gonad was studied by light microscopy in whitefish larvae and juveniles. The first primordial germ cells (PGCs) were observed 47 days after hatching in 15 mm long individuals. Whereas females can be distinguished in fish 103 days old and weight of 0.6 g, cytological differentiation of males could not be observed within the period of the trial (166 days).
Keywords: histology, gonadogenesis, ontogenesis

Introduction

Our objective was to analyse the period of sex differentiation (SD) in whitefish (1) for comparison with other species and for the (2) determination of time and duration of administration of sex steroids which will results in the desired sex inversion. There are several morphological descriptions of gonadal SD in cold- and warm-water fishes (Table 1) but information concerning coregonids is apparently missing (Brusle & Brusle, 1983).

Materials & Methods

Two lots of fish were used in the present study. One group (I) was reared on live zooplankton at a water temperature increasing gradually from 7°C (in April) to 17°C (in June). The second lot was reared on dry, starter diet at constant temeprature of 16°C. Details of the procedure are described elsewhere (Dabrowski & Poczyczynski, 1987). Fish were fixed in Bouin's solution and histological studies were performed on 4-8 µm sections stained with iron hematoxyline of Heidenhein.

Results & Discusssion

For timing of the onset of gonadal development whitefish larvae of 40 days of age were used. However, the first appearance of PGCs was evident 47 days after hatching and at a size of 15-20 mm total length (lot 1). In lot 2 examined first time 46 days after hatching, when fish reached 95-151 mg body weight (1.9-2.5 cm), the indifferent gonad contained only 1-2 PGCs.

In individuals 103 days old (lot 1) at the size of 0.6 g weight (4 cm length), numerous divisions were noticed in the gonads as well as some oogonia in the meiotic prophase. This fact sets the timing of cytological differentiation of the female. The first folicular cells of oocytes were observed 108 days after hatching in fish of 4.8 cm length and 1.1 g body weight.

In several fish 166 days old only PGCs were observed in the gonads and these will probably become the males. In lot 2, fish 95 days old only females were found at the size of 3.2-4.5 g body weight.

The comparison with other species (Table 1) reveals that whitefish gonadogenesis begins far latter than in rainbow trout where normal sex differentiation takes place during the change from endogenous to exogenous feeding. On the other hand, in warm-water fish species, gonad cell differentiation occurs shortly after hatching and in all cases females can be distinguished cytologically much earlier than males.

Table 1. Sex differentiation in fish

Species	T°C	Timing of the onset *,** PGCs	Cytological sex	
Salmo gairdneri	11.5	15 *	50 *	(1)
Perca flavescens	21	5-10 mm	35 mm	(2)
Tinca tinca	18	24 **	151 **	(3)
Oryzias latipes	25	3 *	1 **	(4)
Menidia menidia	21	-	40 *	(5)
C.lavaretus	7	47 **	103 **	present

* days after fertilization, ** days after hatching, (1) van den Hurk & Slof, 1981; (2) Malison et al. 1986; (3) Dlugosz et al. 1983; (4) Kanamori et al. 1985; (5) Conover & Fleisher, 1986.

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CURRENT STATUS OF COD CULTURE

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Abstract

In 1983, a break through was achieved in the mass rearing of cod fry in a seawater pond. The cod produced had been spawned naturally in a closed system, startfed on natural zooplankton and, as fry, received artificial dry food. Along with the generally increasing price for cod on the Norwegian market, there has been an explosion in the interest for cod culture. In 1987 the estimated production is between 1-2 million cod fry shared by six companies, while more than 300 Norwegian fish farmers have applied for licenses for cod culture.

This year will give answers to many questions concerning the pond method of cod fry production. In Hyltropollen, the site of the break through, production has been stable each year since 1983. However, it remains to be seen whether other ecosystems at other locations will yield similar results. 1987 will be a critical year in the process of commercialization of cod as an aquaculture species.
Keywords: cod, mariculture, pond.

THE STEROIDOGENIC GONADOTROPIN OF TILAPIA: ISOLATION AND RADIOIMMUNOASSAY

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The present paper reports on the isolation of the steroidogenic gonadotropin of tilapia (taGTH) and the radioimmunoassay (RIA) established for its quantitative determination.

Pituitaries were collected from fish, mainly male hybrids of Tilapia nilotica X T. aurea. The glycoprotein fraction was isolated from the aqueous pituitary extract by affinity chromatography on concanavalin A Sepharose. The adsorbed fraction (aC) was further fractionated by anion exchange chromatography on DEAE Trisacryl M at pH 7.5 and subsequently by gel filtration on Ultrogel AcA 54. After each step, the GTH-containing fractions were identified employing a bioassay based on the stimulation in vitro of estradiol secretion from the homologous ovary (Bogomolnaya and Yaron, GCE 53,187-196, 1984; Yaron et al., Current Trends in Comparative Endocrinology, Hong Kong Univ. Press, 225-228).

Preparative isoelectric focusing of purified taGTH yielded six peaks at a pH range of 4.20 to 5.35, all showing immunological reactivity and biological activity. They are assumed, therefore, to be GTH isohormones.

An anti-taGTH serum, raised against the DEAE-adsorbed fractions, abolished the GTH activity of the homologous pituitary extract in the bioassay. Immunocytochemical study of the homologous pituitary showed binding of the antiserum to basophilic cells in the proximal pars distalis and in pars intermedia.

A RIA was established using this antiserum and the most purified taGTH fraction as standard. Serial dilutions of either homologous pituitary extract or plasma yielded displacement curves parallel to that of the standard taGTH, indicating the immunological similarity of the GTH form in the circulation and in the pituitary. Serial dilutions prepared from pituitary extracts of T. nilotica, T. aurea, T. mossambica, T. galilea, T. zillii, Cichlasoma nigrofasciata and Mugil cephalus yielded curves parallel to that of the standard, while carp and salmon GTHs yielded curves that were not parallel to that of tilapia. None of the mammalian gonadotropins tested exhibited any cross-reaction in this system.

The RIA established for the steroidogenic gonadotropin of the hybrid tilapia also seems to be suitable for GTH determination in other species of the genus and may support further research on the reproductive physiology of these economically important fish.

GONADOTROPIN SECRETION FROM THE PITUITARY OF TILAPIA: STIMULATION BY GnRH AND POSSIBLE MODE OF ACTION

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The present work is an attempt to study the mechanism of GnRH action on gonadotropin secretion from perifused tilapia pituitary fragments.

In each perifusion experiment, glands were taken from three female tilapia and placed in Eagle's basal medium containing 4 mM $NaHCO_3$, 0.5 mg BSA/ml, 10 IU penicillin, 5 ug/ml streptomycin and buffered to pH 7.4 with 5 mM HEPES. The glands were transferred, together with 0.1 g of preswollen Bio-Gel P-2 (Bio-Rad), into a perifusion chamber and were rinsed at 26°C with the medium at a flow rate of 15 ml/hr. The medium was collected every 15-30 min, and tilapia gonadotropin (taGTH) were determined in aliquots by RIA according to Yaron et al. (this volume). High rate of taGTH output (18.7-22.6 ng/min) occurred initially, but after 12 hr rinsing, a low baseline (0.1 ng/min) was reached. Five min pulses of GnRHa (des Gly^{10}, [D-Ala^6]-LHRH ethylamide) were introduced, at 3-4 hr intervals at each of the following concentrations: 0.0013, 0.013, 0.13, 1.3 nM. The output of taGTH output was dose-dependent, and at concentrations higher than 0.013 nM it exhibited a biphasic pattern: a small peak occurred 20 min after the GnRHa pulse, and a more prominent one was attained about 140 min thereafter. Constant exposure of pituitary fragments to 0.13 nM GnRHa resulted in a continuous high rate of GTH secretion for 15 hr (14 ng/min), followed by a gradual decline for an additional 9 hr. The fact that a high rate of taGTH secretion was maintained for 15 h indicates that, in contrast to mammals, the gonadotrops in the pituitary of tilapia are not desensitized by prolonged exposure to GnRHa.

The mechanism of GnRH action on the pituitary gonadotrops in mammals involves a chain of intracellular mediators including two main second messengers: diacylglycerol (DAG) and inositol triphosphate (IP_3; Nishizuka, Nature 308, 693-698, 1984). The dual control by the hypothalamus on the GTH secretion from piscine pituitary raises the question of whether the mechanism of GnRH action in fish is, indeed, similar to that known in mammals. Preliminary experiments along this line have shown that after the introduction of an unmetabolizable analog of DAG and an activator of protein kinase C, 1 oleyl-2-acetyl-rac-glycerol (OAG; 2.5 mM) the secretion rate of taGTH increased in a biphasic manner similar to that obtained following a pulse of 0.39 nM GnRHa. In mammals IP_3 is recycled by the inositol phosphate cycle culminating in the formation of phosphatidylinositol 4,5- biphosphate (PIP_2). Li^+ is known to reduce the phospholipid turnover by blocking the conversion of inositol-1-phosphate (IP_1) to free inositol. Exposure of tilapia pituitary fragments to 5 mM LiCl had no effect on taGTH basal output. Stimulation of GnRHa during this exposure, however, resulted in a sharp but short peak (19.7 ng/min) of taGTH secretion. Following the withdrawal of the lithium, a high secretion rate was noted for at least 8 hr. These preliminary results indicate the possibility that in fish, as in mammals, diacylglycerol and inositol triphosphate are involved in the mediation of GnRH stimulation of gonadotropin secretion from the pituitary.

PROSTAGLANDIN SYNTHESIS AND ITS INHIBITION BY CYCLIC AMP AND FORSKOLIN IN POSTPARTUM FOLLICLES OF THE GUPPY (*POECILIA RETICULATA*)

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Summary

Prostaglandin synthesis *in vitro* and its inhibition by dibutyryl cyclic AMP and forskolin were examined in postpartum follicles of the guppy. The follicles were cultured for up to 72h under chemically-defined conditions, and the synthesis of prostaglandins was determined by radioimmunoassay. The results show unequivocally that the ovarian follicles devoid of embryos can synthesize PGE2 and PGF from both endogenous precursors as well as added arachidonic acid. That this could be inhibited by indomethacin is indicative of active synthesis rather than release of preformed prostaglandins. Dibutyryl cAMP and forskolin also inhibited prostaglandin synthesis in a dose-dependent manner, as did 3-isobutyl-1-methylxanthine. A similar effect was also evident in vitellogenic oocytes surrounded by follicle layers at the yolk-globule stage of development. While the physiological significance of this action of the cyclic nucleotide remains uncertain at this stage, it is hypothesized that it might be the causative factor for the non-occurrence of ovulation in this viviparous fish soon after final oocyte maturation.

Keywords: prostaglandin, cyclic AMP, forskolin, guppy.

FISH REPRODUCTIVE PHEROMONES

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Although numerous studies of teleost fish have implicated a pheromonal role in a variety of reproductive processes, surprisingly little is known about the chemical identity of pheromones, the sources and control of their production and release, their mechanisms of action, and differences between species. Two studies have indicated that hormones and/or their metabolites may be released to the water as pheromones; androgen and estrogen glucuronides appear to function as attractants in a goby (Colombo et al., 1982) and zebrafish, (Van den Hurk & Lambert, 1983). Recently, using the goldfish as a model, we hypothesized that those hormones which are specifically synthesized at the time of ovulation/spawning are most likely to function as potent reproductive pheromones. Using both endocrine and behavioral bioassays, and electrophysiological recording from the olfactory system (Sorensen et al., 1987), we screened a number of progestins and prostaglandins for pheromonal activity. We found that female goldfish release at least two sex pheromones: a preovulatory steroidal pheromone inducing male endocrine responses and a post-ovulatory prostaglandin pheromone stimulating male spawning behaviour. 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) is released to the water by females during oocyte final maturation where it functions as a potent olfactory stimulant elevating male gonadotropin hormone (GtH) within 15 minutes and increasing milt production within 6 hours (in time for spawning; Stacey & Sorensen, 1986; Dulka et al., 1987). More recently, we found that prostaglandin F2α and one of its metabolites, evidently released by ovulatory females, are potent olfactory stimulants which trigger male sexual behaviour. Together these findings suggest that many species of teleosts may also use reproductive hormones and their metabolites as pheromones.

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THE SPAWNING-INDUCED MILT INCREASE IN GOLDFISH IS STIMULATED BY PRIOR EXPOSURE TO A STEROIDAL SEX PHEROMONE

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Male goldfish appear to possess two mechanisms for increasing milt (sperm and seminal fluid) volume at the time of spawning. The first mechanism is activated by a potent preovulatory pheromone, 17α,20β-dihydroxy-4-pregnen-3-one (17,20P). Female goldfish synthesize 17,20P to promote oocyte final maturation and release this steroid to the water early in the preovulatory period. Males exposed to low concentrations of 17,20P (10^{-11}M) experience increased levels of gonadotropin hormone (GtH) and elevated levels of milt within 6 hours (by the time of spawning). The second mechanism is activated by interactions with a spawning female although the specific cues (behavioural and/or pheromonal) mediating this response are not known. Because strippable milt volumes increase rapidly in spawning males (within 1 hour) and can occur in the absence of a GtH increase, this mechanism may involve neural stimulation of the male reproductive tract.

This study sought to determine whether exposure to water-borne 17,20P (the first mechanism) potentiates milt increases evoked by exposure to spawning females (the second mechanism). Males which had been exposed to 17,20P overnight had more strippable milt than control males. When these males were then allowed to spawn with females and stripped a second time, those males previously exposed to 17,20P once again produced significantly greater quantities of strippable milt than control males. These results indicate that the endocrine response of males to pheromonal 17,20P not only increases the volume of milt in the sperm ducts, but also increases testicular sperm stores which can be rapidly transported to the sperm ducts during spawning. In a species such as the goldfish where males compete for access to spawning females, the volume of sperm immediately available at the time of spawning is likely to be an important determinant of male reproductive success and the evolution of preovulatory "primer" pheromone systems is favored.

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SOCIALLY-STIMULATED GONADOTROPIN HORMONE RELEASE IN MALE GOLDFISH: DIFFERENTIAL DAY-NIGHT SENSITIVITIES TO A STEROIDAL PHEROMONE AND SPAWNING STIMULI

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When injected with prostaglandin F2α (PGF2α), non-ovulated female goldfish perform seemingly normal sexual behaviour (but without oviposition) and release a "releaser" pheromone which stimulates male courtship behaviour. Males spawn with PGF2α-injected females and do not distinguish between them and ovulated females. In addition, males experience rapid increases in blood gonadotropin hormone (GtH) when spawning with either PGF2α-injected females or ovulated females. Several of our recent experiments have shown that the GtH response in spawning males is significantly greater during the night during the day despite the fact that males and females can be induced to spawn at all times of the day and night.

Recently, we have identified a second female pheromone in goldfish which differs from that released by PGF2α-injected females. This pheromone, the gonadal steroid 17α,20β-dihydroxy-4-pregnen-3-one (17,20P), is released several hours prior to ovulation and functions as a "primer" pheromone which rapidly increases GtH secretion and subsequently milt production in male goldfish. This study examined whether males exhibit a day-night sensitivity to the 17,20P pheromone. GtH levels in male fish exposed to either 10^{-10} M 17,20P or PGF2α-injected females were examined over a 24-hour period. Preliminary findings indicate that exposure to water-borne 17,20P elevates GtH at all times of the day while spawning interactions elevate male GtH only at night. These results suggest that endocrine responses to pheromonal 17,20P and spawning females are controlled by different mechanisms in male goldfish.

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ROLES OF PROSTAGLANDIN F2α AND ITS METABOLITES IN GOLDFISH REPRODUCTION: BEHAVIOURAL EFFECTS IN THE FEMALE AND PHEROMONAL EFFECTS ON THE MALE

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Teleost fish, like many vertebrates, synthesize prostaglandin F2α (PGF2α) at the time of ovulation to promote follicular rupture. In goldfish, PGF2α is carried to the brain where it rapidly evokes female spawning behaviour. Recently, we have found that PGF2α injection not only elicits apparently normal female spawning behaviour, but also evokes the release of a pheromone whose effects on male behaviour are similar to those of the pheromone released by ovulated goldfish. PGF2α thus appears to play an important role in goldfish reproduction by synchronizing ovulation with both female and male spawning behaviour.

Where studied in vertebrates, PGF2α has been found to be metabolized very rapidly and we hypothesized that its metabolites might be responsible for some of the actions previously attributed to PGF2α. Similarly, we postulated that PGF2α metabolites may comprise the pheromone released by PGF2α-injected goldfish. Recently completed electrophysiological studies of the olfactory system of male goldfish appear to confirm the latter hypothesis; both PGF2α and one its presumed metabolites are extremely potent olfactory stimulants.

The present ongoing study seeks to determine the relative potencies of PGF2α and two of its presumed metabolites, as hormones evoking female spawning behaviour and as water-borne pheromones stimulating male courtship. Initial results suggest that while only PGF2α evokes female spawning behaviour, both PGF2α and a metabolite have pheromonal effects on male behaviour. In addition, the pheromonal actions of these compounds appear to be synergistic.

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IN VIVO STIMULATION OF HEPATIC ESTROGEN RECEPTORS IN VARIOUS SPECIES OF FISH

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Estrogens stimulate the production of egg-yolk proteins, such as vitellogenin, by the liver cells of oviparous vertebrates including birds and fish. It has also been shown that injection of estradiol (E2) into immature chickens increases the levels of high affinity hepatic estrogen receptors (ER), especially those found tightly bound in the nuclear compartment and extractable only with high salt (Lazier & Haggarty, 1979).

We have also found that a single intraperitoneal injection of E2 (5 mg/kg) will induce very high levels of ER in the livers of immature Atlantic Salmon (Salmo salar) (Lazier et al., 1985). The results of at time course study showed that cytosol ER levels decreased from 0.6 pmole/g tissue in uninjected controls to barely detectable levels by 4 h post-injection. Control levels were gradually restored over the next 16 h and were found to be 1.5-2 times control levels by 48-120 h. Nuclear ER levels, on the other hand, steadily increased with time, from an initial 0.5 pmole/g to over 11 pmole/g by 120 h, the highest concentration of hepatic ER reported to date for any oviparous vertebrate.

We have continued to study the induction and distribution of hepatic ER in several other species of fish. Table 1 shows the results of a preliminary study in which ER levels were measured in control livers and in livers taken 120 h after injection from fish maintained at 9°C. Crude cytosol and nuclear salt extracts were prepared and assayed by exchange with 10 nM ^{3}H-E_2 ± 100 fold excess of diethylstilbestrol (DES) for 18 h at 2°C, followed by charcoal-dextran treatment at 2°C to remove unbound ligand. Specific binding was defined as total ^{3}H bound minus that bound in the presence of DES.

All fractions show some degree of DES-competable binding activity, although the amount and distribution of sites varies among species and none show the high levels of nuclear ER induced in S. salar. Even considering that the cytosolic localization of ER may be an artifact of homogenization, it is clear that livers of different fish respond differently to the same homogenization conditions in terms of apparent intracellular distribution of ER.

A time course study was also undertaken using immature rainbow trout (Salmo gairdnerii) maintained at 4°C. DES-competable binding was found to gradually increase in the livers of these animals over the period of 120 h following injection. In contrast to the results seen with S. salar, 90-95% of the ER was found in the cytosol fraction rather than the nuclear salt extract. Cytosol ER levels rose steadily from 1 pmole/g in controls to 5 pmole/g at 120 h, while nuclear ER levels rose from 15 fmole/g to 800 fmole/g. High levels of cytosol ER were seen whether or not the livers had been frozen prior to preparation of extracts, although there appeared to be a 30% loss of nuclear ER if the tissue had been frozen. The K_d for both nuclear and cytosol ER was 3-10 nM, in agreement with values obtained by Maitre et al (1985) for S. gairdnerii ER and by Lazier et al (1985) for S. salar.

Table 1. Specific binding of E_2 by fish liver extracts

Species	Specific E_2 bound (fmole/g) (±SEM)			
	Control		Treated	
	Nuclear	Cytosol	Nuclear	Cytosol
Sea raven[1]	88±8	1265±8	912±37	1042±52
Trout[2]	31±20	2453±853	410±107	2012±479
Sculpin[3]	44±31	839±143	207±.7	437±121
Ocean pout[4]	15±10	1457±88	34±18	2471±477

1. Hemitripterus americanus 2. Salmo gairdnerii 3. Myoxocephalus octodecimspinosus 4. Macrozoarces americanus

We conclude that the ER system in fish liver shows some species-specific characteristics and that the temperature at which fish are maintained may influence ER dynamics. We are now attempting to look at the regulation at the level of ER mRNA accumulation.

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WHOLE BODY STEROID CONTENT AND IN VITRO STEROID SECRETION DURING SEXUAL DIFFERENTIATION OF COHO SALMON, ONCORHYNCHUS KISUTCH

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A spectrum of potential mechanisms have been proposed to explain sex differentiation in fish, including induction by sex steroids. The objective of the present study is to determine if a sexual "dimorphism" exists in whole body steroid content and in steroid production by gonads and interrenal tissue in vitro during early development of coho salmon that may indicate a role for steroids in the process of sex differentiation.

Whole body steroid profiles for testosterone, 11-ketotestosterone, androstenedione, progesterone, 17α,20β-dihydro-4-pregnen-3-one, and estradiol-17β were generated beginning with unfertilized eggs and ending at 77 days post-fertilization (DPF). Ovarian fluid was also examined for steroid content. At onset of feeding, fish were placed on either a "high" steroid diet (Biomoist) or a "low" steroid diet (casein based).

Steroid profiles of unfertilized eggs essentially paralleled those found in ovarian fluid. Following fertilization, steroid levels declined precipitously until 30 DPF. At hatch (42 DPF), levels of all steroids increased and then declined during yolk absorption. Steroid content of yolk declined during sac absorption. Embryo sex steroid content during this time generally increased or remained constant. At onset of exogenous feeding (60 DPF), Biomoist-fed fish showed a significantly higher steroid body content than casein-fed fish. This difference was evident at 3 and 10 days after the onset of feeding but not at 17 days. Steroid content by this time had begun to increase. It is suspected that steroid synthesis abolished the effects of the different diets. Steroid levels of developing fish showed varying degrees of of bimodality between 42 and 56 DPF, which may be suggestive of a sexual dimorphism. By 101 DPF when presumptive sex could be determined, males had higher levels of testosterone, 11-ketotestosterone, and androstenedione than presumptive females.

At 85 DPF, two distinct gonadal morphologies were readily apparent. The resting output of testosterone in an 18-hr static incubation was similar between presumptive ovaries and presumptive testes; however, the ovaries did not respond to partly purified salmon gonadotropin (SG-G100) whereas the testes demonstrated significant stimulation. Similarly, the resting output of androstenedione was similar between ovaries and testes, but only the testes responded to SG-G100. The resting levels of estradiol were higher in ovarian incubations than in testicular incubations; furthermore, only the ovaries showed significant response to SG-G100.

A second experiment was conducted with combined gonad and interrenal incubations at four times between hatching and 3 weeks after the onset of feeding. We observed significant stimulation of estradiol, testosterone, androstenedione, and cortisol by SG-G100 at several times before the sex of the gonads could be determined from gross examination.

SEX REVERSAL BY DIETARY STEROID TREATMENT OF PACIFIC SALMON

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Summary

Estradiol 17β incorporated into the diets of coho salmon (Oncorhynchus kisutch) and chum salmon (O. keta) fry from the time of first feeding significantly increased the percentage of females in experimental populations. Treatment of coho salmon for 2 weeks with 5 mg estradiol per kg food (5 ppm), fed 3-4 times daily ad libitum, yielded a population in which 72% of the fish were females at 5 months of age. Treatment of coho salmon and chum salmon for 6-12 weeks at 5 or 50 ppm usually produced >95% females. The histological appearance of ovaries in treated fish was normal, although at 50 ppm of estradiol there was a stunting effect on growth and gonadal size. Sex reversal of chum salmon and coho salmon treated with 5 ppm estradiol persisted until at least 10 and 12 months of age, respectively. Dietary treatment of coho salmon with 5 ppm estradiol, beginning 4 or 8 weeks after initiation of feeding and continuing for up to 12 weeks, had no effect on sex ratio.

Dietary treatment of coho salmon with 50 ppm of 11β-hydroxy androstenedione (11βAdione) for 4 or 8 weeks beginning at first feeding decreased to 10% or 4% (n=50 or 100, respectively) the number of fish showing oocyte development at the age of 5 months. Moreover, those fish with oocytes had thin, testes-like gonads, containing only a small number of oocytes. Treatment with 5 ppm of 11βAdione decreased the percentage of females in the population (36%, n=100) when administered for 8 weeks, but not for 4 weeks; however, in fish with oocytes, gonadal size was markedly reduced compared to that in control fish.

Dietary administration of methyltestosterone (5 or 10 ppm), testosterone (5 or 50 ppm), and androstenedione (5 or 25 ppm) to coho salmon and chum salmon and cortisol (5 and 50 ppm) to coho salmon for 8-12 weeeks beginning at first feeding had no effect on sex ratio.

We also conducted experiments on the sex-reversing effects of periodic immersion in solutions of 11βAdione, methyltestosterone, testosterone, and cortisol between the times of hatching and first feeding in coho salmon sac-fry. Results of these experiments will be compared with those from dietary treatment alone.

ANDROGENS IN THE THREE-SPINED STICKLEBACK (GASTEROSTEUS ACULEATUS) - TESTES BIOSYNTHESIS AND BIOLOGICAL ACTIONS

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Summary

Secondary sexual characters and reproductive behaviour disappear after castration and can be restored with androgens like methyltestosterone in the male stickleback (e.g. Wai & Hoar 1963). In order to see which androgens stimulate these traits normally, the androgens formed by stickleback testes in vitro were analyzed and their biological effects were studied by implanting steroid-filled silastic capsules in intact and castrated fish.

Testes from nesting and refractory, post-breeding males were tissue-incubated with tritiated pregnenolone or androstenedione. The steroids formed were identified by thin layer chromatography, derivatization and recrystallization.

The main androgen formed in breeding males was 11-ketoandrostenedione (11-kΔ4), which was formed via the pathway; pregnenolone - progesterone - 17α(OH)-progesterone - androstenedione (Δ4) - 11β(OH)-androstenedione - 11-kΔ4. Testosterone was not formed. Dramatically more 11-kΔ4 was formed in the testes of nesting than refractory males. The formation in the latter was only c. 2% of that in the former.

Silastic capsules filled with Δ4 or 11-kΔ4 were implanted in castrated males. Both androgens stimulated secondary sexual characters such as breeding colours and kidney hypertrophy (the kidney hypertrophies in the breeding season and secretes a "glue", which is used in the building of the nest). 11-kΔ4 was the most effective of the two androgens.

Spermatogenesis is quiescent during the breeding season in the stickleback and starts afterwards, when the secondary sexual characters decline. Methyltestosterone can prevent this onset of spermatogenesis (Borg 1981). Both Δ4 and 11-kΔ4 were also found to have this effect.

Both androgens also stimulated nest-building behaviour, 11-kΔ4 capsules were more effective than Δ4 ones in this respect. 11-kΔ4 treated fish displayed all aspects of reproductive behaviour. They defended territories, built nests, successfully courted females and reared adopted roe to hatching (Borg 1987).

The high effectiveness of an 11-androgen on secondary sexual characters in fish is in agreement with several other studies. The effects of 11-androgens on reproductive behaviour have been little studied. In mammals, usually only androgens that can be converted (aromatized) to estrogens are effective in stimulating sexual behaviour (e.g. Beyer et al. 1973). As 11-kΔ4 is a non-aromatizable androgen (unlike Δ4), the results from the stickleback are in contrast to those from most mammalian studies.

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THE PHOTOPERIODIC CONTROL OF REPRODUCTION IN THE SEA BASS (DICENTRARCHUS LABRAX L)

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Abstract

Previously, it has been shown that the timing of reproduction in the sea bass can be modified by photoperiodic manipulation (Zanuy et al. 1987) suggesting that light is the primary zeitgeber in this species. In salmonids changing daylength does not directly control maturation but serves to entrain an endogenous clock which in turn coordinates the timing of reproductive development. The present work investigates whether similar mechanisms of control also operate in the sea bass.

Beginning in Jan., when ambient daylength was 9L:15D (Castellon, Spain: Lat. 40°N), groups of 2 year-old male and virgin female sea bass (Natural spawning time Jan.-Feb) were maintained in light-proof 2,000 litre aquaria, supplied with aerated sea water, and exposed to the following photoperiod regimes :-

A. 9L:15D (short day) constant
B. 9L:15D until May 2 followed by 15L:9D (long day).
C. 9L:15D until May 2, 15L:9D until June 3 and then 9L:15D
D. 9L:15D until June 3, 15L:9D until July 3 and then 9L:15D
E. 9L:15D until July 3, 15L:9D until Aug 4 and then 9L:15D.

Daylength, which was controlled by electronic timeclocks, provided 1000 Lux at the water's surface. Control fish, maintained in adjacent tanks under ambient conditions, acted as controls. All fish were fed ad libitum on fresh trash fish. Blood samples were taken monthly for analyses of testosterone, oestradiol 17B and vitellogenin (as calcium) and from Sept. onwards biopsies of ovary removed by cannulation for histological examination. At daily and monthly intervals the tanks and fish respectively were examined to establish the timings of egg release and spermiation. The number, percentage-floating and survival of eggs to hatching and as fry was also recorded.

The rates of ovarian maturation and the timing of spawning were advanced in Groups A, C, D and E and delayed in B when compared with the Jan-Feb spawning time of the controls. Maturation was most advanced in Group C where ripe oocytes were present in the ovaries in Oct; this was followed in turn by Groups D, E and A. Group B did not commence spawning until March.

The progressive advancement of maturation in Groups C, D and E by the exposure of fish to one month of long-days in May, June and July respectively, together with the similarity in spawning times of the control fish and those on constant short days suggests that endogenous timing mechanisms may control reproduction in the sea bass. The use of photoperiodic techniques to provide a continuity of supply of eggs and fry for the commerical culture of sea bass is discussed.

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CURRENT KNOWLEDGE OF OVARIAN STEROIDS

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It has been established in a wide range of teleost species that during the stage of vitellogenic growth, the ovaries produce mainly C19 and C18 steroids (androgens and oestrogens), and during the stages of meiotic maturation and ovulation, mainly C21 steroids (progestagens). This paper will mainly be concerned with the nature and role of the latter steroids. More specifically, we will review work and present original data in three areas:

1. effect of synthetic steroids on induction of oocyte maturation;
2. identification of putative oocyte maturation-inducing steroids by radioimmunoassay;
3. identification of steroid biosynthetic pathways using radioactively-labelled precursors.

Among the steroids that will be discussed are:

4-pregnen-17α,20β-diol-3-one (17α,20β-P)

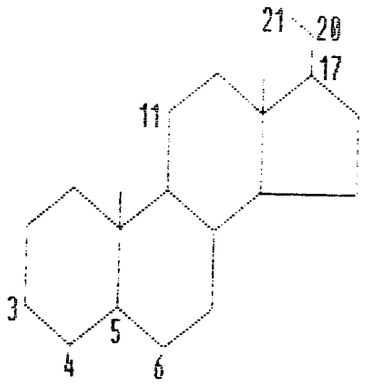
4-pregnen-17α,21-diol-3-one (11-deoxycortisol)

4-pregnen-17α,20β,21-triol-3-one (17α,20β,21-P)

4-pregnen-17α,20α-diol-3-one (17α,20α-P)

17α,20β-P is well known for its role in the induction of oocyte maturation in teleosts belonging to the orders Salmoniformes, Cypriniformes and Siluriformes. 17α,20β,21-P has recently been identified in ovaries of a Perciform fish. 11-deoxycortisol and 17α,20α-P have been identified in the ovaries of Pleuronectiform fishes.

A figure of the C21 skeleton and a brief resumé of steroid terminology are included as an *aide-memoire:*



4-PREGNEN: double bond between carbons 4 and 5.

5-PREGNEN: double bond between carbons 5 and 6.

5 (α or β)-PREGNAN: no double bond. The hydrogen atom at position 5 (which is not present when there is a double carbon bond) points down from the plane of the paper in the α-configuration and up in the β-configuration.

-OL: hydroxyl (-OH) group. (α- and β-configurations as described above).

-ONE: oxygen (= O) group.

HORMONAL CONTROL OF OOCYTE FINAL MATURATION IN PLAICE (*Pleuronectes platessa*) AND DAB (*Limanda limanda*)

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There is now much evidence from a wide range of teleost species that the steroid 4-pregnen-17α,20β-diol-3-one (17α,20β-P) plays a key role in the process of oocyte final maturation. In certain species however elevated levels of this steroid have been difficult to detect. In the plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*) for example, which are temperate marine flatfishes (Order Pleuronectiformes), we have radioimmunoassayed over 300 blood samples from females at different stages of oocyte maturation and ovulation and have failed to detect elevated levels of 17α,20β-P. There may be several reasons for this: (a) levels in the blood are below the limit of detection of the assay; (b) levels are only elevated for a short time and/or at the time when sampling has not been carried out; (c) some other steroid is involved in maturation-induction.

To clarify this problem, four approaches have been taken:

1. *In vitro* bioassay of folliculated oocytes of plaice and dab with synthetic C21 steroids: this has shown that 17α,20β-P is very effective in inducing germinal vesicle breakdown (GVBD). Several other 20β-hydroxylated steroids, however, (including 20β-P and 17α,20β,21-P) have been shown to be equally effective. 20α-hydroxylated steroids show only 20% of the activity of 20β-hydroxylated steroids.

2. Incubation of ovarian homogenates with [H^3]-17α-hydroxyprogesterone as precursor: this has so far revealed the presence of 20α-hydroxysteroid-dehydrogenase (20α-HSD) and 5 - reductase activity. The identification criteria were based on the behaviour of steroids on thin layer chromatography before and after chemical and enzymatic modification, and isopolarity with reference steroids. Plaice and dab sperm was also found to contain 20α-HSD activity.

3. Collections of blood samples at different times of the day and night from mature plaice and dab females at different stages of oocyte maturation and ovulation: bloods have not yet been assayed, but examination of ovarian samples indicates that the actual process of GVBD must be very brief, as only 4 out of ca. 320 fish collected were found to have a significant proportion of oocytes at this stage.

4. Development of RIAs for putative oocyte maturation-inducing steroids: assays for 17α-P and 17α,20β-P have been available for several years. Within the last few months we have developed assays for 4-pregnen,17α,21-diol-3-one (11-deoxycortisol), 4-pregnen-17α,20β,21-triol-3-one and 17α,20α-P. Results of assaying plasma samples and ovarian incubates for these steroids will be reported at the meeting.

PLASMA STEROID PROFILES DURING GONAD MATURATION AND SPAWNING IN THE DACE, *Leuciscus leuciscus* (L.)*

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An understanding of the reproductive cycle of a fish species is an important prerequisite for its artificial culture. The dace, a temperate freshwater cyprinid common in the UK but previously neglected with respect to research and culture, has been studied in the wild over two complete reproductive cycles. Blood plasma samples have been taken for analysis by radioimmunoassay for 17β-estradiol and 17α,20β-dihydroxy-4-pregnen-3-one (17,20αβP), and by fluorometric titration for calcium, as a vitellogenin indicator. Gonad samples have been taken by non-destructive biopsy for germinal vesicle observations, oocyte measurement and subsequent histology.

At spawning there are four discernable cohorts of eggs within the ovary, of diameters ($\bar{x}\pm$s.e.), 1681±12 μm (oocytes prior to ovulation), 359±9 μm (early vitellogenic oocytes), 156±7 μm (stage 2 oocytes) and 83±4 μm (stage 1 oocytes). Development starts in late April in the early vitellogenic oocytes, about one month after spawning, and is rapid over the summer production period reaching up to 1536±119 μm by early November. A gradual rise in plasma calcium has been observed over the summer, with a peak in December, then falling in the prespawning period as vitellogenesis has been completed. The profiles of 17β-estradiol are characterised by two peaks, the first in October to November, and a second, more acute rise immediately before spawning in mid-March. The first peak corresponds to the completion of exogenous vitellogenesis, as confirmed by oocyte and calcium observations. The peak at spawning has been observed in other cyprinids, such as carp (*Cyprinus carpio* L.)[1], and hence may have a maturational role, as vitellogenesis has been completed for two to three months. Profiles of 17,20αβ-P indicate a prolonged maturation of the oocytes before ovulation. 17,20αβP levels are low but detectable from October onwards, peak in December, and remain at a constant level until ovulation in mid-March. Germinal vesicle migration started between early to mid-February when oocyte growth had been completed. Compared to tropical cyprinids, this protracted event may be due to low river temperatures, below 2°C in winter.

These physiological data have facilitated attempts to induce ovulation in prespawning female dace by hormone treatment. Fish that were deemed to be in a receptive condition from the oocyte and plasma observations, were dosed with carp pituitary extract. This induced ovulation in 29 out of 30 fish. The following year, ripe female dace were injected with des-Gly^{10} [D-Ala^{6}]-luteinizing hormone releasing hormone (LHRH-A) (0.1 μg g^{-1}) coupled with an injection of either chlorpromazine (0.1, 1 or 10 μg g^{-1}) or droleptan (0.1, 1 or 10 μg g^{-1}), both dopamine antagonists. Successful ovulations were obtained in all chlorpromazine groups and with droleptan at 0.1 μg g^{-1}.

[1]Kime, D.E. and Dolben, I.P. (1985). Gen. Comp. Endocrinol. 58, 137-149.

*Supported by a grant from the Severn-Trent Water Authority.

STIMULATION BY ESTRADIOL OF mRNA LEVEL FOR PITUITARY GLYCOPROTEIN HORMONE ALPHA SUBUNIT IN THE EUROPEAN FEMALE SILVER EEL, ANGUILLA ANGUILLA L.

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In the European silver eel (at the stage of the catadromous migration), the gonads are still immature and remain at this juvenile stage as long as the reproductive migration to Sargasso sea is prevented. This blockade of gonadal development results from a deficiency in pituitary gonadotropin (GTH) synthesis and release. The administration of sexual steroids strongly stimulates the synthesis of GTH in silver eel, as demonstrated by histological studies, bioassay and radioimmunoassay. Such a positive effect also occurs in juvenile salmonids.

In mammals, gonadal steroids negatively regulate gonadotropin synthesis via inhibitory effect on messenger ribonucleic acid (mRNA) synthesis for alpha and beta (LHβ, FSHβ) subunits : e.g. castration increases and steroid administration decreases mRNA levels for these subunits in several species.

The objective of the present work was to examine the changes in the levels of mRNAs coding for GTH subunits in the pituitary of the female silver eel after a chronic treatment with estradiol.

Total RNA was extracted from pituitaries of normal female eels and from eels treated with estradiol 17 β (9 injections over 3 weeks of 2 μg/g body weight) and translated in a wheat-germ cell-free system in the presence of (^{35}S) methionine and cysteine. After reduction and carboxymethylation of the translation products, labeled polypeptides were specifically immunoprecipitated by antisera against reduced carboxymethylated bovine α and LHβ and characterized by SDS-PAGE and fluorography.

We demonstrated that, in both control and estradiol-treated eels, one of the translated polypeptides cross-reacted with the antiserum to denatured bovine α subunit. Its apparent Mr (18.5 K) was close to that of the rat α precursor (17 K). The specificity of immunoprecipitation was confirmed by inhibition of cross-reaction with an excess of bovine α but not LH β nor FSHβ. These data indicate that this polypeptide represents the precursor of the α subunit of eel glycoprotein hormone.

For quantitative determinations, the radioactive bands were excised from the gel, solubilized and counted. The putative eel α subunit precursor represented 0.17 % and 0.22 % of the total protein translated from pituitary mRNA of control eels in the two experiments, values very close to those found in normal rat. Chronic treatment of the eels with estradiol increased the values to 1.45 % and 1.76 % for the two experiments: thus estradiol increased by 8.5 and 8.0 fold the translational capacity of mRNA coding for the α subunit in RNA preparation from eel pituitary. In contrast, such an effect was produced by castration in the rat.

Concerning the β subunit, data from a radioimmunoassay specific for GTHβ showed for the two experiments a 10 fold increase in pituitary GTH content of estradiol-treated eels. This stimulation is probably mediated by an increase in mRNA level for GTHβ, as recently indicated by Trinh and coworkers in the testosterone-treated juvenile trout. Using antiserum to denaturated bovine LHβ, we could not specifically detect the presumed eel GTHβ precursor, probably due to species specificity.

In conclusion, our results demonstrate that a 3 week-treatment with estradiol induces a 8 fold increase of the eel pituitary mRNA level for glycoprotein hormone α subunit. This increase probably results from stimulated gene transcription but may also involve increased mRNA survival. This strong positive effect of estradiol on GTH subunit mRNA level, opposite to that demonstrated in various mammals, probably participates in the positive control exerted by sexual steroids on GTH synthesis in some juvenile teleosts.

INTESTINAL NUTRIENT UPTAKE DURING THE REPRODUCTIVE CYCLE OF THE RAINBOW TROUT: PRELIMINARY INFORMATION

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University of California, Los Angeles and Davis

Summary

Reproductive activity heightens the requirements for energy and nutrients, particularly for the female due to the high costs associated with gamete formation. However, despite over three decades of research on the nutrient requirements of salmonids, there exists little information on the nutrient requirements associated with reproduction. The two principal questions to be addressed are (1) what are the specific requirements and (2) when do the requirements change. Regarding when, other vertebrates respond to the increased requirements by hyperphagia and a hypertrophy of the intestine which results in an increased ability to absorb the additional nutrients (Karasov and Diamond, 1987). Thus, we investigated the intestinal uptake of the amino acid proline to determine if, and when, the rainbow trout might exhibit intestinal adaptations characteristic of the increased nutrient uptake associated with reproductive activity. Such information will be essential for elucidating when to concentrate efforts on improving the nutrition of broodstock.

Utilizing an in vitro method adapted for use with fish (Buddington et al. 1987) we measured the intestinal uptake of proline at three stages of the reproductive cycle of trout (10, 16, and 20 months). Uptake rates were determined in the caeca, proximal intestine and distal intestine. In addition, we observed intestinal morphology, gonadal histology and plasma concentrations of alkali-labile phosphoproteins (ALPP).

In females, intestinal uptake capacities (calculated from the product of uptake per cm and post-gastric alimentary canal length), as a function of metabolic size of the females were lowest during advanced stages of vitellogenesis (Figure 1). Maximal rates of proline uptake (nM/mg-min) were observed during the onset of vitellogenesis (16 months). Uptake rates were actually lower during advanced vitellogenesis (20 months) when nutrient requirements would apparently be higher based on significant elevations of plasma ALPP and increased oocyte diameters. Males did not exhibit a corresponding decline in uptake capacities. Also in females, intestinal weight and surface area were lower on a weight specific basis during advanced vitellogenesis. In contrast, in males undergoing spermatogenesis the intestinal weight increased.

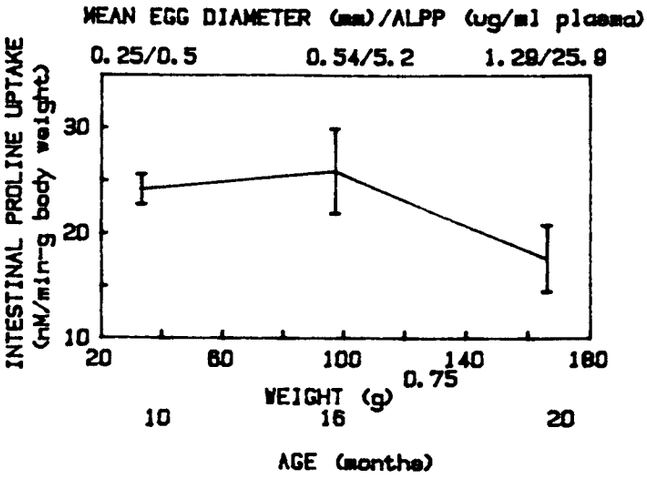

Figure 1. Intestinal proline uptake capacity of female trout as a function of metabolic weight, age, mean egg diameter, and ALPP

Although it is presently unclear how trout, as well as other fish, partition endogenous and exogenous nutrients for the specific needs of vitellogenesis, these preliminary data suggest that the intestine of female trout may be regulated to maximize nutrient uptake prior to, but not during, the period of vitellogenesis. Thus, a reduction in follicular atresia causing an increase in fecundity may be accomplished by increasing feed availability during the early phases of oogenesis. This would allow the fish to accumulate the energy and nutrients for the period of active vitellogenesis when demands are greater than can be provided by exogenous sources. Now that we know when nutrient requirements are apparently maximal, based on intestinal adaptation, further research will be needed to elucidate specific requirements for the design of effective broodstock diets which will improve fecundity and gamete quality.

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The Current Status of Oocyte Growth

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Teleost oocytes, arrested in meiotic prophase, initially arise within germinal regions of the ovarian luminal epithelium. Prefollicle cells surround each oocyte and the resulting complex buds off the germinal nest as a primordial follicle. The oocyte then grows within the follicle by accumulating normal cytoplasmic components to some extent; however, the preponderant mechanisms contributing to oocyte growth are the endogenous synthesis of cortical alveoli, the accumulation of exogenously derived yolk protein (vitellogenesis) and, in some (particularly marine) teleosts, a pronounced water uptake concomitant with the resumption of meiosis (maturation). These cellular events do not sequentially replace one another, but rather are sequentially initiated and remain active throughout oocyte development. *In vitro*, oocytes grow without restraint and pass through internally programmed stages (except maturation) without external cues. *In vivo*, however, major differences in follicle recruitment phenomena exist among teleost species, so that final egg laying, which may occur once or repeatedly each year, corresponds with the most propitious environmental circumstances. A major challenge thus remaining is to define the cellular and hormonal events that regulate recruitment phenomena within the ovary as a response to environmental signals.

Maintenance of Fundulus heteroclitus with sexually mature ovaries outside of the normal breeding season.

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The ovaries of Fundulus heteroclitus regress toward the end of the breeding season, which normally lasts from March to September in northern Florida. The gonadotropic activities of the pituitaries collected from fish outside of the breeding season show a corresponding decrease. The aim of this study was to develop a routine husbandry procedure so as to maintain a population of F. heteroclitus with sexually mature ovaries throughout the year. Field fish brought into the laboratory in August and maintained at warm temperature (25°C) and long photoperiod (14L:10D) possessed mature ovaries and showed no sign of regression throughout the winter months (September to February). Contrary to the more northern population of F. heteroclitus, cold temperature (15°C) and short photoperiod (10L:14D) pretreatments were not required to promote premature recrudescence. Prematurational follicles (1.3-1.5 mm) obtained from such laboratory-maintained fish underwent germinal vesicle breakdown normally in vitro upon stimulation by pituitary extract and 17α-hydroxy-20β-dihydro-progesterone. Pituitaries obtained from such fish also maintained high gonadotropic activities throughout the year.

REGULATION OF OOCYTE MEIOTIC MATURATION IN *FUNDULUS HETEROCLITUS*

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Abstract

Teleost oocytes are arrested in meiotic prophase I during much of their active growth period, and only resume meiosis (mature) towards the end of this period in response to a specific hormonal signal. The mechanism by which the proper hormonal signal is translated into the proper response (maturation) is poorly understood. Results from recent *in vitro* culture experiments in this laboratory with *Fundulus heteroclitus* oocytes suggested that hormones may act in part by removing an inhibition of meiosis imposed on the oocyte by the innermost layer of somatic (follicle) cells that surround the oocyte during the growth period. Oocytes denuded of these follicle cells frequently undergo spontaneous maturation during *in vitro* culture in the absence of hormonal stimuli, similar to the response of mammalian oocytes to removal from their follicles. We here present evidence from additional studies showing that exogenously-supplied hormones can potentiate spontaneous maturation of denuded *F. heteroclitus* oocytes by greatly accelerating the rate at which this process proceeds. Thus, these studies considered together suggest that there are both inhibitory and stimulatory aspects to the regulation of oocyte maturation in this species that need to be considered in future investigations.

ISOLATION AND CHARACTERISTICS OF 17α,20β-DIHYDROXY-4-PREGNENE-3-ONE RECEPTOR ACTIVITY IN OVARIAN TISSUE OF THE BROOK TROUT, SALVELINUS FONTINALIS

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Receptor activity for 17α,20β-dihydroxy-4-pregnene-3-one (17α,20β-DHP) has been isolated and characterized in brook trout ovarian tissue cytosolic extracts during various stages of terminal oocyte maturation. The association constant K_a equals $1.39 \pm 0.67\ 10^8 M^{-1}$(n=7). Competitive inhibition studies shows the following hierarchy of binding, testosterone > 17α, progesterone > 17α,20β-DHP > R5020 > progesterone > estradiol > pregnenolone. Cortisol showed no competitive inhibition. Cytosolic extracts when pre-equilibriated with various labelled steroids and eluted from a Sephacryl S-300 column gave similar multiple specific binding peaks. The receptor activity was lost when incubated with various proteases, but DNase and RNase had no effect on the binding activity.

The recent research on action of 17α,20β-DHP to bring about GVBD fish oocytes indicate it is via external plasma membrane. In goldfish oocytes external application of 17α,20β-DHP attached to high molecular weight polymer caused GVBD, while microinjection had no effect (Nagahama 1986). In yellow perch oocytes the GVBD caused by 17α,20β-DHP in vitro incubation studies was inhibited by cAMP and also by inhibitors of phosphodiesterase and activators of adenylate cyclase. Both of which increase in vivo the levels of cAMP (DeManno and Goetz 1986). Similar studies were also carried out in rainbow trout (Jalabert and Finet 1986).

The low affinity and non-specificity of 17α,20β-DHP receptors are in contrast with criteria used by physiologists and endocrinologists to characterize somatic cell steroid receptors. This ambiguity in 17α,20β-DHP receptor properties complements its physiological function, since conditions for the action of 17α,20β-DHP membrane associated receptors are different from that of classical somatic cell steroid receptors. In salmonids the granulosa cells which secrete 17α,20β-DHP are in direct contact with the oocytes (Nagahama and Adachi 1985), and the steroid hormone is locally delivered and probably present in high concentrations at the receptor site. This eliminates the necessity of the receptor having high affinity (K_a). Also the absolute specificity for the receptor is not a necessary requirement, since other steroids probably either do not reach oocytes membrane at high concentrations in vivo or their action is complementary.

The quantity of receptors in the cytosol (N_{max}) decreases during final maturation. We hypothesise that 17α,20β-DHP receptors are synthesized in the cytoplasm and migrate to the plasma membrane for incorporation and physiological function during the later stages of oocyte maturation. This hypothesis is supported by the observation that plasma levels of 17α,20β-DHP are maximal in salmonids during the final stages of oocyte maturation (So et al. 1986).

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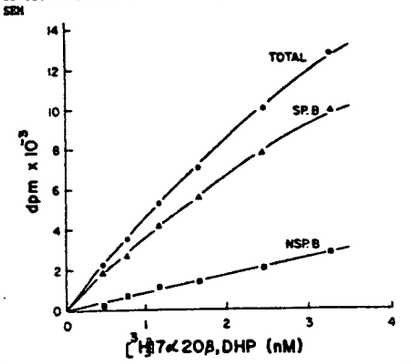
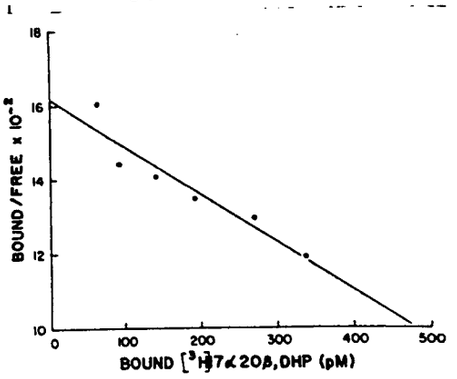
N_{max} values from brook trout cytosol at various stages

Stages oocytes	N	$K_a \times 10^8$ + SEM(M^{-1})	N_{max} f mol/mg protein
1	5	0.216 ± 0.019**	1928 ± 247.61
2	3	0.313 ± 0.008	1161 ± 70.57
3	5	0.209 ± 0.030	1180 ± 118.32
4 and 5	6	0.253 ± 0.048	516 ± 95.40
6 and 7	8	0	0

* number of fish used for determination
** mean ± SEM





Travail sur membranes en cours
Ka + élevé
pas d'étude de spécificité

UPTAKE OF VITELLOGENIN INTO CULTURED OVARIAN FOLLICLES OF RAINBOW TROUT.

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Abstract

Oocyte growth in the rainbow trout, Salmo gairdneri, results primarily from the sequestration of the glycolipophosphoprotein, vitellogenin (VTG). During the 6 months prior to ovulation VTG deposition accounts for an increase in the gonadosomatic index from less than 1% to 20% and above. Recent studies in-vivo have shown that oocytes selectively sequester VTG (Tyler et al., unpublished data.) and indicate that uptake occurs by receptor-mediated endocytosis in a similar fashion to that already demonstrated for the chicken, (Cutting and Roth, 1973.) and Xenopus laevis (Wallace et al., 1970). In fish, however, little is known about the mechanism(s) controlling VTG uptake; control may arise by altering the supply of VTG in the blood and/or by manipulating the competence of the follicle for its sequestration. As in-vivo studies are sometimes difficult to interpret because of the complexity of the system their is a need for an in-vitro technique to study VTG uptake. Such a technique is used in the present study to investigate the environmental parameters affecting VTG uptake into trout follicles.

Vitellogenic follicles (diameter 1.4 to 3.9mm) were cultured for upto 24 hours at 18°C in a defined, serum supplimented, medium containing either iodinated (I^{125}) VTG or in-vivo labelled ^{3}H-VTG. Equivilent rates of VTG sequestration were found using the different labelling techniques and subsequently both forms were used in the experiments reported below. Sepharose 6B column chromatography on the culture medium showed that VTG remained intact with no label dissociation during the incubations; this confirmed that the follicles were sequestering intact VTG.

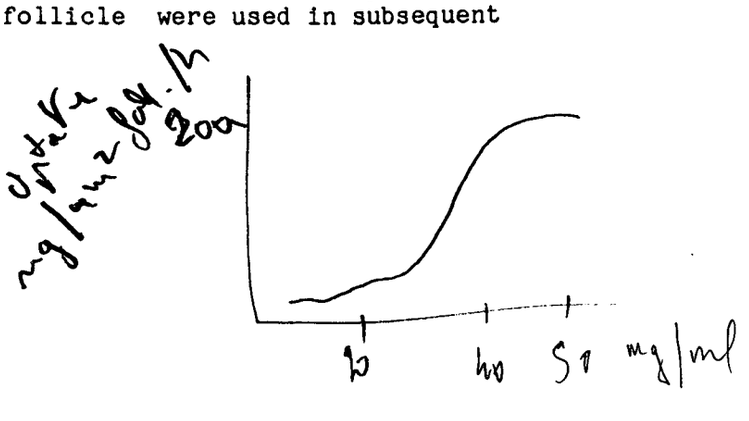
Initial experiments indicated that there were no differences in the rate of VTG uptake between intact follicles (possessing the zona radiata, granulosa and theca, but divested of the external connective tissue and its underlying epithelium) and those in which the theca had been removed by dissection; these follicles are sometimes referred to as denuded. Ovulated eggs and follicles cultured with the interstitial connective tissue intact, although binding VTG to the external surfaces, did not sequester it. Intact follicle were used in subsequent incubations because only minimal preparative handling was required.

The rate of VTG uptake was dependent on the external VTG concentration, with uptake not saturated up to a concentration of 13mg/ml. VTG levels in the culture medium ranging from 0.175 to 12.5mg/ml produced uptake rates from 2.6 to 63ng/mm^2 follicle surface area/hour, respectively. Subsequent follicle incubations were carried out in a medium containing VTG between 5 and 15mg/ml. Rates of VTG uptake, when expressed as ng VTG /mm^2 follicle surface area/hour, were similar across the size range of vitellogenic follicles investigated. EDTA (5mM) in the medium reduced the rate of uptake by 70%. There was a clear temperature dependence for VTG uptake, with greater amounts of protein being sequestered with increasing temperature, upto and including 25°C. At temperatures as low as 5°C VTG was still sequestered, although the rate was only 20% of that observed at 25°C.

These data illustrate that cultured trout follicles sequester VTG at rates comparable to those occurring in-vivo in the rainbow trout (Tyler et al., unpublished data) and to the rates established in-vitro for Xenopus oocytes (Wallace et al.,1970). The observation that VTG uptake was not saturated upto an external VTG concentration of 13mg/ml is consistent with high blood levels of VTG (upto and exceeding 50mg/ml) which occur in vitellogenic trout, and further illustrates the rapid turnover that VTG undergoes during vitellogenesis. The reduced ability of follicles to sequester VTG in the presence of EDTA is a feature also observed in Xenopus (Wallace et al.,1973) and similarly with EGTA in the locust (Rohrkasten and Ferenz,1985) and probably reflects the inhibition of receptor-mediated endocytosis caused by these agents chelating Ca^{++}.

This communication shows that an in-vitro system can be used as a model for detailed studies of VTG uptake into trout follicles. Other investigations on the mechanisms controlling VTG sequestration, in particular the hormonal control of this process, are currently being made.

This work was supported by a SERC grant to JPS and NB.



Rearing Environment Modifies Age at Sexual Maturity in Atlantic Salmon (Salmo salar)

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Atlantic salmon from Western Arm Brook (WAB), Newfoundland, which mature in nature exclusively as grilse (one sea winter) were artificially spawned near the river; fertilized eggs from four single pair (family) matings were moved to the St. Andrews, N.B. Biological Station, incubated and reared to the smolt stage. Fourteen month old (1+) smolt were identified as to family by fin clip, moved to a commercial salmon farm in S.W. Bay of Fundy and grown in a sea cage. After 17 months, 67.5% of males and 47.6% of females were mature as grilse. Whereas all WAB salmon mature as grilse in nature, only 56.5% matured under cage culture conditions. In September, both mature and immature individuals took on the dark color of mature salmon. In another stock of salmon (Saint John River) reared at the same farm, immature fish were bright and silvery. The sex hormones, testosterone, 11-keto testosterone and 17 alpha, 20 beta dihydroxy progesterone were assayed for WAB fish samples in late November. Levels of these hormones are discussed in relation to the spawning colors observed in immature fish. There were differences between families in respect to survival to various life stages, incidence of sexual maturity, and size. Mature males and females were consistently larger than immature fish of the same sex in respective families. WAB salmon were much smaller at comparable mature and immature stages than Saint John River fish reared under similar conditions. These observations confirm earlier conclusions regarding genotype-environment interaction in respect to age at maturity.

HORMONAL CORRELATES OF SEX CHANGE AND COLOUR PHASE CHANGE IN A FREE-LIVING POPULATION OF THE STOPLIGHT PARROTFISH (Sparisoma viride, Scaridae)

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Summary

Sex steroids were measured by RIA in plasma samples obtained from free-living stoplight parrotfish. Gonadal sex was determined histologically and individuals classified as female, intersex or male. Sex change (female to male) and colour phase change ("initial" to "terminal" colour phase) are not always simultaneous in this species, resulting in two colour (and behavioural) types of males. However, initial colour phase males eventually transform into terminal phase males. The results suggest that brief surges of 11-ketotestosterone (11kt), but not testosterone (T) accompany both sex change and colour phase change. Furthermore, sex change seems to be accompanied by decreased production of estradiol (E), although males with initial phase colouration had higher plasma levels of E than males with transitional or terminal phase colouration.

GONADOTROPHIN LEVELS IN RAINBOW TROUT (SALMO GAIRDNERI) DURING SEXUAL MATURATION

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Many reports have detailed the changes in blood gonadotrophin (GTH) levels accompanying the annual reproductive cycle of trout. A few have also examined the level in the pituitary gland. Although these studies have provided much information, they have also raised many questions, mainly because the results have often not been those anticipated. As part of a broader study investigating endocrine and morphological changes during sexual maturation, we have determined both the blood and pituitary GTH level in two strains of rainbow trout, an autumn-spawning strain and a winter-spawning strain. Blood samples were collected monthly from at least 10 males and 10 females, beginning when the fish were 6 months old and continuing until they were 2½ years old. We used a very sensitive GTH RIA capable of detecting 0.1 ng GTH/ml. If there are two or more GTHs in trout, this assay measures the one referred to as the steroidogenic or ovulatory GTH.

Changes in pituitary GTH content were indistinguishable between males and females. Pituitary GTH levels were very low, or undetectable (5 ng/pituitary) in all fish up to 1 year of age. Thereafter there was a very clear distinction between those fish that would have spawned for the first time as 2-year olds (nearly all of the males and about half of the females) and those that would have spawned for the first time as 3-year olds. In the former group GTH levels began to rise approximately 12 months before their first spawning season. Thereafter they increased exponentially, approximately trebling each month, such that each pituitary contained about 500 ng GTH 6 months before the spawning season, 1500 ng GTH 3 months before the spawning season, and 100,000 ng GTH at spawning. The GTH content of the pituitary gland remained very high for a long time, and even 6 months after spawning, at which time gonadal recrudescence for the next spawning season was well under way, the level had fallen only to about 4000 ng GTH. In the fish that would have spawned for the first time as 3-year olds, the pituitary GTH content remained low throughout their second year. It did increase slowly during this time, but only in proportion to the increase in body size of the fish. Thus when these fish were 2 years old their pituitaries contained about 500 ng GTH, around 0.1% of that present in the pituitaries of fish of similar size that did spawn that year. From 2 years onwards the pituitary GTH level in these fish began to increase dramatically. It had reached about 4000 ng when the fish were 2½-years old, the same value as that present in fish of the same age that had spawned previously as 2-year olds.

Blood GTH levels did not undergo such dramatic changes. In males GTH levels were very low (0.3 ng/ml) in immature fish. There was a slow increase during the last few months of sexual maturation, but the highest mean level reached, at spermiation, was only about 3-fold that recorded in immature fish. The GTH level in the blood fell rapidly after the spawning season, to return to that observed in immature fish. In many respects a similar pattern was observed in females. Blood GTH levels remained very low (0.3 ng/ml) for much of the reproductive cycle, and rose only in the last 3 months, during the phase of very rapid oocyte growth. Just before ovulation blood GTH levels began to rise very rapidly, to reach around 25 ng/ml at ovulation, a value much higher than that found in mature males. As observed in males also, the GTH level fell quite quickly after spawning, especially when compared with the pituitary GTH level, which remained very high for many months.

There were no differences between the two strains, with the exception that all changes occurred two months earlier in the autumn-spawning strain than in the winter-spawning strain. Although we measured the GTH level in over 1000 blood samples, there was no sign of any episodic release of GTH at any stage of the reproductive cycle.

In summary, whereas pituitary GTH content provided a good indication of the degree of sexual maturing, blood GTH levels did not. This latter observation is perhaps surprising in light of the fact that rainbow trout grow very large gonads, growth that is presumably driven by gonadotrophin. Possibly the very high levels of sex steroids present in these fish for much of the reproductive cycle keep the blood GTH level very low, or, alternatively, there may be a second GTH not detected by the RIA used in this study.

THE INTERACTION OF STRESS AND REPRODUCTION IN TROUT

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It is well established that many forms of environmental stress can inhibit reproductive processes in higher vertebrates, and a reasonable amount is known about the mechanisms underlying the phenomenon. However, although there is a considerable amount of circumstantial evidence, obtained from the study of both natural and laboratory populations, that stress can depress the reproductive performance of fishes, there is very little direct evidence. This communication describes the results of experiments undertaken to assess two aspects of the potential interaction of stress and reproduction; firstly, the effects of environmental stress of the reproductive hormones of the brown trout, Salmo trutta, and, secondly, the effect of sexual maturity on the stress response.

Sexually mature male brown trout were subjected to an acute stress, which consisted of netting the fish from their rearing tanks, transferring them to a small confinement tank for a period of 1 hour, and then returning the fish to their own rearing tanks. This procedure caused a marked stimulation of the hypothalamic-pituitary-interrenal (HPI) axis; ACTH and cortisol levels had risen from basal levels of 50 pg/ml and 13 ng/ml, respectively, to 120 pg/ml and 70 ng/ml at the end of the 1 hour confinement. Upon return to the rearing tanks the ACTH level fell rapidly to that of the control fish, and remained there for the following 48 hours. Cortisol levels took longer to return to unstressed values; they were still elevated after 8 hours, but back to basal by 24 hours. This expected stimulation of the HPI axis was accompanied by a suppression in plasma androgens. Testosterone levels were significantly depressed, compared to the unstressed fish, after the 1 hour of confinement, and remained depressed for at least 8 hours, before returning to the unstressed level. Plasma 11-ketotestosterone levels were also significantly depressed, compared to the control fish, but only at the 4 hour sampling point. Plasma gonadotrophin (GTH) levels were high at the beginning of the experiment, because the fish were fully mature. The 1 hour of acute stress caused a significant increase in the blood GTH level, from 2 to 4 ng/ml. This elevated level was maintained for a further 3 hours at least, but had fallen back to the level in unstressed, control fish by 8 hours.

The effect of chronic stress on the reproductive hormones of mature male brown trout was assessed by confining fish in small glass aquaria for one month, while unstressed, control fish remained in their rearing tanks. The confined fish did not fully acclimate to the conditions, and even after one month they still had significantly elevated plasma cortisol levels (14 ng/ml) compared to the unstressed fish (2 ng/ml). The stressed fish also had significantly depressed plasma testosterone and 11-ketotestosterone levels after 1 month of chronic confinement. As observed in the experiment assessing the effects of acute stress, this suppression of plasma androgens was more pronounced for testosterone than for 11-ketotestosterone.

In experiments carried out to investigate various aspects of the stress response of trout, we have observed that the degree of sexual maturity of the fish affected the stress response. When the trout were laparotomized at the end of the experiments, some of the males were found to be immature, while others were maturing or mature (depending on the time of year when the experiment was conducted). In three separate experiments, two with brown trout and one with rainbow trout, the maturing or mature fish showed a significantly reduced cortisol response to stress compared to the immature fish. For example, acute stress elevated the cortisol level of immature brown trout to 81.4 $\pm$ 5.1 ng/ml, whereas in the same experiment it reached only 34.3 $\pm$ 5.7 ng/ml in mature fish.

In summary, we have provided direct evidence that the HPI axis and the hypothalamic-pituitary-gonadal axis are linked in male trout; our results demonstrate that both acute and chronic stress can affect the reproductive hormones, and that the degree of sexual maturity affects the stress response.

The relationship of yolk vesicles and cortical alveoli in teleost oocytes.

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The relationship of yolk vesicles and cortical alveoli in oocytes of teleost fish has been unclear. Recent studies on growing oocytes of the cyprinodont, Fundulus heteroclitus, have been directed at clarifying this relationship. Yolk vesicles and cortical alveoli are morphologically indistinguishable, membrane-limited vesicles that show identical staining properties with both classical dyes for acidic glycoproteins and with several fluorescent-labeled lectins for specific carbohydrate moieties. Lectin (Dolichos biflours agglutinin)-affinity chromatography was used to isolate from yolk vesicle-stage follicles a >200-kDa glycoconjugate that had been shown previously to be synthesized within the oocyte and to reside within yolk vesicles. Antiserum was prepared against the >200-kDa glycoconjugate and antibody specificity was verified on immunoblots. Indirect immunolabeling of sections prepared from various sized follicles and eggs demonstrated that the antibody reacted with a substance present only in yolk vesicles and cortical alveoli. These studies substantiate earlier morphological observations that suggested that yolk vesicles of small oocytes give rise to cortical alveoli of eggs. As a corollary, they also indicate that the term "yolk vesicle" is a misnomer since these structures, when mature, ultimately release their contents into the perivitelline space at fertilization, rather than serve as true yolk, which provides a source of nutrients for the embryo.

STEROIDS IN MALE REPRODUCTION

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I - TISSUES IDENTIFIED AS ABLE TO SYNTHETIZE OR TO METABOLIZE STEROIDS IN MALE FISH

- The testes = Steroidogenic cells in the fish testes. Is there need for a specific terminology ?
Identification of the true Leydig cell homolog.
Arguments for steroidogenic potentialities in Sertoli cell homolog.
- The tractus
- Other tissues.

II - STEROIDOGENIC POTENTIALITIES OF THE TESTIS, PERIPHERAL METABOLISM AND PLASMA SEX STEROIDS DURING THE REPRODUCTIVE CYCLE

- Is there a $\Delta 4$ or $\Delta 5$ predominant pathway ?
- The now "classical" steroids in male :
  11 - oxygenated compounds.
- The neglected $5\alpha/5\beta$ reduced compounds.
- New interest for progestins in male.
- Is there oestradiol in male fish ?
- Conjugations.

III - REGULATION OF SEX STEROID PRODUCTION

- Pituitary factors
- Tissues cooperation
- Temperature

IV - BIOLOGICAL SIGNIFICANCE OF STEROIDS IN MALE REPRODUCTION

- Androgens : their activity on secondary sexual characters is well shown. Their role on spermatogenesis is not so clear.
- Progestins :Is 17α-hydroxy-20β-dihydroprogesterone a specific hormone in spermiation control ? Is 20β-oxydoreductase a key enzyme in male as it is in female reproduction ?

V - CONCLUSION

CHARACTERIZATION AND GtH REGULATION OF MICROSOMAL OVARIAN AROMATASE ACTIVITY IN RAINBOW TROUT

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Introduction

The maturational gonadotropin (GtH) has been claimed to depress aromatase activity in fish ovary, especially at the end of the sexual cycle. These conclusions were based on *in vivo* analysis of plasma oestradiol (E2) levels (Kraak *et al.*, 1984), *in vitro* measurements of E2 output (Fostier & Jalabert, 1986) and *in vitro* studies of androstenedione metabolism by ovarian tissues (Sire & Depeche, 1981).

To confirm GtH action, we partially isolated aromatase activity by subcellular fractionation for further characterization and kinetic studies.

Experimental

Microsomes were obtained by differential centrifugation of homogenates from preovulatory or vitellogenic (oocyte mean diameter : 2.5 mm) ovaries. They were incubated 10 min. with NADPH and 3H androstenedione ($\Delta 4$) or testosterone (T). Oestradiol and oestrone production was measured after extraction and chromatography. Oestrogens identity was checked in two cases by crystallization to constant isotopic ratio.

To study aromatase activity regulation, ovaries were preincubated with or without GtH (60 ng/ml) for 24 h before their processing for subcellular fractionation.

Results

The aromatase specific activity was compared in the various fractions collected during subcellular fractionation. It was mainly located in the microsomal pellet (195000g pellet : 92 pmoles oestrogens/min.mg ; total homogenate : 0.2 pmoles/min.mg). Aromatization was therefore studied in the microsomes.

The activity was linear with time during at least 10 minutes. Specific activity was not affected by microsomal protein concentration between 1 and 5 mg. Androstatrienedione, known as an aromatase inhibitor in mammals, was also a powerful inhibitor of oestrogens synthesis in our preparations (I 50 : 90 nM, for a 200 nM 4 concentration).

Kinetics of aromatization was investigated in microsomes issued from GtH treated or control ovaries. In most cases, the Michaelis constant (Km) as not affected while Vmax was lowered (table) by GtH treatment.

Table : Effect of GtH pretreatment (60ng/ml) of trout ovaries on their microsomal aromatase activity
* : significantly different from the control

Sexual cycle period	substrate	Vmax (pmoles/min-animal) control	+GtH
preovulatory	$\Delta 4$	8	8
		20	7 *
		11	7 *
		4	3 *
vitellogenesis	$\Delta 4$	46	47
		39	25 *
	T	46	31 *

Conclusion

As in mammals, trout ovarian aromatase appears to be mostly located in microsomes. Its partial isolation provides a new tool to study the regulation of its activity.

We confirm GtH inhibitory effect on ovarian aromatase activity during the second half of the sexual cycle.

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ANDROGEN BINDING PROTEIN IN TELEOST TESTIS (Salmo Gairdneri)

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Introduction

In several mammalian species the action of androgen on the genital tract is believed to be modulated by an androgen binding protein, ABP, specifically produced in the testis by the Sertoli cells (Lobl T.J., 1981) and present in the seminal plasma (Jegou & Le Gac,1978). Such a protein has,to our knoweldge, never been looked for in lower vertebrates, although it would provide an excellent tool to study Sertoli cell function and regulation.The aim of the present study was to investigate sex steroid binding in trout testis.

Results and discussion

A factor binding tritiated testosterone was detected using "steady state" polyacrylamide-gel electrophoresis. It migrated with a Rf identical to that of rat ABP. This binding was thermolabile, and was competitively inhibited by unlabelled testosterone. The dissociation of the steroid-protein complex was rapid (t 1/2 = 2 min.).

The steroid binding protein was found in :
1) cytosols from trout testis, wich had been previously perfused to reduce plasma contamination
2) in trout seminal plasma
3) in the testicular explant incubation media (in larger quantities than could be measured in the explants at the beginning of incubation).

Using a quantitative assay which utilizes DEAE bio-gel (Johnson et al., 1985) and Scatchard analysis, the following results were obtained with a spermiating testis :

	number of sites	affinity constant (4°C)
cytosol	357 pmoles/2gonads	$3.5\ 10^{8}\ M^{-1}$
incubation media	13.5 pmoles/g/16hrs	$3.9\ 10^{8}\ M^{-1}$

Hormonal specificity was studied by the competition of 3H-T binding with several concentrations of unlabelled competitors and the following order for affinities was obtained : 5α-dihydrotestosterone > testosterone > androstenedione>> oestradiol ≅progesterone ≅ 11-cetotestosterone≅ 17 α hydroxy 20 ß dihydroprogesterone>> cyproterone acetate>cortisol.

Dissociation kinetics, electrophoretic mobility, affinity and steroid specificity decribed here differe widely from a classical androgen receptor's characteristics, but are typical of extracellular binding protein. A steroid binding protein (SBP) has been demonstrated in trout plasma (Fostier & Breton, 1975) but certain arguments ruleout blood contamination. High testicular cytosol and seminal plasma concentrations and apparent in vitro production indicate that the testis may synthesise an "ABP-like" protein in the trout. Such a factor would complicate testicular steroid receptor measurments. However, it would provide a unique marker of sertoli cell activity in various physiological or experimental situations.

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BINDING AND ACTION OF s-GtH ON RAINBOW TROUT TESTIS AT VARIOUS STAGES OF GAMETOGENESIS

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Introduction

The steroidogenic response of the trout testis to maturational gonadotropin was studied at various stages of gametogenesis, in relation to the binding characteristics of GtH.

Results and discussion

The effect of Salmon GtH (provided by B. Breton) on steroidogenesis was studied during in vitro incubations of testicular tissue.

S-GtH stimulated production and output of 11-cetotestosterone (11KT) and 17α-hydroxy-20 β dihydroprogesterone (17α-20 β-OHP) at all stages studied. This effect was a function of incubation duration and of GtH concentration.

The steroid production under GtH stimulation was analyzed as "relative response" ("stimulated" versus "basal" production) ans as sensitivity (defined by determination of the GtH concentration necessary to induce half-maximal stimulation : ED50)

For both steroids, the relative response to GtH increased during maturation.

The sensitivity of 11K production to GtH increased during spermatogenesis : It was minimal during early gametogenesis (ED50=40 ng/ml) and maximal at the beginning of spermiation (ED50=6 to 10 ng/ml).

For 17 20 OHP production, the sensitivity to GtH increased during maturation and was greatest at the time of full spermiation (beginning of gametogenesis : ED50=100 ng/ml ; spermiation : ED50=3 ng/ml).

GtH receptors were studied by Scatchard analysis of specific ^{125}I-s-GtH binding on testicular membrane preparations. The high affinity component of this binding was studied in terms of affinity and binding capacity expressed per pair of gonads.

Significant changes in the affinity constant values at the different stages could not be demonstrated (Ka : 1 to 4 x 10^{10} M^{-1}). However, the maximum number of sites measured in these membrane preparations increased dramatically during gametogenesis.

The increase in plasma 11 KT and 17α20β OHP observed at the end of the reproduction cycle, which could play a role in initiation and maintenance of spermiation, (Baynes & Scott, 1985 ; Fostier et al., 1982 Ueda et al., 1983) could be linked to an enhanced sensitivity to GtH. This could be due in part to an increase in GtH receptor number during this period.

Stage	Regressed	Beginning of gametogenesis	Prespermiation
Ka M^{-1}	1,3 10^{10}	1,8 10^{10}	2,5 10^{10}
B max fmoles 2gonads	detection limit	93	830

stage	spermiation	end of spermiation
Ka M^{-1}	2,7 10^{10}	1,4 10^{10}
B max fmoles/2 gonads	300 to 2000	153

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REGULATION OF GONADOTROPIN HORMONE SECRETION IN RAINBOW TROUT BY GnRH AND STEROID HORMONES - AN IN VITRO STUDY

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In rainbow trout, variations of plasma and pituitary gonadotropin hormone (GtH) contents, of gonadal steroid hormone circulating levels (Billard et al. 1978), of pituitary responsivness to gonadotropin-releasing hormone (GnRH) (Weil et al.,1978) have been studied in our laboratory throughout the sexual cycle.

The purpose of the present work is to study in vitro the regulation of pituitary GtH secretion by salmon GnRH and steroid hormones.

Primary cultures of pituitary cells, collected from fish at different stages of gonadal development, were obtained by dispersion with collagenase 0,1 % (20 hr - 12°C) followed by a 3-day preincubation period as previously described (Weil et al., 1986). The 6. 2 x 10^4 cells initially plated were submitted to increasing doses (0-10^{-6} M) of salmon GnRH (sGnRH) during a 24-hour incubation period.

The main steroids, involved in oocyte maturation (17 α-hydroxy - 20 β-dihydroprogesterone), in vitellogenesis (oestradiol -17 β), in spermiation (11 ketotestosterone, 17 α-hydroxy - 20β-dihydroprogesterone) were tested by adding them to the preincubation medium.

In steroid free cultures (control cultures),basal GtH release (dose 0 of sGnRH during the incubation period) and sGnRH - induced GtH release were variable throughout the sexual - cycle, in a characteristic pattern as predicted from previous in vivo experiments (Weil et al., 1978). For females, the highest values for basal GtH release and for responsivness to sGnRH were found on the day of ovulation. For males they were recorded at the end of spermatogenesis and at spermiation.

In steroid pretreated cultures, the response to sGnRH was modified in comparison with control cultures, depending on the ovarian stage. It was found that a steroid may desensitize or sensitize pituitary gonadrotrophs to sGnRH.

The results obtained, indicate that the variation of circulating GtH levels may be partly due to a direct action of gonadal steroid hormones on pituitary gonadrotrophs by modulating their responsivness to GnRH.

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BIOLOGICAL ACTION OF LAMPREY GONADOTROPIN-RELEASING HORMONE IN LAMPREYS

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Gonadotropin-releasing hormone (GnRH) is the major hypothalamic peptide known to control the pituitary-gonadal axis in mammals. The presence of GnRH in the hypothalamus in many different species of vertebrates signifies a functional role that has been conserved throughout vertebrate evolution. We have recently determined the structure of GnRH in the sea lamprey (Petromyzon marinus), as pGlu-His-Tyr-Ser-Leu-Glu-Trp-Lys-Pro-Gly-NH_2 (Sherwood et al., 1986). The structure of the lamprey GnRH differs in five amino acids compared with mammalian GnRH and chicken GnRH I and in four amino acids with salmon GnRH and chicken GnRH II.

Lampreys belong to the class of Agnathans, the oldest living vertebrates. Although lampreys lack vascular or neural connections between the hypothalamus and adenohypophysis, the control of the hypothalamus by the pituitary is probable by diffusion of GnRH across the connective tissue to the adenohypophysis (Gorbman, 1965; Nozaki et al., 1984). Physiological studies utilizing mammalian GnRH analogues which stimulated various reproductive processes (Sower et al., 1982, 1983, 1985) have provided evidence for the regulatory influence of the hypothalamus on the pituitary-gonadal axis. More recently in our first reported paper on the biological activity of lamprey GnRH, we demonstrated that ovulation had occurred in 80% of the lampreys treated with either a single injection or two injections of lamprey GnRH at 0.2 or 0.1 ug/kg (Sower et al., 1987). This present paper examines the biological activities of lamprey GnRH, a lamprey GnRH putative antagonist ([D-$Phe^{2,6}$, Pro^{3}] lamprey GnRH) and a mammalian GnRH superagonist ([D-Ala^{6}, Pro^{9} NEt] mammal GnRH) on steroidogenesis as an indicator of pituitary function in female and male adult sea lampreys in two different reproductive stages to further enhance our understanding of the role of hypothalamic GnRH in reproduction in lampreys.

The lamprey GnRH or the mammalian GnRH superagonist significantly stimulated plasma estradiol and progesterone in male and female lampreys undergoing the final maturational processes (Figs 1 and 2). In contrast, in male and female lampreys in the parasitic phase, plasma estradiol decreased and progesterone increased in response to lamprey GnRH (0.1 or 0.05 ug/g) or the mammalian GnRH superagonist (0.05 or 0.025 ug/g). The putative lamprey antagonist which significantly inhibited ovulation in our earlier study (Sower et al., 1987) had no effect on estradiol levels in the present study except at a dose of 0.05 ug/g in the female lamprey in which estradiol levels were significantly higher than controls at 24 hr. However, this putative lamprey antagonist at doses of 0.3, 0.15, or 0.075 ug/g, stimulated estradiol levels but had no effect on progesterone levels in the male lampreys.

In summary, lamprey GnRH is biologically-active in stimulating the pituitary-gonadal axis and its activity is dependent upon reproductive stage of the lamprey. The lamprey GnRH molecule has retained the length and NH_2- and COOH-termini of the GnRH molecule and has been conserved in its function in terms of its ability to stimulate the reproductive system in the lamprey. Increasing our understanding of the structure and function of the vertebrate GnRHs may contribute to our understanding of the evolution of the reproductive system in vertebrates.

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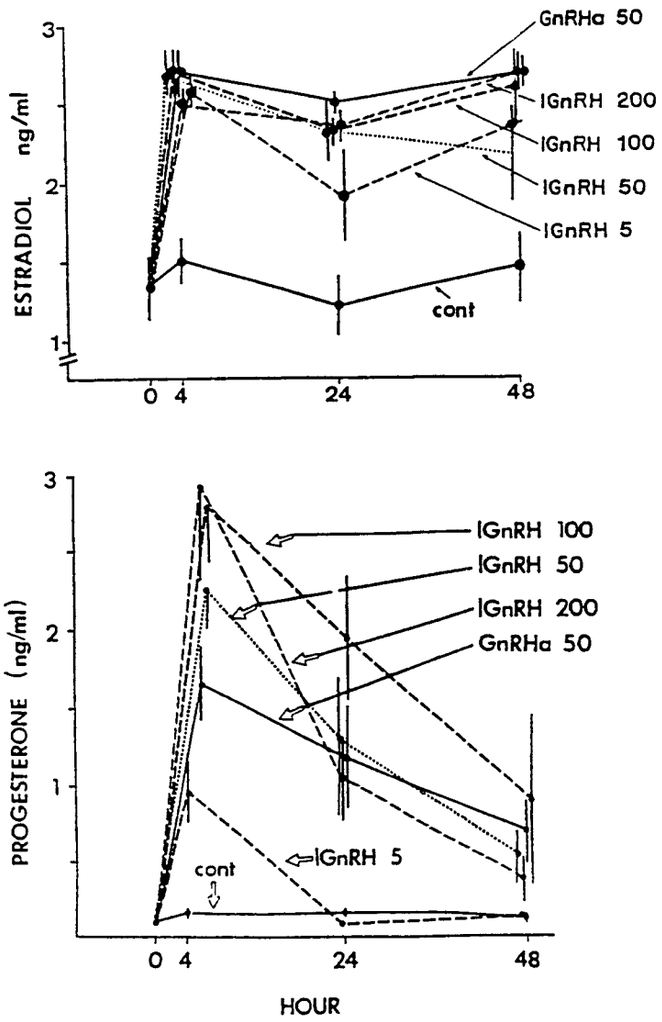

Figure 1. Plasma estradiol (ng/ml) and progesterone (ng/ml) of male lampreys injected at 0 hr with saline (cont), lamprey GnRH (lGnRH) at 200, 100, 50, or 5 ug/kg, or [D-Ala^6, Pro^9 NEt] GnRH (GnRHa) at 50 ug/kg. Plasma samples were taken at 0, 4, 24, and 48 hr after the injection.

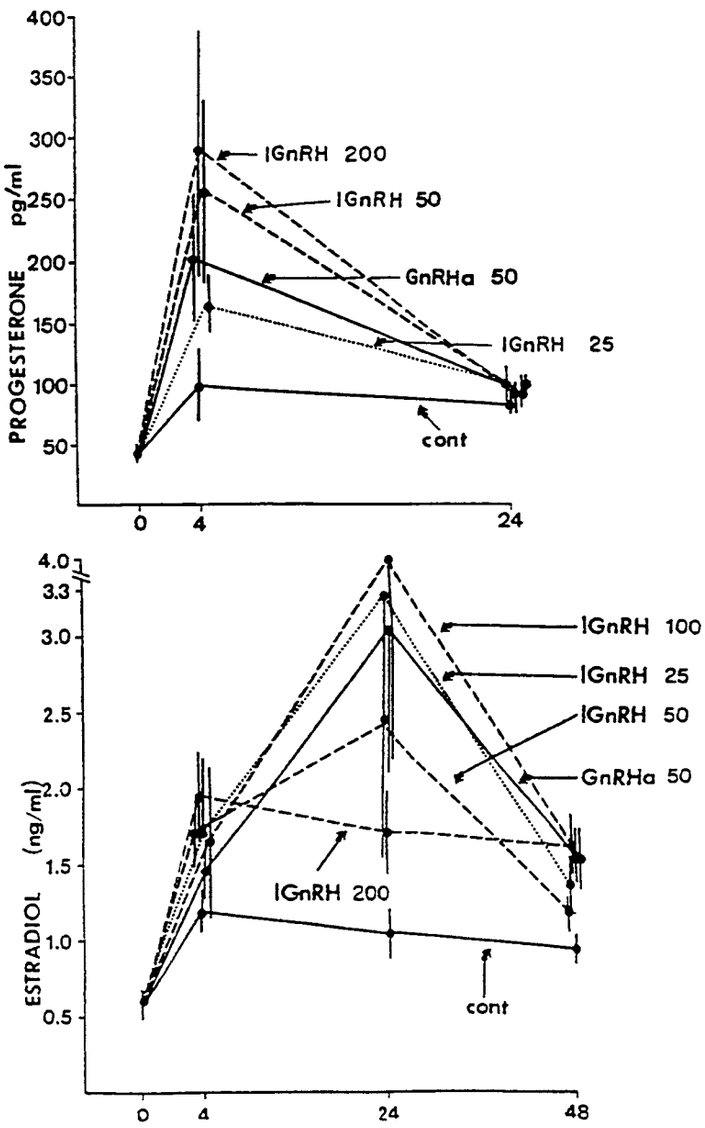

Figure 2. Plasma estradiol (ng/ml) and progesterone (pg/ml) of female lampreys injected at 0 hr with saline (cont), lamprey GnRH (lGnRH) at 200, 100, 50, or 25 ug/kg, or GnRHa at 50 ug/kg. Plasma samples were taken at 0, 4, 24, and 48 hr after the injection.

17 α -hydroxy-20 β -dihydroprogesterone in the duct fluid of sex-inversed rainbow trout

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Adult rainbow trout (Salmo gairdneri) that are genetically female, but have developed functional testes following methyltestosterone treatment at the time of first feeding (sex-inversed), very often develop vasa deferentia that are not continuous with the testes nor the cloaca. During the time of maturation of spermatozoa within the testes, the ducts may become distended with fluid that has K^+ and Na^+ concentrations comparable with normal seminal fluid, but no spermatozoa (or occasionally very few) enter the duct. Although seminal fluid containing spermatozoa has 2-20 ng ml^{-1} of the steroid 17α -hydroxy-20 β - dihydroprogesterone, when spermatozoa are absent the steroid is at a concentration of less than 1-2 ng ml^{-1}.

COMPARATIVE IMMUNOLOGICAL RESPONSES BETWEEN CATLA, CYPRINUS AND MYSTUS PITUITARY GTH AND THEIR RELATIVE BIOLOGICAL ACTIVITY AFTER PURIFICATION.

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The profile of immunological responses displayed by pituitary GTH of Catla catla, Cyprinus carpio and Mystus seenghala, though belonging to the same order, did not show parallelism in their cross - reactions when tested in heterologous RIA systems. C. catla GTH demonstrated some relatedness with C. carpio GTH but neither C. catla nor C. carpio GTH showed any affinity with GTH obtained from M. seenghala. Despite the insignificant immunological cross - reactivity between GTH of different fishes similar biological activity was recorded as judged by testosterone and estradiol - 17B production from oocytes in vitro. Purified GTH of each fish was almost equally potent in stimulating the production of these steroids in in vitro culture of oocytes either from the same or other species. However, the biological activity varied after the different methods and degree of purification but pituitary GTH of each fish obtained through similar method of purification gave identical biological response. The apparent spectacular declension in biological potency could be correlated with the purity of GTH preparation. Parallelism in biological activity of purified pituitary GTH between different fishes of same order was noticed which was not reflected by immunocrossreactivity.

Thus RIA assesment of GTH from one species in heterologous system does not necessarily correspond with its biological response.

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Variations in hepatic metallothionein, zinc and copper levels after estradiol treatment of rainbow trout, Salmo gairdneri.

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Metallothioneins (MT), a class of heavy-metal binding proteins, are characteristically of low molecular weight and enriched in cysteine. MTs have been widely observed among eukaryotes and are especially noted for the induction of their synthesis by heavy metal ions and glucocorticoids. The biological role of MT has been subject to numerous investigations but the physiological function is still unclear. MT appears, however, to be involved in the metabolism of zinc and copper. Several investigations have focused on the normal variations in MT content of the liver during foetal and neonatal development in mammals. A recent study demonstrated that MT may be involved in the regulation of zinc during the annual reproductive cycle in rainbow trout. The objective of the present study was to determine the variations in hepatic metalloathionein, zinc and copper levels, in rainbow trout, after intraperitoneal injections of estradiol. The fish were injected with 10 mg estradiol / kg body weight and sampling was performed after 2, 7, 14, 21, 28 and 35 days. The amount of zinc and copper was determined using flame atomic absorption spectrophotometry after digestion with (70%) nitric acid of the liver homogenates. Samples for MT quantification were treated by centrifugation at 10.000 x g at 4°C during 10 min prior to and after heat denaturation at 95°C for 5 min. The MT levels were determined by differential pulse polarography. The total hepatic zinc levels rose in the estradiol treated fish and peaked after 21 days to return to normal levels after 35 days. No change was observed in copper levels of the liver. The Vitellogenin mRNA levels rose in the estradiol treated fish to peak after 21 days and decreased thereafter. The MT levels increased after 21 days in total liver and remained high after 35 days. The present results provide evidence for a zinc regulatory role of MT during exogenous vitellogenesis in rainbow trout, thereby ensuring the animal of a control mechanism to keep the pool of available zinc at an appropriate level.

CURRENT STATUS OF HORMONES AND SEXUAL BEHAVIOUR IN FISH

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Correlational studies combined with traditional 'removal and replacement therapies' have confirmed the involvement of the endocrine system in the regulation of reproductive behaviour in fish. Nevertheless, the precise nature of the role of hormones is far from clear, and there remain a number of puzzling anomalies in the data obtained thus far.

Two important developments promise to increase our understanding of hormone-behaviour mechanisms in fish. First, radioimmunoassay techniques have made it possible to describe in considerable detail the relationship between changes in specific hormone levels and the onset, maintenance, and completion of reproductive behaviour. Second, these techniques are being combined with social and endocrine manipulations and applied to an increasingly diverse array of species of fish reproducing under natural or semi-natural conditions.

The application of this combination of procedures will be illustrated by reference to current investigations involving the rainbow trout (Salmo gairdneri) and a sex-changing parrot fish (Sparisoma viride). In both cases an attempt has been made to identify the hormones associated with particular phases of reproductive behaviour. In addition, both studies demonstrate the impact of social stimulation by sexual partners (trout) or territorial competitors (parrot fish) upon endocrine activity.

I

DEGRADATION OF GONADOTROPIN RELEASING HORMONE AND ITS ANALOGS IN THE GILTHEAD SEABREAM SPARUS AURATA: AN IN VITRO STUDY.

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ABSTRACT

In recent years there is an increasing interest in the use of GnRH analogs for the induction of ovulation and spawning in fish of commerical importance. One of the major factors controlling the biological activity of GnRH and its analogs is the rate of their metabolism. We report here the pattern and kinetics of *in vitro* degradation of salmon GnRH (sGnRH) and different analogs in the gilthead seabream *Sparus aurata*. 10 nmoles of hormone were incubated with cytosolic or crude membrane preparations of pituitary, liver or kidney at 20°C. The reactions were stopped at different times and their products were identified and quantified by reverse-phase HPLC and amino-acid analysis; as reference, fragments of sGnRH which were sythesized by the solid-phase method were used.

Results using pituitary tissue show degradation of sGnRH by the cytosolic fraction and not by the membrane fraction. There is a very high preference for a primary cleavage at the Tyr^5-Gly^6 bond. The 1-5 GnRH fragment accumulates whereas the 6-10 fragment is further degraded into single amino-acids. However, in the liver, the 1-5 fragment is further degraded into the 1-3 fragment. sGnRH and LHRH show a similar pattern and kinetics of degradation, e.g. 60% being degraded after 90 minutes. The analogs [D-Ala^6-Pro^9-NET]-LHRH, [D-Trp^6]-LHRH and [D-Arg^6-Pro^9-NET]-sGnRH are highly resistant to degradation, e.g. less than 10% being degraded after 90 minutes of incubation. Preliminary studies show similar results in the liver.

This and additional data (Zohar et al., present meeting) indicate a correlation between the resistance to degradation of LHRH, sGnRH and analogs and their potential to stimulate GtH release, ovulation and spawning in *Sparus aurata*. Thus it is demosnstrated that the resistance to degradation is a key factor in determining the superactivity of the GnRH analogs. We are currently studying the role of the degrading enzymes in the mechanisms of action of GnRH.

Acknowledgements

This research was supported by a BARD grant No. I-772-84. The [D-Arg^6-Pro^9-NET]-sGnRH was a gift of Dr. B. Breton, INRA, Rennes.

STUDIES ON GONADOTROPIN ISOLATION AND BIOLOGICAL ACTION FROM A SOUTH AMERICAN TELEOST FISH, *Semaprochilodus* sp. (1)

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Summary

Gonadotropic hormones (GtH) have been isolated in varying degrees of purity from the hypophysis of several teleosts. Otherwise, there are very few Knowledgements concerning to South American species GtH. A parcial characterization of *Prochilodus* scrofa GtH, using isoelectric focusing and gel filtration methods, and *in vitro* testosterone production by mature carp testis bioassay, has been recently performed (Val-Sella *et al.*, submitted). In order to develope an homologous radioimmunoassay (RIA) system for *Semaprochilodus* GtH, a better understanding of the reproductive cycle hormonal control in South American species, and the application on fish culture, this preliminary work was carried out using affinity chromatography (Con A-Sepharose), gel filtration (Ultrogel AcA54) and ion exchange chromatography (DEAE-Cellulose). Heterologous RIA systems for carp and salmon GtH, and SDS+PAGE electrophoresis monitored all steps of the GtH purification scheme.

The hypophysis were collected from mature animals of both sexes, caught in the Amazonas river during the spawning migration. The glands were dehydrated by acetone, preserved at 4 °C during two months, then lyophilised. About 1080 hypophysis (± 0.93 mg dry weight each) were homogenized in Tris-Buffer, then chromatographed on Con A-Sepharose column (3 X 20 cm). The adsorved portion (glycoproteins) was eluted after inclusion of 0,15M α-methyl-D-glucoside in the Buffer. The protein distribution in the obtained fractions was determined spectroscopically (OD_{280nm}). The 12 fractions included in the peak were concentrated by ultrafiltration using PM-10 membrane (Amicon), then applied on Ultrogel AcA54 column (2.5 X 100 cm). For the estimation of molecular weight (MW), standard proteins of known MW (ribonuclease, chymotrypsinogen and ovalbumin) were used as markers. There were obtained 35 fractions with higher OD, distributed in four peaks (1rst peak MW > 43,000; 2nd MW 25,000 - 43,000; a big 3rd peak and 4th MW 13,700 - 25,000). The fractions containing GtH were detected mainly in the 2nd and 3rd peaks, differently of others studies on fish GtH (Idler & Ng, 1983 for references). The correspondent fractions of 2nd and 3rd peaks were, separately, jointed and dyalised against 0.01M Tris HCl pH 7.8 Buffer and applied to DEAEC column (0.9 X 20 cm). The fractions obtained from each chromatographed sample showed to contain GtH through RIA and electrophoresis methods employed. The gonadotropic activity was evaluated by *in vitro* trout oocyte maturation (Jalabert *et al.*, 1973). The DEAEC fractions from 3rd peak were more effective, since lower doses induced higher percentage of activity.

(1) FAPESP (Proc.nº 86/0369-5)

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USE OF DOPAMINE ANTAGONISTS AND GnRH ANALOGS IN INDUCED BREEDING OF CULTURED FRESHWATER FISH IN CHINA

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Summary

Gonadotropin secretion in teleosts is primarily regulated by gonadotropin-releasing hormone (GnRH) and dopamine acting as a gonadotropin release-inhibitory factor (GRIF). Drugs that deplete catecholamines (e.g., reserpine) or dopamine receptor antagonists (e.g., domperidone, pimozide) block the GRIF actions of dopamine at the level of the pituitary, thereby potentiating the actions of enogenously administered GnRH. Injection of the combination of [D-Ala^6, Pro^{9}NEt]LHRH (LHRH-A) or [D-Arg^6, Trp^7, Leu^8, Pro^{9}NEt]LHRH (sGuRH-A) plus domperidone, or pimozide, or reserpine is highly effective in stimulating gonadotropin secretion, relative to the actions of LHRH-A on sGnRH-A alone, or any of the catecholaminergic drugs alone, in common carp, bream and loach. These combinations have also proven to be highly effective in inducing ovulation in common carp, silver carp, mud carp, bream, grass carp, bighead carp, black carp, loach and African catfish. During the spring of 1987 field trials were successfully conducted involving approximately 30,000 kg of brood stock females induced to ovulate with domperidone plus sGnRH-A or LHRH-A, and approximately 15,000 kg of brood stock fish induced to ovulate by treatment with reserpine plus sGnRH-A or LHRH-A. Following ovulation, brood stock fish were usually allowed to spawn, thereby achieving natural fertilization. This new technique for induced ovulation and spawning of cultured freshwater fish, called the Linpe method, can be judged effective by several criteria: cost effectiveness in terms of labor and reduced stress on brood stock as fish have to be handled only once to give a single set of injections, the induction of a high rate of ovulation throughout the full spawning season, ovulations are complete, the time to ovulation following injection is short and predictable, the fertility of ovulated oocytes is high, fry are normal and have high viability, and subsequent reproductive cycles of brood fish appear unaltered by the treatment to induce ovulation. The Linpe method has a growing acceptance by workers in hatcheries for freshwater fish in China; trial commercialization of the Linpe method during 1987 involved approximately 400,000 kg of brood stock fish.

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Stressful manipulation obscures signalling of sexual identity in the weakly electric fish, Gnathonemus petersii

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Several species of weakly discharging electric fish exhibit sexually dimorphic electric organ discharges (EODs) used in social communication (Kramer, 1979; Moller, 1980; Hopkins, 1983) and sexual identification (Hopkins, 1972) under laboratory and field recording conditions. The sexually dimorphic EODs are typically hormonally manipulable (Bass, 1985, 1986; Hagedorn & Carr, 1985) such that fish treated with heterologous hormones exhibit heterotypical EODs. Gnathonemus petersii, a weakly discharging African electric fish was recently reported not to show a sexually dimorphic EOD. In order to investigate environmental effects on the EOD waveform, G. petersii were confined, restrained, or allowed to freely rest. An unexpected sex difference ($\chi^2 = 2$, $p = 0.032$) in the peak power frequency of the Fourier spectrum in the free condition was eliminated by the aversive manipulations. Further, there was a dramatic decrease in variability between individual peak power spectral frequencies for both males and females in the 'free' condition compared to the relatively large variability between individuals in the 'restrained' condition ($F(1,7) = 6.15$, $p < 0.05$). It appears that a sex difference in mean peak power spectral frequencies was eliminated by the high variability in the 'restrained' condition, which resulted in greater overlap among individual male and female means. Under the 'free' condition, male and female peak power spectral frequencies were clumped together in distributions which only slightly overlapped. However, in the 'restrained' condition, individuals of both sexes shifted their peak power spectral frequencies to either the low end or the high end of the frequency spectrum. Changes in environmental factors have been reported to alter the EODs of other electric fish species and influence the sexually dimorphic characteristics of their electric signals (Meyer, 1983; Bass & Hopkins, 1985; Hagedorn & Carr, 1985; Bass, 1986; Bratton & Kramer, 1986). Stressors such as capture and confinement increase circulating catecholamines and corticosteroids in many fish species (Mazeaud et al., 1977; Strange et al., 1977; Mazeaud et al., 1981). It is likely that the confinement procedures in the present study constituted stressful events for the subjects and resulted in a hormonal stress-response which, in turn, altered the EODs emitted by these fish. Stress may affect all levels of the hypothalamic-pituitary-gonadal axis and is deleterious to reproduction in many species. Corticotropin-releasing-factor has been shown to mediate the inhibition of luteinizing hormone resulting from stressful manipulation (Rivier et al., 1986). Somatic stressors have also been shown to result in decreased plasma testosterone levels, and changes in social dominance status have resulted in changes in plasma testosterone and plasma cortisol concentrations in a variety of species (Bardin & Peterson, 1967; Dessypris et al.,1976; Sapolsky, 1982). Similar hormonal changes in electric fish may influence the EOD behavior and thus the message broadcast by these signals. The EOD waveform of G. petersii could thus serve to signal information about the individual's current physiological status, be it exposure to stressors or readiness to mate. The effects of stress are deletorious to reproductive behavior across a wide range of animals. As the EOD is used in social communication and for sexual identification, the effects of aversive environmental factors on the EOD may result in stress-produced reproductive restraints on the fish.

Effects of 17∝ -methyltestosterone and mate size on sexual behavior in Poecilia reticulata

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In a variety of species, androgen administered to genetic females produces masculinized behavior patterns. In fish, the extent of masculinization resulting from such treatment appears to vary according to the subject's age and the species studied. Usually, the most pronounced effects occur when females are treated very early in life. However, without data on the effects of treating adult fish, it is not possible to assess whether the early treatment with heterologous hormones resulting in heterotypical behavior has organized the neural substrates for the behavior or simply activated the existing substrate. Testosterone fed to Poecilia reticulata for sixty days after birth behaviorally masculinized only some genetic females (Clemens et al., 1966). While 90% of the control males exhibited courtship behavior and 100% sired young, only 7% of the treated fish exhibited male sexual behaviors and 14% sired young. Further, 78% of the treated females which did not sire young produced sperm, leading the authors to conclude that there was a behavioral deficit in mating. To date, no studies involving hormone administration to adult fish reported detailed behavioral effects compared to untreated controls. The present studies quantify the behavioral effects of feeding methyltestosterone to adult female Poecilia reticulata. Treated females were paired with untreated females for 8 min behavioral tests following 24 h of isolation. The mean duration of male courtship (e.g., posturing, sigmoids) and copulatory behaviors (e.g., copulation attempts, gonopodial swings) exhibited by the treated fish were compared with the same behaviors exhibited by untreated males also paired with untreated females ($\alpha = .05$). In the first study, 100% (n=8) of the treated females and 100% (n=8) of the control males exhibited at least some courtship behaviors. Overall, treated females exhibited significantly more courtship behavior than did control males. However, analyses of individual courtship behaviors revealed that control males exhibited significantly more sigmoid displays, while treated females spent significantly more time posturing. Control males (100%) displayed significantly more copulatory behavior than treated females (62.5%). The results of this study indicated that adult treatment with methyltestosterone induced masculine behavior in genetic females, but other than preliminary courtship behavior (e.g., posturing), control males exhibited both courtship and copulatory behaviors more frequently. Observation of behavior during testing in the first study revealed that the treated females appeared to behave differently according to the size of the normal female with which they were paired. As male guppies are naturally smaller than females, this suggested that female size may be an important parameter modulating male behavior. The second study was designed to investigate the effects of pairing methyltestosterone-treated adult females with larger or similarly sized untreated females. An additional group, pairing treated females with other treated females of different size was included. Fish were treated, isolated and tested in the same manner as the first study. Treated females (100%) paired with larger untreated females exhibited significantly more posturing, sigmoids, and copulation attempts than treated females paired with untreated females of the same size. Treated females with larger untreated female partners exhibited more posturing, no difference in sigmoid displays, and significantly more copulation attempts than normal males (100%). Finally, when treated females of different sizes were paired, except for posturing, the smaller of the pair invariably assumed the male role, exhibiting significantly more sigmoids, gonopodial swings, and copulation attempts than its partner. This study suggests that hormonal treatment in the adult female guppy can induce the full complement of male courtship and copulatory behavior. In addition, it appears that mate size may be an important factor in the regulation of sexual behavior in this species. In contrast to other species, treated adults show more behavioral plasticity than treated immature females.

THE ENTRAINMENT AND FREE-RUNNING OF THE ENDOGENOUS CIRCANNUAL CLOCK WHICH COORDINATES REPRODUCTION IN THE FEMALE RAINBOW TROUT (SALMO GAIRDNERI)

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Abstract

It has been proposed that the annual cycle of maturation and spawning in the trout is controlled by an internal timing mechanism which under natural conditions is entrained by the seasonal changes in daylength (Duston & Bromage, 1987). Abrupt reductions in photoperiod e.g. 18L:6D to 6L:18D during the early stages of maturation are able to phase advance this clock or rhythm and hence the timing of maturation (Duston & Bromage, 1987).

A characteristic feature of endogenous rhythms is that they free-run under constant conditions and for trout maintained on constant short days this results in the fish spawning with a circannual periodicity (Duston & Bromage, 1986). The present study investigates whether this free-running behaviour is also exhibited by trout whose spawning time has been initially advanced by photperiodic change.

Groups of 2+ year-old rainbow trout, with a natural spawning time in December, were maintained over a two year period in light-proof 1.5m diameter circular tanks on artificial lighting. Daylength, controlled by electronic timeclocks, provided 25 Lux at the surface of the water. Water temperature was a constant 8.5 - 9°C and the fish fed on a commercial dry pellet at the rate of 0.5% of body weight day^{-1}. During the first year spawning was advanced by 3-4 months into August and September by exposing the fish to constant long days (18L:6D) from mid-January to March or April and then constant short days (6L:18D). Half the fish failed to spawn as a result of this photoperiod regime. Subsequently, all the fish, irrespective of whether they had spawned or not, were maintained on the same short days for the remainder of the experiment.

Between July and September during the second year of photoperiod treatment, a further maturation occurred in which all the fish spawned; this was approximately one year after the time in which half of the fish had undergone their first maturation.

These data show that during the first year of the experiment the 'long to short' reduction in photoperiod phase-advanced the internal clock which coordinates maturation and spawning. Subsequently, the clock proceeded to free-run under constant short days, approximately 4 months out-of-phase with the natural cycle. Similar results were also recorded in a further series of experiments using another strain of trout whose natural spawning period was January/February.

Although a proportion of the fish failed to spawn during the first year of the experiment, clearly their internal clock(s) were being phase-advanced by the 'long-to-short' photoperiod because in the following year all the fish spawned after a similar circannual period. Importantly, these results indicate that the internal clock can be dissociated from the neuroendocrine mechanisms which are responsible for the control of reproduction. It is proposed that the dissociation of the clock and the failure to spawn in a particular season occurs to prevent undersize fish from undergoing the energetically-demanding process of oocyte maturation.

This work was supported in part by a NERC award to Dr N. Bromage

THE ADVANCEMENT OF PUBERTY OR TIME OF FIRST-SPAWNING IN FEMALE RAINBOW TROUT (SALMO GAIRDNERI) MAINTAINED ON ALTERED-SEASONAL LIGHT CYCLES

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Abstract

Although there is overwhelming evidence that the processes of maturation and spawning in the rainbow trout are timed and synchronized by the seasonally-changing light cycle, it is not clear whether the timing of first spawning (i.e. puberty) which in many strains of trout occurs at the end of the second year of life, can also be modified by photoperiodic change. An advancement of puberty would be of considerable advantage to commercial hatcheries because it offers the possibility of a reduction in the time that broodstock have to be maintained on farms before egg production can begin. The effects of photoperiod on the timing of first maturation are investigated in the present study.

Beginning in December rainbow trout, 4 months after fertilization were maintained over a 3-4 year period in a series of circular tanks rendered light-proof with black butyl covers and exposed to a series of two out-of-phase compressed seasonal light cycles. Daylength (50 lux at the water's surface) was adjusted once per month by electronic time clocks so that during the first and second years of treatment the longest and shortest days occurred on April 1 & October 1 and February 1 & June 1 respectively. Subsequently, the fish were maintained on a 12-month seasonal light cycle, 7 months out-of-phase/in advance of the changing natural photoperiod. Control fish received ambient daylength (latitude 51.8°N) and otherwise equivalent conditions. Water temperature was a constant 10°C and all fish were fed throughout at rates recommended by the feed manufacturers.

In the control group of female fish first-spawning or puberty occurred in early August exactly two years after they themselves had been produced as eggs. In contrast in the fish maintained on the advanced seasonal photoperiods, spawning commenced in April, 102 days before that of the controls, when the fish were only 20 months of age. Well over 90% of the the experimental and control groups of fish matured at the time of first-spawning, at which point they averaged 1.3 and 1.9 Kg in weight respectively. During the following year the control fish spawned again in early August whereas the experimental group matured 6 months earlier in late January.

The finding that the time of first spawning in the two groups occurred when the fish were on quite different daylengths confirms the view that reproduction in the trout and possibly all other fish is controlled by an endogenous circannual clock or rhythm. It is proposed that the endogenous clock which coordinates maturation and the timing of puberty is entrained by photoperiod from an early stage of development. Consequently compression of the seasonally-changing 'light' cycle advances this clock and in turn the timing of puberty providing the fish have attained a certain critical size.

OOCYTE DEVELOPMENT IN THE RAINBOW TROUT WITH SPECIAL REFERENCE TO ATRESIA AND VITELLOGENIN INCORPORATION.

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Abstract

Light and electron microscopical techniques together with counts of oocytes at the different stages of development were used to study the structural and compositional changes in the ovaries of three Oct/Nov, Nov and Dec/Jan spawning strains of rainbow trout (Salmo gairdneri) over the first three years of their lives. Concurrently, the seasonal changes in ovarian histology were correlated with circulating levels of oestradiol-17B and testosterone measured by radioimmunoassay and vitellogenin (as calcium) analysed by fluorimetry.

A methacrylate plastic embedding technique enabled 1-2 u sections to be produced even of the largest yolky oocytes. Seven stages of oocyte development were identified, together with primary and secondary oogonia and hypertrophic and non-hypertrophic (oolytic) atresia. Oogonia were present throughout the reproductive cycle with the largest numbers occurring immediately after sex diffentiation and also during the post-ovulatory period. After the first spawning, stage 4 oocytes were present at all stages of the subsequent reproductive cycles indicating that there is no discrete stage or phase of vesicle (endogenous 'yolk') formation in previously matured fish. In contrast, for each strain and age-group of trout, vitellogenin incorporation (true or exogenous vitellogenesis), assessed histologically and also by using horseradish peroxidase as a tracer, occurred during a specific period of the annual cycle. In 1 + year-old fish, vitellogenin sequestration by the oocytes commenced during July/August, whereas in 2+ year-olds this process started in April.

Atresia was a common phenomenon during the vitellogenic phases of oocyte development (stage 4, 5, 6 and 7), with atretic oocytes numbering up to 700 $100g^{-1}$ body weight, i.e. 5-10% of the total numbers. There were also significant reductions in the numbers of previtellogenic oocytes although the presence of interfollicular spaces was the only evidence that this was due to atresia. It is suggested that atresia is an important determinant of fecundity in the trout.

THE INFLUENCE OF RATION SIZE ON THE PRODUCTIVE PERFORMANCE OF FEMALE RAINBOW TROUT (SALMO GAIRDNERI)

John Jones & Niall Bromage

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Abstract

Despite the widespread belief that reproductive performance is affected by nutrition few studies have properly differentiated the direct effects of ration on fecundity and egg size from those solely due to the size and growth of the parent fish. Those studies involving wild stocks offer only circumstantial evidence since dietary influences are difficult to quantify and to separate from other environmental variables. Although some laboratory investigations point to a direct effect of nutrition on both fecundity and egg size much of the evidence is obscure and in some instances contradictory. The present study examines the effects of five different rations on the reproductive performance of rainbow trout broodstock.

Approximately 4000 one year old, all-female rainbow trout were distributed between five sea cages and maintained under commercial conditions on separate rations corresponding to either 0.4%, 0.75%, 1.0%, 1.2% or 1.5% body weight day^{-1}. Throughout the maturation period prior to first spawning the mean daily water temperature and salinity were 11.7°C ± 3.6 (mean ± S.D.) and 30.3 ppt ± 2.1 (mean ± S.D.) respectively. At spawning ripe fish were anaesthetized with 2-phenoxyethanol and the eggs manually-stripped, fertilised and water-hardened in freshwater. Subsequently ova diameter, total fecunidty, total egg volume and percentage of fish spawning from each treatment group were determined. Covariance analysis was used to distinguish the effects of ration on egg production parameters from those merely relating to fish size.

As expected the serial weight increases of fish during the year in each of the five cages was directly proportional to the size of ration received with the highest ration producing the heaviest mean fish weight at spawning.

Both within group and between group total fecundity, relative fecundity, total egg volume and relative egg volume were positively correlated with post-stripped weight. Partitioning of the effects of ration and fish size by covariance analysis revealed that when comparing fish of similar weights those receiving the two lowest rations produced significantly fewer eggs and a lower total egg volume than those fish which were maintained on the upper three ration levels. In addition little advantage was gained as far as egg number and total ova volume were concerned in feeding broodstock above 1.0% suggesting that an optimum ration exists between 0.75% and 1.0% body weight day^{-1}. Surprisingly, regression analysis failed to demonstrate a significant correlation between ova diameter and fish weight in any of the five different groups suggesting that ration has no effect on egg size in fish spawning for the first time.

This work shows clearly that diet has a significant effect on the production of eggs over and above that due to the growth and size of the parent fish.

This work was supported by grants from six commercial fish farms, the Ministry of Agriculture, Fisheries and Food and B.P. Nutrition (UK) Ltd.

FINE STRCTURE OF *Sparus aurata* L.OOCYTE DURING MATURATION

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Summary

The gonad morphology and function, as well as the ultrastructural differentiation of the gametes was studied in *Sparus aurata* L., under laboratory conditions and in the wild environment.

The sucessive stages of meiotic prophase, and the ultrastructural modifications of the nucleus and cytoplasm are related with sex reversal fase and the age composition of the species. In the nucleus, the cromossoma appearance and the nucleolus evolution indicates the stages of RNA transcription. In the cytoplasm, the organization during oocyte growth, is intimately related with specific adaptations to the metabolic and endocitic activity characteristic of final stages of oocyte maturation.

The existence of endocitic compartments and an highly specialized cortex allows the internalization of vitellogenin.

This study indicates that the patterns of oocyte maturation follows in *Sparus*, a tipical organization of most vertebrate

EFFECT OF SALMON GROWTH HORMONE ON STEROIDOGENESIS IN MUMMICHOG, FUNDULUS HETEROCLITUS

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In the course of study of growth hormone effects on hepatic function in fish, we observed a steroidogenic action. The availability of pure teleost prolactin and growth hormone as cloned products (Kawauchi et al., 1986) gave us the opportunity to study this action in different fishes. The effects of cloned salmon growth hormone (s-GH) and cloned salmon prolactin (s-PRL) were compared with effects of salmon pituitary extract and salmon gonadotrophin (s-GtH) on plasma sex steroid levels and gonadal function in F. heteroclitus. In the first experiment, male F. heteroclitus were hypophysectomized, which significantly reduced the GSI and plasma testosterone and estradiol levels. Replacement therapy with pituitary extract or s-GH elevated the GSI of hypophysectomized fish. Plasma testosterone and estradiol levels were also significantly elevated following treatment with pituitary extract or s-GH. The effects of pituitary hormones on in vitro steroidogenesis by gonadal tissue from hypophysectomized male and female fish that had been given s-GH supported the results of in vivo experiments. Whole pituitary extract stimulated testosterone and estradiol production by ovaries and testes, and s-GH stimulated significant production of testosterone by testes and of estradiol by ovaries. By comparison, purified s-GtH showed significant stimulation of testosterone by either gonad. Cloned s-PRL was without any effect. Results of a third experiment further indicated that s-GH could directly stimulate testosterone production by testes of hypophysectomized Fundulus. In that experiment there was an indication of steroidogenic action by s-PRL also. These results demonstrate that s-GH and possibly s-PRL, has steroidogenic activity in F. heteroclitus, the significance of which is yet to be understood. We are presently examining steroidogenic enzyme activities in hormone treated fish to address the mechanism of steroidogenic action of these hormones.

EFFECTS OF METOPIRONE ON OOCYTE MATURATION IN FUNDULUS HETEROCLITUS. Malcolm H. Taylor, School of Life and Health Sciences, University of Delaware, Newark, DE 19716.

Evidence for participation of cortisol in female reproduction exists for a number of teleosts. In Fundulus heteroclitus this steroid is a moderately effective stimulator of final oocyte maturation and circulating levels are elevated at the spawning peak in the semilunar reproductive cycle.

Metopirone, a blocker of cortisol synthesis, was used in a series of experiments designed to determine the importance of cortisol in oocyte maturation. In vivo metopirone treatment (50 mg/l in holding tank water) suppressed serum cortisol levels and prevented oocyte final maturation in fish injected with 50 IU/day Human Chorionic Gonadotropin (HCG). After 9 days treatment, ovulated eggs were stripped from the fish which received only HCG. The metopirone-treated fish had no mature oocytes when autopsied on day 13.

Exposure of oocyte cultures to metopirone (10 μg/ml) slowed both spontaneous maturation of large oocytes (1.50-1.69 mm) and HCG induced maturation of smaller oocytes (1.10-1.39 mm). Cortisol (1 μg/ml) or 17α,20ß-dihydroxyprogesterone (0.1 μg/ml) reversed this inhibition. Effects of metopirone on oocytes whose maturation was induced by cortisol or 17α,20ß-DHP alone were less consistent. Steroid stimulated oocytes usually were unaffected by metopirone. Hirose (1973) found similar effects of metopirone in HCG and cortisol-treated medaka.

Since both 17α,20ß-DHP and cortisol-stimulated oocyte maturation in the presence of metopirone, it seems probable that metopirone acts at an early step in the follicular steroid synthesis pathway. There is evidence that metopirone inhibits cholesterol side chain cleavage in mammals (Carballeira et al., 1974). This would affect the entire steroid synthesis pathway and could account for the inhibition of oocyte maturation observed in F. heteroclitus and the medaka.

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A COMPARISON OF TESTICULAR FUNCTION IN MATURE SALMONIDS (*SALMO* SPP.) AND WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*) WITH PARTICULAR REFERENCE TO PUTATIVE SITES OF STEROIDOGENESIS

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Testicular recrudescence and maturation are compared in iteroparous salmonids and a pleuronectid. Potential sites of steroidogenesis have been investigated using a procedure designed to stabilise steroids *in situ*. Fresh testicular tissue was reacted with sodium borohydride in Tris buffer, then with ethanolic digitonin and postfixed by the gradual introduction of formalin. Control samples were subjected to pentane extraction after the borohydride incubation. Fixed tissue was dehydrated in ethanol, embedded in Paraplast and sectioned. An indirect immunofluorescent technique was used to localise steroids in these sections and in sections of Bouin fixed gonads.

The pattern of distribution of steroids detected after the borohydride digitonin treatment is reported. Results obtained with this novel methodology are compared to those obtained with other procedures. Possible sources of variance in steroid distribution are differential steroid retention in producer and target tissues. A temporal shift in steroidogenic sites during recrudescence and maturation is also discussed, in association with the histochemical localization of enzymes associated with steroidogenesis.

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CURRENT STATUS OF TURBOT CULTURE

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During the last fifteen years, research institutes and commercial organizations in Europe have devoted considerable resources to the development of techniques for the rearing of turbot (Scopthalmus maximum L.). In spite of this effort, only about 10 commercial plants (in Spain, France, U.K., and Norway) have been established and the total production from these plants is less than 1000 T/year.

Rearing methods for juveniles and larger fish are fairly well established. Turbot accept both fresh fish meat and dry food pellets, with a food coefficient of 2.5 - 3.5 for fish meat and 0.6 - 1.5 for food pellets. In northern Europe, rearing takes place in cooling water from power plants etc. In southern Europe the fish are reared at ambient temperature. At 17°C turbot reach a size of 200-300 gm at one year and approx. 2 kg when marketed at age 2 years. Disease is no longer a restricting problem as the most important disease, vibriosis, may be treated with antibiotics and a vaccine is presently being developed. Production of turbot eggs is now possible over most of the year through light and temperature manipulation of the brookstock.

The bottleneck for increasing production, is the rearing of the larvae from hatching to day 20-30, when weaning to dry food is possible. Traditionally, turbot larvae are reared in the laboratory on the marine rotifer, branchionus and the brine shrimp, artemia. This rearing system, however, yields only limited survival (less than 10%) and produces manformations and mispigmentation. Alternative rearing systems are, therefore, sought and interest is presently focused on:

1) Increasing the nutritional value of branchionus/artemia by feeding them unicellular algae with a high content of polyunsaturated fatty acids.

2) Cultivation of chopepod nauplii, which are the natural food for the larvae. The copepods acartia tonsa and tisbe holothuriae are presently being used and survival rates of approx. 30% have been achieved with these food sources.

3) Rearing in large, predator-free tanks and enclosures, stocked with natural plankton. In principle, this method is simple, but the results are dependant on the complex interaction between nutrients, phytoplankton, zooplankton and fish larvae. A survival of up to 70% has been achieved, and the larvae seem to be healthier than those reared in the laboratory.

4) Rearing on dry food. Despite considerable effort in this area, it has not been possible to produce food pellets suitable for start-feeding of turbot larvae. The main problems seem to be the digestability, chemical composition and physical properties of the pellets. Solution of these problems is dependant on an increased knowledge of the digestion process.

It is expected that within 2-4 years it will be possible to produce sufficient turbot larvae to meet market demand. At that time, turbot production will increase significantly. This increase is expected to occur mainly in southern Europe, due to the limited heat resources in northern Europe.

STEROID HORMONE FEEDBACK ON PITUITARY GONADOTROPIN SECRETION

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Hypophysial gonadotropin secretion is subjected to a complex regulatory system. Most clearly established are the roles of neuropeptides, classical neurotransmitters, both of hypothalamic origin and gonadal sex steroid hormones and the gonadal peptide inhibine.

This paper will exclusively deal with the role of steroid hormones on the regulation of gonadotropin secretion.

Already fifty years ago it has been described that certain cells in the pituitary show signs of hyperactivity after castration. These experiments were carried out in adult male rats. Since than, these cells are referred to as castration cells, or, due to an often characteristic excentrically situated big vacuole as signed ring cells.

The development of suitable differential staining techniques, the introduction of the electron microscope in studies on the classification of pituitary cells and gonadotropin assays made it possible to correlate the hyperactivity of certain cells in the pituitary after castration with enhanced plasma gonadotropin levels.

Such studies have not been limited to mammals and now it is well established that in all vertebrates, including fish, gonadal sex steroids excert a negative feedback on hypophysial gonadotropin secretion. For many species this has been confirmed by sex steroid replacement experiments following castration.

As for the mechanism of action of steroid hormones on gonadotropin secretion a number of possibilities are proposed, all of them more or less supported by experimental data:

1) gonadal steroids stimulate the enzymatic breakdown of gonadotropin-releasing hormone (GnRH); 2) gonadal steroids influence the synthesis of GnRH-mRNA; 3) regulation of gonadotropin release (in connection to synthesis) via an influence on the synthesis of gonadotropin-mRNA; 4) gonadal steroids cause a a down regulation of GnRH-receptors on the gonadotropin producing cells; 5) gonadal steroids interfere with the catecholamine metabolism and control the release of gonadotropin via catecholamines.

Most of the hypotheses explaining the mechanism of action of the negative feedback by sex steroids originate from investigations carried out on mammals. So, this chapter in the proceedings of a symposium on reproductive fysiology of fish might look as a foreign body, since it contains more information about mammals than fish.

Under certain circumstances gonadal steroids do not (only) have negative effects of gonadotropin secretion but the reverse. In mammals it is well established that estrogens cause a preovulatory LH release. In several teleost fish species, especially at immature stages, it has been demonstrated that steroid hormones stimulate the maturation of the hypothalamic GnRH generating system, the development of the gonadotropic cells in the pituitary and the synthesis of gonadotropic hormone. This positive influence of sex steroids might play a role in the process of sexual maturation.

PRELIMINARY GONADIC AND HORMONAL STUDIES ON REPRODUCTIVE CYCLE AND SEX REVERSAL IN CHRYSOPHRYS AURATUS FROM SOUTHEAST TUNISIAN SEA

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The gilt-head Chrysophrys auratus is a protandrous hermaphroditic fish. The young fishes are males and in their gonads only the testicular zone is functional. The female area contains oocytes which do not have reached stages of maturation leading to spawning. In big fishes , in most cases, the female area is functional whereas the male one is not. Testicular area looks like small degenerative edges.

Histological study demonstrates that in every fish, the two areas, male and female are simultaneously present but cnly one is functional at once. We do not have observed a realy functional simultaneous hermaphroditism.

Sex reversal occurs by degenerative process of testis. Disorganization begins along peripheral zone of testicular area and then progresses to the internal part. This disorganization is characterized by the presence of crystal like granules in male cells.

This general scheme of protandrous sexuality gets complicated by the occurence of big males, in classes of weight which would normally include only females, if sex reversal would be a standard rule. In these big males, the study of gonads demonstrates that an attempt of sex reversal had occured but it failed. In young males indeed, oocytes present in the non functional female area do not have begun previtellogenesis. On the contrary, in big males, the female area of the gonad contains atretic oocytes which had begun vitellogenesis but which get into an abortive process.

Histological study of sex reversal in Chrysophrys auratus is associated to the hormonal mechanism investigation.

The first results show that the brain of Chrysophrys auratus contains neuropeptide like factors which can be determined by radio immunoassay (RIA) using mammalian standard.

The whole brain contained $587^{\pm}$ 54 pg of LHRH like factor, $442^{\pm}$ 130 pg of SP like factor and $417^{\pm}$ 65 pg of β endorphine like factor. A very wide and heterogenous distribution of these substances is registered over the brain.

Quantitatively, the highest percentage is found in the di and mesencephalon : 65 % of LHRH factor content, 77 % of SP like factor content and 57 % of β endorphine like factor content. These peptides are essentially localized in the intermediate part of the di and mesencephalon. This region is known to be important in the control of reproduction.

Experiments are in progress in our laboratory in order to determine profiles of di and mesencephalon content of these neuropeptides during the reproductive cycle and sexual reversion of Chrysophrys auratus., and to establish correlation between hormonal cycle and histologicaly spotted evolution of the gonads.

THE HORMONAL CONTROL OF VITELLOGENIN UPTAKE INTO CULTURED OOCYTES OF THE RAINBOW TROUT

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Abstract

Oocyte deposition of the hepatically-derived yolk-protein precursor vitellogenin, (VTG) represents the principal means of ovarian growth in the rainbow trout, Salmo gairdneri, and other oviparous vertebrates. Recent studies in-vivo have shown that vitellogenic trout follicles selectively sequester VTG (Tyler et al., unpublished data). However, little is known about the mechanism(s) controlling this uptake. An array of hormones have been implicated in the control of VTG sequestration especially the gonadotropin(s), including both the CHO-rich and CHO-poor fractions described by Ng and Idler (1978). The majority of studies, however, have been conducted in-vivo and the actions of the hormones are difficult to interpret because their involvement with VTG uptake may be either direct, affecting follicular receptivity and competence, or indirect, their actions being relayed by other endocrine tissues. This study addresses the hormonal control of VTG uptake in trout follicles in-vitro using a series of hormones including; growth hormone (chinook salmon GH, LeBail, Rennes, France), insulin (Pacific salmon, E. Plisetskaya, University of Washington, Seattle), thyroxine (T4) and tri-iodothyronine (T3) (Sigma Chemical Company, England), gonadotropin (GtH, a CHO-rich fraction from chum salmon) and a pituitary extract (PE) obtained from the fish which supplied the follicles for culture in the current investigation. The GH, insulin and GtH were all considered to be of high purity.

Intact vitellogenic follicles (mean diameter 3.2 ± 0.1mm) were cultured at 18°C for 18 hrs in a defined medium containing 3H-labelled VTG. The external concentration of VTG, which included both labelled and non-labelled fractions, was 13 mg/ml. Each purifed hormone was added at 100 ng/ml. The PE. was added at 0.16% of the total soluble extract per ml of culture medium.

Vitellogenic control follicles sequestered 22 ± 0.9 ng.VTGmm^{-2} hr.$^{-1}$, a rate similar to that observed in-vivo (Tyler et al., unpublished data). Statistical evaluation of the different hormone treatments showed significant differences in the rate of VTG sequestration (ANOVAR: P 0.001). Multiple comparison of the means using the GT-2 method modified by Gabriel (1978) clearly demonstrated a significant stimulation of VTG uptake by insulin, PE and GtH, with increases in the rate of VTG sequestration of 27%, 22% and 34% respectively. GH, T_3 and T_4 showed no significant stimulatory effects on VTG sequestration. A similar increase in VTG uptake into follicles cultured with insulin was obtained by Wallace and Misulovin (1978) working with Xenopus laevis oocytes. The stimulation of VTG sequestration by GtH, the CHO-rich gonadotropin, confirms the in-vitro observations by Breton and Derrien-Guimard (1983) and clearly illustrates that GtH has a vitellogenic role as well as the well-established maturational and ovulatory roles in ovarian development. It is our intention to present further work at the meeting on the hormonal control of VTG sequestration.

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ESTROGEN RECEPTORS AND REGULATION OF VITELLOGENESIS

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In oviparous vertebrates, estrogens induce the hepatic synthesis of the vitellogenins, the precursors of the egg yolk proteins. Analysis of the molecular mechanisms involved has provided a useful model for understanding aspects of the steroidal regulation of specific gene expression. Studies in teleosts show that liver contains an estrogen receptor system with some characteristics similar to and some distinct from the receptor system in the widely studied avian and amphibian species. Similarly, fish vitellogenins have some unique and some conserved properties.

In this lecture the comparative aspects of estrogenic regulation of vitellogenesis will be discussed in detail. Some particular advantages of the use of teleost liver as a model system for analysis of estrogen receptor dynamics and regulation of vitellogenin gene expression will be pointed out.

EFFECTS OF ADVANCED PHOTOPERIOD ON REPRODUCTION CYCLES OF RAINBOW TROUT IN COLD ENVIRONMENT

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Abstract

It is widely known that the onset of reproductive cycles of salmonid fish are easily induced by light exposure at temperatures near 10 °C.

By advancing the photoperiod with three months we were able to induce complete, advanced reproduction cycles in two-year-old rainbow trout from a spring spawning stock, inspite of the prevailing extreme winter temperature (0.1 - 0.5 °C).

The induction was most prominently manifested in ovarian growth and plasma hormonal levels as well as calcium concentration. The hormonal changes were also reflected in skin structure.

BRAIN PEPTIDES AND THE CONTROL OF REPRODUCTION IN FISH

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Abstract

Gonadotropin-releasing hormone (GnRH) is an important mediator for neural control of reproduction in fish from lamprey to teleosts. A member of a family of peptides, GnRH has different forms, even within one species. The GnRH family members appear to have evolved from DNA changes and not from differential splicing of mRNA or differential processing of the translation product. Rather, the identified forms of GnRH have the same posttranslational changes, which occur at their termini. The primary structures of 5 GnRH peptides are known for vertebrates, two of which are fish forms. However, indirect evidence suggests several additional and probably distinct forms of GnRH exist in cartilaginous, ganoid and teleostean fish. GnRH polymorphism within a single species is observed in many fish, but only tentative evidence suggests the physiological roles of the peptides vary. The possibility exists that in fish a GnRH gene-associated peptide (GAP) is present similar to the one identified in mammals, but the structure and function of a fish GAP remain to be determined.

The phylogenetic distribution of GnRH in fish is broad. Various forms of GnRH have been identified with immunological and HPLC techniques in several classes of fish: Agnatha (lamprey), Chondrichthyes (dogfish, ratfish) and Osteichthyes (ganoids: sturgeon, garfish; teleosts: herring, goldfish, salmon and others). The jawless hagfish is the only vertebrate in which GnRH has not been detected clearly. However, distinct forms of GnRH appear to be present in the protochordates suggesting the presence of GnRH at the invertebrate-vertebrate transition. The distribution of GnRH within the fish brain includes not only the hypothalamus and pituitary, but the telencephalon, preoptic area, midbrain, hindbrain and, in more recently evolved fish, the olfactory areas and retinae.

The classical role of GnRH, induction of release and synthesis of pituitary gonadotropins (GtH), appears to have been conserved throughout fish from lamprey to teleosts. The routes by which GnRH reaches the pituitary gonadotropes, however, shows considerable variation in fish representatives such as lamprey, ratfish, sturgeon and salmon. Likewise, the role of a number of factors such as dopamine and length of exposure to GnRH varies among fish in effective stimulation of ovulation. Other putative functions of GnRH that may be associated with reproduction are suggested by the location of GnRH in the fish brain. GnRH neurons in the olfactory area may be associated with detection or mediation of pheromonal signals related to reproductive events or behavior. A neurotransmitter function has been suggested for GnRH fibers in the midbrain and hindbrain; the high concentration of salmon II GnRH compared to salmon I GnRH in these brain areas may be correlated with a transynaptic function. Finally, the ontogenetic pattern of GnRH appearance suggests the peptide(s) may be important at certain stages for differentiation of gonadotropes in the pituitary.

The presence of GnRH receptor subtypes within a species or individual remains to be proven. However, the structure of GnRH receptors in different species has changed during evolution: lamprey GnRH neither binds to pituitary membranes nor effectively stimulates GtH release in birds and mammals; salmon GnRH does effectively stimulate GtH release in birds, but not in mammals; and mammalian GnRH causes release of GtHs in birds, amphibians and fish. An explanation at the molecular level for coordinated changes in the structure of receptors and their peptides during evolution has been advanced by Blalock.

IMMUNOLOGIC PROPERTIES OF GONADOTROPIN SUBUNITS OF RUSSIAN STURGEON

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Summary

A comparative investigation of antigenic properties of α-and β-subunits of gonadotropic hormone (GTH) of Russian sturgeon Acipenser güldenstädti Br. was done by a double immunodiffusion, immunoelectrophoresis and radioimmunologic analysis (RIA). By using antisera raised in rabbits against GTH and separate subunits (GTHα and GTHβ) a pronounced immunologic difference between GTHα and β was found and a similarity in antigenic properties of dimeric molecule and GTHα was shown. A conclusion has been drawn on different structure-function role of GTHα and β in the formation of antigenic determinant set of GTH molecule.

Chromatographically pure subunits appeared to be strong antigens giving raise to antiserum with a relatively high antibody concentration. The sensitivity of GTH and subunit antisera (as) in the double diffusion at a stepwise dilution of antigens corresponded to 7.5 μg of preparation per ml in all the cases.

In RIA cross reactions and double immunodiffusion a complete antigenic difference between GTHα and β was found. Similar discrepancies in immunological characters of complementary subunits were also revealed in immunoelectrophoresis. A comparative immunological estimation of GTH and separate subunits was done in the following RIA systems: GTH-asGTH, GTHα-asGTHα and GTHβ -asGTHβ. It was found that GTHα in the system GTH-asGTH partially crossreacted, while GTHβ showed no activity with asGTH.

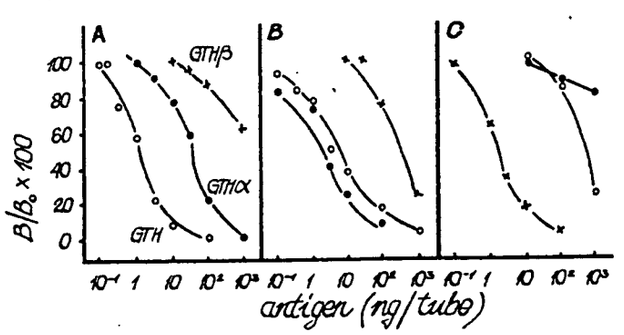

fig.1. Inhibition curves for sturgeon GTH α and GTH β in homologous GTH(A), GTHα (B) and GTHβ (C) systems.

Obviously, that antiserum against GTH contained a fraction of antibodies specific for antigenic determinants of GTHα, but the antibodies to GTHβ were absent. That allowed us to suppose that at a complementary recombination of counterparts in GTH molecule, GTHβ is rather densely embraced by conformationally more labile GTHα(Zenkevich et al., 1985), thus eliminating the antigenicity of GTHβ.

A complete antigenic incompatibility of GTHα and β was found also in RIA cross reactions in systems GTHα -asGTHα and GTHβ -asGTHβ. However, the native GTH acted with asGTHα almost on the level of immunoreactivity of the homologous antigen, i.e. GTHα, while its reaction with asGTHβ was at least 50 times lower.

Thus, it is obvious, that α- and β-subunits of the Russian sturgeon GTH differ as antigens and they are not equal as components of the whole antigenic structure of the dimeric GTH molecule. Besides that, GTHα carries the majority of determinants while GTHβ, due to its pronounced conformational rigidity, mainly determines (fixes) the species stereospecificity of ionogenic groups - antigenic determinants not only in GTHα, but in the whole molecule as well.

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Evidence For Another Yolk Precursor Protein, Distinct From Vitellogenin, In Winter Flounder (Pseudopleuronectes americanus).

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The process of vitellogenesis in teleosts involves the ovarian uptake of a hepatically derived glycolipophosphoprotein termed vitellogenin (Vg). It is known that Vg obtained by the ovary is cleaved in the oocyte to form the yolk proteins lipovitellin and phosvitin. Until recently, following work with amphibians, Vg was believed to be the sole precursor of ovarian yolk. However, another serum protein (Pk A[1]) has been isolated from the peripheral blood of winter flounder (Pseudopleuronectes americanus) and radiolabelled (I^{131}) Pk A protein is taken up by the developing ovary during vitellogenesis and incorporated into ovarian lipoprotein (So and Idler, 1987). This is the first evidence in teleosts that native serum protein other than Vg is sequestered by the ovary. The present study sought to examine Pk A protein and Vg in terms of their relationship to ovarian yolk.

Pk A protein (1, 170 K Mr), similar to Vg (515 K Mr), is a glycolipophosphoprotein staining with Coomassie Blue and Sudan black B on polyacrylamide gels (PAGE). It is held by Concanavalin A Sepharose affinity media indicating a carbohydrate content and contains 7.4 µg P/mg protein compared to 11.8 for flounder Vg. However the electrophoretic mobilities of Pk A on native-PAGE and SDS-PAGE are quite unique suggesting that it is not related to Vg.

Antisera to Pk A protein, raised in rabbits, was used to develop a radioimmunoassay (RIA) that permitted a comparison of immunoreactivity between Pk A and Vg. Flounder serum Vg does not cross-react in the Pk A RIA. Ovarian yolk proteins extracted with 0.5 M NaCl, precipitated with 5 mM $CaCl_2$, were separated on Sephacryl S-400 gel filtration media and incubated with Pk A and Vg antibodies (Ab). The different patterns of immunoreaction between Pk A Ab and Vg Ab with ovarian yolk proteins indicate that Pk A protein is involved with ovarian yolk and that the Pk A Ab recognizes yolk proteins exclusive of those reacting with the Vg Ab.

Finally using RIA annual serum profiles for Pk A protein and Vg were derived from female flounder to show the seasonal prevalence of thse two proteins in the blood providing evidence that Pk A protein is available, in addition to Vg, during vitellogenesis.

[1] denoted Pk A since it is found in the first peak when serum is chromatographed on Sephacryl S-300 gel filtration media.

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This research was supported by the Natural Sciences and Engineering Research Council of Canada, grant # A6732 to D.R.I.

EFFECT OF PELLET SIZE AND THAWING TEMPERATURE ON FERTILITY OF FROZEN-THAWED RAINBOW TROUT (SALMO GAIRDNERI) SPERM

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This investigation was conducted to establish the most favourable pellet-size and thawing temperature when freezing trout semen.

Experiment 1: Pooled semen from 5 milters was frozen on dry ice as described by Büyükhatipoglu and Holtz (1978) to give pellet sizes of 0.02, 0.035, 0.05, 0.1 and 0.2 ml, rsp.. Pellets were thawed at 9° C and used to fertilize freshly stripped eggs. This procedure was repeated 6 times. Fertilization amounted to 5.8 ± 11.0 %, 19.1 ± 17.8 %, 37.3 ± 13.9 %, 48.5 ± 11.9 % and 31.6 ± 6.4 ($\bar{x}$ ± SD) eyed eggs, rsp..

Experiment 2: Pooled semen from 5 milters was frozen at pellet size 0.1 or 0.03 ml. Before thawing, part of the 0.1 ml-pellets were chopped up into 0.03 ml fragments. Fertilization tests were conducted after thawing at 5° C, 10° C or 20° C. The experimental design was a 3 x 3 factorial with five replicates. Results obtained with pellets frozen and thawed at size 0.1 ml and those frozen at 0.1 ml and thawed at 0.03 ml were virtually identical (38.5 ± 1.4 vs. 39.0 ± 1.5%). Pellets frozen and thawed at 0.03 ml were significantly inferior (32.0 ± 1.4, $P < 0.05$).

Thawing temperatures 5, 10 and 20° C amounted to 33.4 ± 1.4 %, 34.7 ± 1.4 % and 41.4 ± 1.5 %, rsp. ($\bar{x}$ ± SD), 20° C being significantly superior to lower temperatures ($P < 0.05$). There were no significant interactions.

These results indicate that 0.1 ml is the most suitable pellet size, a thawing temperature of 20° C is more favourable than lower temperatures and pellet size is critical during freezing, not during thawing.

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GROWTH RATES OF DIPLOID AND TRIPLOID RAINBOW TROUT (SALMO GAIRDNERI R) OVER THE SPAWNING SEASON

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Abstract

The growth rates of diploid and triploid rainbow trout in mixed-sex or mono-sex female populations were compared over a 14 month period which included the first sexual maturation. The commercial advantages of sterile, female triploids were clearly demonstrated.

Mixed-sex and female-only stocks of 2 strains of rainbow trout were produced by heat-shock of ova for 10 minutes at 28°C, starting 30 minutes after fertilization. Triploid and diploid control fish were reared under semi-commercial conditions, in replicated trials at two sites for 28 months.

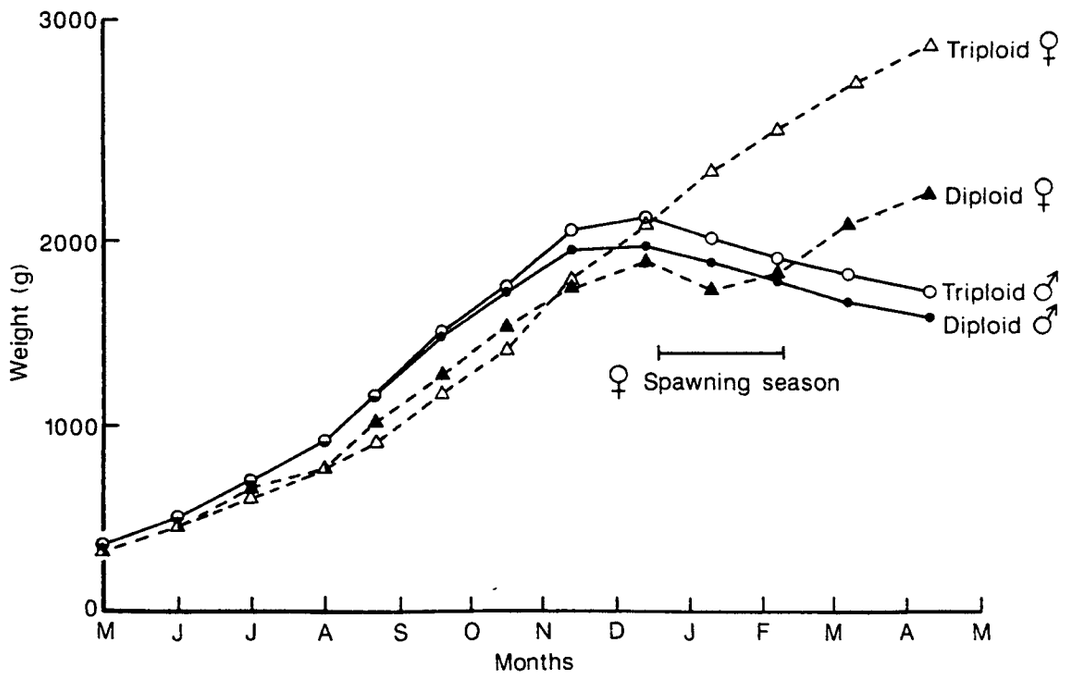
When diploid and triploid female trout were reared together the growth rate of the triploids was significantly lower than that of the diploids. However, when the female diploids and triploids were reared in isolation, in separate tanks, their growth rates were not significantly different.

In mixed-sex populations with the diploids and triploids reared separately, from the onset of sexual maturation the growth performance of the female triploids was superior to that of the male triploids and both male and female diploids (Figure 1). The latter 3 groups stopped feeding and growing and therefore lost condition for 3 months or more around the spawning period whilst the sterile triploid females continued to feed and grow and maintained their condition. The mortality of the triploid females was lower than for the other 3 groups.

In rainbow trout strains spawning in winter the enhanced performance of female triploids over the spawning period is less pronounced since low temperatures suppress growth in all fish.

This prevents the sterile fish gaining substantially in weight at the time when the maturing fish have stopped feeding.

We conclude that farming of sterile female triploid trout should eliminate the problems of poor growth, inferior flesh quality and higher mortality associated with the sexual maturity which is inevitable when trout over 1500 g are required. However, in order to obtain the maximum growth advantage it is essential to rear the triploid females in isolation and an advantage to use strains which spawn between April and November.



ENVIRONMENTAL MANAGEMENT OF MARINE FISH REPRODUCTION

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Abstract

The commercial culture of non-salmonid marine fish has developed rapidly in Europe over the past 5 years with a total production of around 1000 tonnes anticipated for 1987. A major reason for this sudden expansion is improved control of broodstock reproduction resulting in year-round availability of better quality eggs and fry.

The species of most interest for aquaculture at present are turbot Scophthalmus maximus, gilthead sea bream Sparus auratus, sea bass Dicentrarchus labrax and Dover sole Solea solea. All are cultured commercially although production of the sole is very low. The cod Gadus morhua can be reared successfully but is likely to be used for ranching rather than intensive farming and the considerable research on the halibut Hippoglossus hippoglossus and the wolffish or steinbit (Anarhichas lupus) is not likely to yield commercially viable rearing methods for 4 to 5 years.

All the fish species of interest to fish farmers in the temperate zones do not spawn throughout the year but have a restricted spawning period occurring at a particular season. It appears probable that the timing of annual maturation is under the control of an endogenous endocrine rhythm which is synchronized to the seasonal cycle by responses to environmental cues. Although temperature has a limiting role, in that gonad development does not proceed if the ambient temperature is outside a relatively narrow range, the main synchronizing cue appears to be the seasonal cycle of changing daylight duration.

Aquaculture requires a year round availability of juveniles and Bye and Htun Han (1979) were able to achieve this in marine flatfish by manipulation of photoperiod under relatively constant temperatures. Subsequently, systems have been developed for sole, turbot, sea bass and sea bream in which broodstock maintained in controlled photoperiod conditions can be induced to spawn at any season. Under commercial conditions 4 broodstocks controlled to give peak spawning at approximately 3-month intervals yield a virtually continuous supply of eggs. In practice the spawning time is advanced or retarded by exposing mature fish to compressed or extended cycles of changing photoperiod. Out of season spawning is then maintained by rearing in a normal 12-month cycle of increasing and decreasing daylength which is out of phase with the natural rhythm.

Environmental control of broodstock maturation is now in widespread use in Europe to provide continuity of egg supply for turbot, sea bass, sea bream and sole on commercial aquaculture farms. Similar control systems are being investigated for other species.

The methods employed for controlling the timing of marine fish reproduction are described and the light which this throws on the natural control of maturation is discussed. The interaction of photoperiod, temperature and other environmental conditions including salinity and nutrition are considered.

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POSTSPAWNING TESTICULAR REGRESSION IN SOME TELEOSTS

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There are numerous descriptive studies on annual teleost reproductive cycles which divide testicular development (recrudescence) into a series of discrete stages depending upon the maturation of germ cells. Recent histological investigations have demonstrated that recrudescence is a continuous process in seasonally breeding teleosts which involves the generation of spermatogenic tubules, formation of an extensive duct system and propagation of interstitial myoid cells. The pattern of testicular recrudescence is similar in Oreochromis aureus (Grier and Abraham, 1983) and Sciaenops ocellatus (Grier et al., 1987). The reverse process, postspawning testicular regression, is poorly understood.

During testicular regression, residual sperm are resorbed (Billard, 1986). However, the fate of Sertoli and duct cells, myoid and Leydig cells, which reside within the intratubular and interstitial testicular compartments, is not documented following seasonal reproductive activity. Postspawning testicular regression in Centropristis striatus, Strongylura notata and S. marina, Fundulus heteroclitus and F. grandis involves the appearance of periodic acid-Schiff-positive structures which appear to be composed of necrotic cell debris and nuclei. They have been termed "accumulation bodies" (Grier, 1987). Accumulation bodies appear to represent focal points of tissue degredation and are considered important to the overall process of testicular regression.

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EFFECTS OF OLFACTORY SYSTEM OR NERVUS TERMINALIS ABLATION ON GONADAL RECRUDESCENCE, SEXUAL BEHAVIOUR, AND RESPONSES TO PHEROMONES IN THE MALE GOLDFISH (*CARASSIUS AURATUS*).

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The nervus terminalis (Nt) is a small cranial nerve that is present in members of all vertebrate classes. The function of the Nt is unknown, but it has been suggested that in teleost fishes the Nt regulates various aspects of reproductive development and behaviour, including responses to sex pheromones. In goldfish, the bilateral Nt ganglia are embedded within the olfactory nerves and fibres project rostrally toward the olfactory epithelia and caudally via the medial olfactory tracts to various forebrain and midbrain targets and the retina. Because of this close physical association of the olfactory and Nt systems, traditional stimulation and lesioning experiments have not been able to separate olfactory and Nt effects on reproduction.

In this study, we attempted to remove the nervus terminalis of goldfish without significant olfactory damage by ablating the olfactory nerves and anterior edge of the olfactory bulbs and maintaining the animals for one year following surgery. A pilot experiment indicated that the olfactory nerves regenerated and re-formed their connections with the olfactory bulbs within this time, while the Nt ganglia and Nt retinal fibres did not reappear. A preliminary report will be presented on the effects of this treatment (Nt removal) or removal of both the olfactory and Nt systems on gonadal recrudescence, sexual behaviour, and responses to a sexual pheromone (17_{alpha},20_{beta}-dihydroxy-4-pregnen-3-one) in the male goldfish. This work is supported by the Natural Sciences and Engineering Research Council of Canada.

MAMMALIAN, SALMON AND CHICKEN-LIKE LHRH'S FROM HYPOTHALAMI OF WINTER FLOUNDER (*Pseudopleuronectes americanus*) AS EVIDENCED BY CHROMATOGRAPHIC MOBILITY AND IMMUNOREACTIVITY.

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An earlier report presented chromatographic, immunologic (Antisera 743, A. Arimura) and bioassay evidence for an LHRH-like substance in winter flounder (1). The current status of LHRH's in fish has been reviewed (2).

Two antibodies, one built agaist mammalian LHRH (Arnel) and the other against salmon LHRH, have been used to show that the hypothalamus of a winter flounder has three LHRH-like substances which behave chromatographically and immunologically as synthetic forms of chicken LHRH (P-Glu-His-Trp-Ser-Tyr-Gly-**Leu-Gln-Pro**-Gly-NH_2), mammalian LHRH (P-Glu-His-Trp-Ser-Tyr-Gly-**Leu-Arg-Pro**-Gly-NH_2), salmon LHRH (P-Glu-His-Trp-Ser-Tyr-Gly-**Trp-Leu-Pro**-Gly-NH_2). Purification was achieved by acetic acid extraction, dialysis (molecular weight <2000), salmon LHRH IgG affinity chromatography, and three HPLC systems: ion exchange, gel filtration and C_{18} reverse phase. Surprisingly, the gel filtration column (TSK-GEL, G-2000 SW), separated the three LHRH's even though their molecular weights are nearly identical; the same separation was achieved at this stage with flounder LHRH's.

Fig. 1. The separation on reverse phase HPLC of the three immunoreactive peaks from hypothalami of ca.1000 winter flounder previously dialysed and purified by affinity chromatography. A gradient of buffer A (0.01% trifluoroacetic acid (TFA/water) and buffer B (0.01% TFA/acetonitrile (ACN)) was used. Aliquots of the fractions were assayed with salmon LHRH antibody. When another buffer system was used, buffer A (0.02 M triethylammonium phosphate (TEAP) pH 3.0) and buffer B (0.2 M TEAP pH 3.0:ACN [1:9]), under the same conditions, the u.v. peaks from chicken LHRH and salmon LHRH standards appeared in reverse order as did the first two immunoactive peaks from flounder.

Fig. 2. Reverse phase HPLC separation of a u.v. absorbing immunoreactive peak was isolated from an extract of ca.1500 flounder hypothalami; absorbance at 280 nm was recorded at 0.01 units full scale. This immunoreactive which had been through the entire purification procedure, was initially separated on cation exchange and gel filtration columns. The peak co-eluted with mammalian LHRH in all three HPLC systems.

Fig. 3. Cation exchange HPLC isolation of a u.v. absorbing immunoreactive peak from a flounder extract containing ca.3000 hypothalami. Absorbance at 280 nm was recorded at 0.05 units full scale. The third peak was initially isolated on the reverse phase column (Fig. 1) and had been through the complete purification procedure. Elution was isocratic with 0.15 M ammonium acetate pH 3.5 for 20 min. The immunoreactive peak was detected by salmon LHRH antibody. The first immunoreactive peak (Fig. 1) purified in the same manner as Fig. 3 was isolated and ran with chicken LHRH in all three HPLC systems.

When cross-reaction studies were performed using salmon LHRH and mammalian LHRH antibodies, the three immunoreactive peaks behaved as the three LHRH standards. The best example would be the immunoreactive peak chromatographically resembling mammalian LHRH. This peak was at least 4X as large when detected with the mammalian LHRH antibody as with the salmon LHRH antibody.

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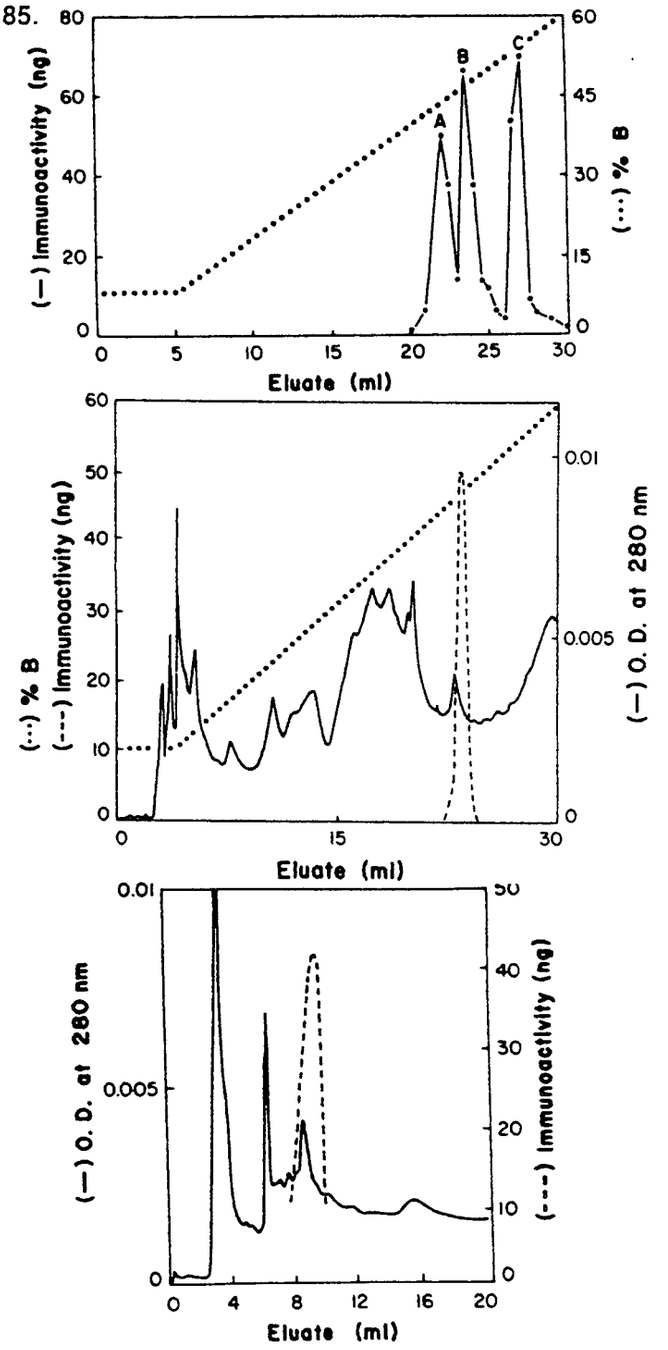

RESPONSE OF FEMALE RAINBOW TROUT (SALMO GAIRDNERI) TO 6 MONTHS-LIGHT CYCLES WITH CONTINUOUS OR INTERRUPTED DAY LIGHT PERIODS

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By way of light rainbow trout may be successfully stripped more than once per year (Pohl, 1986). This experiment was conducted to investigate if a continuous light phase may be replaced by 2 or 5 one h light impulses.

Each of 3 circular 1600 l tanks was stocked with 17 spawners after these had been stripped. Within a 6 months light program group 1 was subjected to a continuous light phase varying between 8 and 17 h.

Instead group 2 received five 1 h light impulses, and group 3 merely 1 h of light at the beginning and the end of the day light phase. Each fish was tested for mature eggs at biweekly intervals. The experiment was continued for 2 successive years.

Group 1 responded at 6 month intervals (Fig. 1): 100 %, 92 % and 82 % of the females spawned, fertilization rate was 88 %, 64 % and 71 %, respectively. In group 2 the corresponding figures were 100 %, 63 % and 73 % and 86 %, 74 % and 75 %. The 2nd season was skipped by 31 % of the fish. All females of group 3 responded the 1st time, though with a 4 week delay. The next spawning occurred 12 months later spread out over more than 5 months. These obviously did not recognize the light impulse but obeyed an endogenous annual rhythm.

Differences among groups or seasons with regard to number and weight of eggs were not substantial, though statistically significant.

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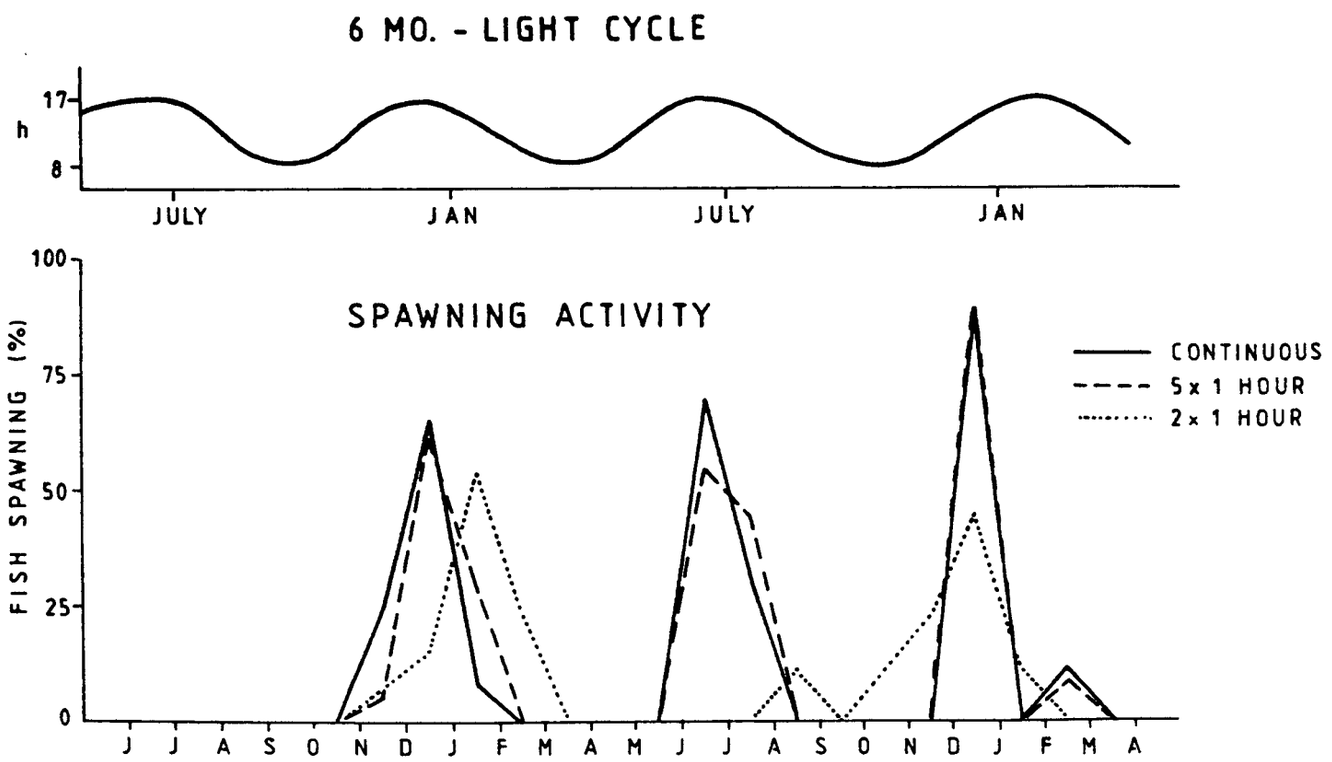

Isolation of tuna (Thunnus thynnus) carbohydrate-rich gonadotropins.

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Tuna pituitaries are routinely extracted and acetone-dried from a local fishery in Barbate (Spain) shortly before the spawning season. Extracts of their material are highly effective in inducing gametogenesis and spawning in the sole Solea seuegaleusis (1) and production of 11-ketotestosterone (11-KT) in hypophysectomized (hypex) mature male winter flounder.

Tuna pituitaries were extracted and chromatographed through Con A-Sepharose 4B and then Ultrogel AcA 54 with modified solvent system F:0.4 mM DTT, 0.4 mM PMSF, 0.15 M NaCl (2). Prior to gel filtration, proteins with molecular weights (MW) larger than 100 kDa were removed by membrane filtration. Two peaks with MW corresponding to 68 kDa and 34 kDa were obtained. Gonadotropic activity in these peaks were tested by determining 11-KT production in hypex flounder. The 34 kDa fraction increased serum 11-KT significantly in 24 hr while the 68 kDa fraction brought 11-KT only to the level of the sham-operated animals.

The 34 kDa proteins were fractionated by HPLC on a C4 phenyl-silica reverse-phase column using a linear gradient of water-acetonitrile at low pH. Fractions were tested for their ability to induce $^{32}PO_4$ uptake by the testis of one-day old chicks as previously described (3), using ovine LH (NIH-LH-S18) as standard. Fraction 4 showed no activity, and fractions 2, 3, 6 and 7 were equivalent in activity to 0.25-0.40 µg of LH, when tested at 1 µg of protein; fraction 8 was equipotent with ovine LH, and 1 µg of protein from fraction 5 was more active than ovine LH.

The results provide evidence that tuna pituitaries contain six or more carbohydrate-rich proteins with gonadotropic activity. It remains to be determined if these represent one or more than one family of isohormones.

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LHRH CHARACTERISTICS IN THE FISH PITUITARY

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Among many potential methods for achieving control of fish reproductive cycles, the traditional technique of hypophysation remains popular for inducing spawning in captive fish. More recently, it has been demonstrated that superactive analogs of LHRH (Gonadotropin-Releasing Hormone) are capable of stimulating release of pituitary gonadotropin (GtH) and inducing spawning in the number of teleost species (see review of Crim et al., 1987).

Binding of LHRH to pituitary plasma membranes is the initial step towards LHRH-stimulated release of gonadotropin in mammals and a number of post-receptor events are known to follow (see review of Conn, 1987). In the case of the fish pituitary, little is known about the mechanism of action for induced GtH release by LHRH analogs except for the presence of pituitary LHRH receptors which have been recently characterized in in vitro studies of the goldfish (Habibi et al., 1987 and the winter flounder (Crim et al., in press). When the radioactive LHRH analog was given in vivo intravenously to female winter flounder, selective uptake of labelled LHRH analog occurred only in the pituitary gland providing no evidence for extra-pituitary LHRH binding sites in the flounder. From both the goldfish and flounder LHRH receptor studies mentioned above, it was demonstrated that, compared with the native LHRH peptide hormones, LHRH analogs have higher binding affinities to fish pituitary receptors which presumably partially accounts for their superagonist GtH release properties.

In our continued studies of the flounder reproductive cycle, we have noted a decline in the binding of labelled LHRH analog to flounder pituitaries collected from post-spawned, sexually regressed fish. In subsequent studies of reproductively active flounder, it was found that there is an inverse relationship between steroid levels and LHRH receptor binding, e.g. when gonads are removed from sexually mature male winter flounder, binding of labelled LHRH analog is increased. The latter observations agree with the effects of castration reported on LHRH receptors in the rat. It is suggested by our flounder pituitary data that LHRH receptor levels in the winter flounder are under a complex hormonal regulation.

Our many attempts to demonstrate and characterize LHRH receptors in the salmonid pituitary (rainbow trout, speckled trout and fall chinook salmon) indicated that binding of labelled LHRH analog is very low under the standard radioreceptor assay conditions successfully utilized for goldfish and the winter flounder.

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THE EFFECT OF CASTRATION AND STEROID REPLACEMENT ON GONADOTROPIN-RELEASING HORMONE (GnRH) RECEPTOR BINDING IN THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*

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Introduction

In adult teleosts with a functionally active brain-pituitary-gonadal axis, gonadal hormones exert a negative feedback effect on gonadotropin (GTH) release. In the African catfish castration of males leads to an increase of plasma GTH levels, a decrease of pituitary GTH content, and a degranulation of the gonadotropes (De Leeuw et al., 1986). Both testosterone and androstenedione restored these castration effects, whereas, the non-aromatizable androgens 5α-dihydrotestosterone and 11ß-hydroxyandrostenedione did not. From these results De Leeuw et al. (1986) concluded that an aromatization of androgens into estrogens might be essential in the feedback regulation of GTH release

The purpose of the present study was to investigate the effect of castration and steroid replacement on GnRH receptor binding, i.e., affinity and capacity, in the African catfish.

Materials and methods

Adult male catfish (n=36) were castrated, i.e., testes and seminal vesicles removed, under anesthesia. Sham-operated animals (n=12) were exposed to similar surgical conditions but the gonads were left in place After two weeks the castrated catfish were divided into three groups of 12 animals each. One group received silastic capsules with androstenedione (Δ^4); one group received capsules with 11ß-hydroxyandrostenedione (11ßOHΔ^4); the third group of castrated fish served as control and received empty capsules. Three weeks after the operation, i.e., one week after the steroid implantation, the animals were killed by decapitation and the pituitaries were collected. Blood samples were taken once a week, starting just before operation.

In order to determine GnRH receptor binding affinity (Ka) and binding capacity (Bmax), pituitary membrane fractions of the different groups (six pituitaries per group per determination) were incubated with increasing concentrations of the iodinated salmon GnRH (Trp^7-Leu^8-LHRH) analog, D-Arg^6-Pro^9-sGnRH-NEt (sGnRHa) for 2 hr at 25°C. Non-specific binding was measured in the presence of 10^{-6} M unlabeled sGnRHa and subtracted from total binding. The Ka and Bmax were calculated by means of a Scatchard plot analysis of the saturation data.

Results and Conclusions

Castration caused a significant increase of plasma GTH concentrations (day 14: 1.98±0.2 ng/ml) compared to sham-operated animals (day 14: 0.82±0.13 ng/ml). After Δ^4 implantation the plasma GTH levels were back to normal (day 21: 0.65±0.12), whereas after 11ßOHΔ^4 implantation, these levels remained elevated (day 21: 2.02±0.25). Correlating with these results, castration caused an increase of GnRH binding capacity (238±20%) compared to sham-opeated animals (100%). After Δ^4 implantation GnRH binding capacity was back to normal (126±15%). After 11ßOHΔ^4 implantation GnRH binding capacity remained high (246±34%). Castration nor steroid replacement had any effect on GnRH binding affinity (Ka=0.37-0.62 x $10^9 M^{-1}$).

These results indicate that the feedback regulation of gonadal steroids on GTH release might involve a GnRH receptor regulation. Furthermore, an aromatization of androgens might be essential in this feedback action.

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MILKFISH CULTURE AND ARTIFICIAL PROPAGATION

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ABSTRACT

Milkfish (Chanos chanos Forsskal) is widely distributed in the Indo-Pacific region and one of the most extensively cultured fish in Southeast Asia. In the Philippines, Indonesia and Taiwan, close to 500,00 hectares of brackishwater ponds and freshwater bodies are used to culture milkfish for food and as baitfish. Annual production in these countries was about 280,000 metric tons in 1983.

For centuries, milkfish was cultured in shallow brackishwater ponds where natural food sources was sufficient to support growth of relatively few fish. In recent years, various culture methods have been developed to increase production per unit pond area. These include: (1) improved methods of pond fertilization, (2) stock manipulation, (3) adoption of deep-water pond systems and (4) supplemental feeding.

Fry needed to stock milkfish ponds come largely from the wild. Fluctuations in fry supply due to seasonal, climatic and biological factors however has been partly responsible for the slow growth of the milkfish industry. To augment and stabilize the fry supply, efforts to breed milkfish in captivity was initiated in several institutions. These efforts have resulted in: (1) development induced spawning techniques by hypophysation and administration of gonadotropin-releasing hormone analogues (GnRH-A); (2) spontaneous maturation and spawning of captive milkfish: (3) hormonal induction of gonad development; and (4) development of larval rearing techniques.

While milkfish culture has considerably improved and artificial propagation has been achieved, numerous problems still have to be addressed particularly those related to (1) the development of new culture techniques (2) controlled breeding.

CARBOHYDRATE-RICH GONADOTROPIC ISOHORMONES OF CHUM SALMON

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Pituitary glands from chum salmon were homogenized and carbohydrate-rich (Con A II) fraction was run on Sephadex G-75 to obtain the 41 kDa gonadotropin (GtH) fraction (1) which was chromatographed on CM Biogel-A. The GtH fraction eluted from CM Biogel-A with 3 mM ammonium acetate (CM I) was further fractionated by preparative electrophoresis (Prep-PAGE) using a LKB 7900 Uniphor Column Electrophoresis System, and a 7.5% gel prepared according to the Davis method (2). The fractions were tested by analytical PAGE and pooled into seven fractions according to the Rf values on analytical PAGE. They were designated, from the fastest to the slowest moving band, as Fraction 1 to Fraction 7, and their electrophoretic patterns and Rf values determined (Fig. 1).

The original CM I fraction had two minor bands with Rf values less than 0.3. These two bands were not collected in the Prep-PAGE fractions because the slow moving bands diffuse during prolonged Prep-PAGE.

The activities of the Prep-PAGE fractions were tested by using a cAMP assay (3) and radioimmunoassay (RIA) (4). The fast moving fractions, 1 and 2, did not produce a significant increment of cAMP production over the control, while the remainder of the fractions were all active in the cAMP assay. The protein content of the fractions in mg were: 1, 0.6; 2, 1.9; 3, 5.1; 4, 2.4; 5, 3.0; 6, 3.5; 7, 1.6. The fraction which had the highest cAMP activity was fraction 6. The cAMP assay, both in the immature male and female trout gonad assay, revealed that the "specific activity" was higher for the slower moving fractions (Rf 0.32 - 0.53).

The RIA results showed that all seven fractions cross-reacted to the antibody which was produced against the total carbohydrate-rich GtH fraction. However, fractions 1 and 2 exhibited no parallelism with the rest of the fractions. The most RIA-active fractions were the same as for the cAMP assay.

Prep-PAGE fractions were analyzed for amino acid composition by HPLC and will be compared with the "carbohydrate-poor" gonadotropin(s) isolated by Prep-PAGE from the appropriate fraction of the proteins unadsorbed on Con A-Sepharose.

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CALCITONIN APPEARS TO HAVE A FEMALE-SPECIFIC FUNCTION RELATED TO LATE MATURATION IN SALMONIDS

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The physiological function of calcitonin (CT) in teleosts remains yet to be defined. While large efforts have been directed towards a possible regulatory role of CT in calcium homeostasis, conclusive experimental data for this are still lacking. An alternative function in the reproductive physiology is suggested by the increased activity of the ultimobranchial glands and the higher plasma levels of CT during sexual maturation.

Plasma was collected from male and female rainbow trout (Salmo gairdneri) each month throughout an annual reproductive cycle. Profiles of CT, determined by a radioimmunoassay for salmon CT, and of total, free and proteinbound Ca, analyzed by an ultrafiltration method and subsequent atomic absorption spectroscopy, were obtained. In an additional experiment, only females were studied, as males showed no significant change in plasma CT or in the plasma distribution of Ca. Hence, maturing females were serially sampled every week around the time of ovulation in order to obtain a more detailed picture.

In females sampled each month, plasma CT was found to be increased during three months, representing the late stage of maturation and ovulation. The peak values of 1676±304 pM CT (n=9) were observed in the group of females where five out of nine were ovulated. In females sampled weekly, a sharp rise in plasma CT occurred the week before ovulation, followed by a rapid decline immediately after ovulation. Free plasma levels of Ca were not affected, while total plasma Ca increased during maturation and peaked 1-2 months before ovulation. This increase was due to the binding of Ca to vitellogenin and was not correlated to plasma CT levels.

The appearance of several steroid hormones together with CT in plasma and the complexity of the hormonal changes during maturation made it of interest to investigate the possible involvement of steroid hormones in CT regulation. As estradiol-17β occurs only in females and has an important function in maturation by inducing vitellogenin synthesis in the liver, juvenile Atlantic salmon, (Salmo salar) and rainbow trout were administered estradiol. A single intraperitoneal injection of 1-10 mg estradiol/kg resulted in an increase of plasma CT within 5-10 days in both species. On the other hand, repeated estradiol treatment over a period of 30 days caused a dose-dependent decrease of plasma CT levels in rainbow trout.

The results from these experiments suggest that CT may have a function during late maturation and ovulation in salmonids. This function is female-specific, as there was no change of plasma CT in males during the reproductive phase. The changes of plasma CT in females were not correlated to free plasma Ca levels, suggesting that there is no direct feedback regulation between plasma CT and Ca in rainbow trout. Estradiol can be a physiological regulator of CT secretion in salmonids during maturation.

SUBLETHAL PENTACHLOROPHENOL DEPRESSES SERUM VITELLOGENIN LEVELS IN MATURE FEMALE AND ESTRADIOL INJECTED IMMATURE RAINBOW TROUT (SALMO GAIRDNERI): Preliminary Observations

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The effect of sublethal pentachlorophenol (PCP) on serum levels of the ovarian yolk precursor, vitellogenin, was studied in mature female rainbow trout (Salmo gairdneri) during active vitellogenesis and in an experimental model system in which vitellogenin synthesis was artificially induced in immature rainbow trout by 17β-estradiol (E_2) injection. Serum vitellogenin levels in mature female controls increased from 8.9 to 18.7 mg/ml over 18 days, which did not occur in fish exposed to sublethal PCP. After an 18 day exposure to 21 µg/L PCP serum vitellogenin levels were significantly lower by 50%, than control fish. This would critically reduce available vitellogenin for incorporation into the yolk mass of the developing oocytes. There were reduced serum E_2 levels and gonadosomatic indices in females exposed to sublethal PCP. In the experimental model serum vitellogenin was significantly lower after 5 days of exposure to 25 µg/L PCP in E_2 injected immature rainbow trout and remained below the level of the non- exposed E_2 injected group for the duration of the experiment. The experimental model was useful in examining the mode of action of sublethal PCP in affecting serum vitellogenin levels and could be utilized in studying the effect of water pollutants on reproductive mechanisms.

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In 1975 we introduced the use of Concanavalin A-Sepharose for the isolation of the carbohydrate-rich gonadotropin(s) from pituitaries of Pacific salmon (1). The ability to separate the carbohydrate-rich (CR) from the carbohydrate-poor (CP) pituitary proteins encouraged us to look for biological activity in the latter fraction. Particularly, we searched for a previously undescribed gonadotropin to explain the rapid uptake of vitellogenin by the ovary at a time when CR-GtH was not abundant in the circulation and we reported the first such activity using hypophysectomized flounder in 1976 (2). Over the next 5 or 6 years we reported the isolation of highly purified CP-GtH's from plaice, carp, salmon and flounder (e.g. 3). While the fish CR-GtH's resemble mammalian LH in that they display steroidogenic and maturation-ovulatory activities the CP-GtH's have very weak or no such activities. The CP-GtH's bear superficial resemblance to FSH in that both stimulate follicular growth, albeit by a different mechanism since vitellogenesis does not occur in viviparous mammals. It is not possible to state with certainty the relationship, if any, between CP and CR-GtH's in fish but there is no compelling evidence to suggest that the former is a partially deglycosylated form of the latter or that one arises from the other. Chemical and biological properties of CP and CR gonadotropins will be briefly reviewed with respect to carbohydrates; sites of pituitary production; steroidogenesis; exogenous and endogenous vitellogenesis; and maturation and ovulation.

Until recently most of our studies on the stimulation of exogenous vitellogenesis by CP-GtH's employed radioactive leucine and phosphate because the preparation of undenatured radioactive vitellogenin by iodination, using classical methods, had eluded us. The use of Iodogen reagent has made possible the successful labelling of trout (4) and salmon vitellogenin (5) among other sensitive substances. New data will be presented on ovarian uptake of labelled vitellogenin and its control by CP-fractions of the pituitary. Antibodies to a salmon CP-GtH, which was homogenous on analytical PAGE, were used to demonstrate that prolonged binding of the CP-GtH *in vivo* during active exogenous vitellogenesis resulted in a significant reduction in GSI of landlocked salmon compared to controls. A similar study using antibody to CR-GtH during exogenous vitellogenesis failed to significantly reduce GSI in the same species (6). This is evidence that while administered CR-GtH stimulates exogenous vitellogenesis it may not have physiological significance. Partial suppression of gonadosomatic growth by anti-CP-GtH was accompanied by a rise in plasma CR-GtH and in plasma vitellogenin. In a similar experiment for a shorter time anti-CP-GtH reduced ovarian uptake of vitellogenin and increased plasma levels.

The antibodies to electrophoretically homogenous CP-GtH have been used to develop an R.I.A. for CP-GtH in plasma of landlocked Atlantic salmon. The plasma levels of the CP-GtH closely parallel the seasonal pattern for plasma vitellogenin and the levels are very much higher than we have found for CR-GtH during exogenous vitellogenesis. Thus, it seems not unreasonable to suggest that the CP-GtH and not the CR-GtH exerts the principal control over exogenous vitellogenesis. A second CP-peptide may be closely related to the CP-GtH involved with exogenous vitellogenesis as evidenced by cross-reactivity to the respective antibodies. This peptide begins to rise when vitellogenin is decreasing and peaks during endogenous vitellogenesis. It is known that suppression of CR-GtH with antibody during this period impairs early ovarian development (7), and the second CP-peptide may also be involved in this process.

Evidence will be presented that major plasma proteins other than vitellogenin are also taken up by the ovary under the influence of pituitary CP-peptide(s).

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SECONDARY SEXUAL CHARACTERISTICS DEVELOPED BY 60-CO STERILISED ATLANTIC SALMON

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In a sterilisation experiment, eyed eggs of Atlantic salmon (Salmo salar L.) of River Almond origin were exposed to an acute dose of 10Sv gamma irradiation from a 60Co source at the Universities Reactor Centre, East Kilbride, Scotland, in February 1983. Growth of these fish through the freshwater and the first year after transfer to sea cages was normal, and sterility levels of c.93% were achieved (Thorpe et al., 1986).

On 29 July 1986, after 14mo at sea, 41% (114 out of 278) of the untreated control fishes and 40% (78 out of 195) of the treated fish were judged to be maturing, on the basis of external appearance - enlarged heads, deepened bodies, and darkened flank colouration - and the farmer harvested them. Whereas 17 of 21 (81%) of these supposedly maturing control fish sampled had enlarged gonads, only 2 of 22 (9%) similar treated fish had gonad enlargement. On 29 September 1986, 123 of 149 (82.5%) remaining control fish were judged mature, and 41 of 108 (38%) of remaining treated fish. The mature control fish were harvested, but the immatures and the treated fish were retained.

On 25 March 1987 gonadal steroids were assayed from blood of 14 fish - 6 controls, 6 steriles, and 2 dark-coloured "maturing" steriles. All 6 controls were maturing (testosterone >0.5ng/ml), 5 females (oestradiol >0.5ng/ml) and one male (11-ketotestosterone at 4.4ng/ml). The treated fish were immature (steroids <0.5ng/ml), except for one of the 2 coloured fish, which had 0.6, 25.0, and 40.0ng/ml testosterone, 11-ketotestosterone, and oestradiol respectively, and appeared to be maturing as a hermaphrodite.

The remaining fish were harvested on 25 April 1987. Seventeen fish were sampled: 6 controls, 6 treated, and 5 treated showing secondary changes. All the control fish were maturing females, with significant quantities of oestradiol (0.6 - 1.1ng/ml). In the treated fish, 11-ketotestosterone levels were significant in all those showing secondary changes (2.8 - 29.0ng/ml), and in 2 of those without secondaries (2.6ng/ml). Anatomical examination showed little testis development. However, histological examination revealed that whereas gametogenesis had not occurred in the irradiated fish, the secretory tissue appeared functional, which was in accordance with the androgen levels recorded.

We conclude that a 10Sv acute dose of 60Co gamma irradiation blocked gametogenesis but not steroidogenesis, and did not alter the production of secondary sexual changes associated with maturation, or the normal pattern of seasonal growth characteristic of maturing fertile fish. A follow-up experiment is now in progress, in which eggs were irradiated at a succession of developmental stages to determine the precise time at which such treatment has maximal sterilant effect.

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ENVIRONMENTAL CONTROL OF REPRODUCTION IN TELEOSTS: AN OVERVIEW

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An overview is presented focusing on problem areas. First, environmental control has often not been studied in relation to the different phases of the reproductive cycle (e.g. in the female: oogonial proliferation and first growth phase of oocytes, endogenous and exogenous vitellogenesis, final oocyte maturation and ovulation, oviposition or spawning, regression or termination of spawning season, and refractoriness). The environmental factors involved may be different for the different phases, or some phases may be dependent on environmental cues while others are not. It is conceivable that the environmental factors which foretell the advent of the spawning season and stimulate the preparatory gonad growth (so-called approximate factors) should differ from the more precise (so-called proximate) cues which signal the arrival of suitable spawning conditions conducive to larval survival and trigger/synchronise the final phases.

Second, environmental factors are often studied singly with other factors kept constant. In nature, interactions among factors are likely to occur. Such interactions have not been studied in most species. They should include not only physical factors (e.g. photoperiod and temperature in temperate species) but also social and dietary/nutritional factors.

Third, how breeding is terminated in species which spawn many times during the spawning season needs more studies. The basis of post-spawning refractoriness encountered in some species also requires attention.

Fourth, very few tropical species have been studied experimentally. Environmental factors involved, if any, are more difficult to identify compared to temperate or subtropical species. Experiments conducted in our laboratory on a few species are discussed.

Fifth, very few studies distinguish environmental control of puberty (first cycle) from that of recrudescence (subsequent cycles). This is of particular interest in species which attain first maturity (puberty) only after a few years. Of equal interest is the question: why are the fish refractory to the environmental conditions in the pre-pubertal years?

Sixth, are there sex differences in the environmental control? This is likely to be dependent on species. In nature, males of many species are observed to mature earlier than the females.

Seventh, how plastic is the environmental control? Can the environmental factors involved be altered as a result of changed circumstances (e.g. domestication and introduction)? Examples are discussed.

Last but not the least, the relative importance of endogenous rhythms versus environmental control needs careful evaluation. Endogenous rhythms have been studied in only a few species and the situation is still not fully clear.

SYNTHESIS OF 17α-HYDROXY,20α-DIHYDROPROGESTERONE BY TELEOST OVARIES

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17α-Hydroxy,20α-dihydroprogesterone (1720αP) has been identified as a major gonadal steroid in dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*), both of the order Pleuronectiformes.

Incubation of mature dab and plaice ovarian homogenates with tritiated 17α-hydroxyprogesterone or pregnenelone, yielded a major compound which was identified, after chemical modification and silica gel thin layer chromatography (TLC), as 1720αP. (The same steroid was produced by dab and plaice sperm incubates.)

A radioimmunoassay for 1720αP was applied to plasma samples of female dab and plaice after separation on TLC. This showed large amounts of two immunoreactive compounds - one corresponding to 1720αP and the other to a steroid that we have tentatively identified as 5α-Pregnane-3β,17α,20α-triol. High amounts of both steroids were also found in a conjugated form (glucuronide and/or sulphate). Levels of both steroids, in free and conjugated form, were markedly raised (up to 200-500 ng/ml) by the injection of HCG into mature female dabs.

In *in vitro* incubates of dab ovaries, production of 1720αP and its metabolites showed a dose-dependent response to HCG.

The role of this steroid, which is found at levels far exceeding those of other known ovarian steroids (e.g. Testosterone, Estradiol, 1720βP) has not yet been established. Since the steroid is poorly active in *in vitro* bioassays, it is unlikely to be involved in the induction of oocyte final maturation. We hypothesise that it may play a behavioural/pheromonal role (cf. 1720βP in goldfish).

It is likely that 1720αP will be found in several other species, as 20α-hydroxysteroid dehydrogenase activity has been demonstrated, by other workers, to be present in the ovaries of *Trachurus mediterraneus*, *Perca flavescens*, *Mugil cephalus*, *Heteropneustes fossilis*, *Gobius jozo* and *Serranus cabrilla*. 1720αP is not present, however, in the plasma of mature female rainbow trout, *Salmo gairdneri* (own observations).

I

USE OF PELLETED LHRH ANALOGUE TO INDUCE SPAWNING IN ATLANTIC SALMON

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In recent years, Atlantic salmon broodstock has been raised to maturation in commercial sea cages. Synchronized spawning of such fish would reduce the time required for gamete collection. Advancement of spawning time may also improve egg quality due to reduced seawater exposure of maturing females. LHRH analogues have been shown to be powerful inducers of ovulation. D-Ala6, des-Gly10-LHRH ethylamide was incorporated into cholesterol pellets giving a dose of 100µ. This pellet was inserted, via the egg pore, into the ovary. Commercial broodstock females were implanted at the cage sites and egg samples subsequently collected to measure percent fertilization or percent survival to eyed stage. Results indicate similar egg survival for treated (eg. 79.3% fertilization n=14) and untreated (eg. 77.3% fertilization, n=13) fish. Untreated freshwater broodstock showed higher fertilization (84.1%, n=5). LHRH implanting of Grilse in fresh and seawater showed reduced egg survival compared to placebo groups. Plasma hormone profiles of gonadotropic hormone and 17α-hydroxy,20β-dihydroprogesterone showed low values compared to previous studies on Atlantic salmon. The reason for these unexpected results is still unclear. Osmolarity measurements of ovarian fluid collected from all experimental fish will provide information on their osmoregularity status at ovulation.

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