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Investigations on accelerating sexual maturity of eel

/Anguilla anguilla L./ males

by

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Summary

Studies on accelerating sexual maturity of European eel males have been conducted. The fish were kept in fresh or artificial sea water at the temperature 20°C, and hormonal injections were applied. From among hormones used, the positive results were obtained with HCG. After six weeks of the experiments all the eel males injected with HCG gave sperm containing living spermatozoa. The quality of sperm of the males kept in the fresh water was much poorer in comparison with the sperm of the males kept in the artificial sea water.

Introduction

Successful attempts with accelerating sexual maturity of European eel males have been made so far in France /Fontaine, 1936; Olivercau, 1961/, Denmark /Boëtius I. and Boëtius J., 1967/ and Germany /Meske, 1973/. Fully mature Japanese eel males have been obtained by means of hormonal injections in Japan /Yamamoto et al., 1971/. Similar experiments undertaken in Poland in late 1974 were crowned with success after six weeks. In contrast to the earlier studies, the emphasis was put on:

1. general semen characteristics, i.e., quantity of sperm, concentration and motility of spermatozoa;
2. estimating of differences in semen quality depending on the medium in which experimental eels were kept /fresh or artificial sea water/;
3. possibilities of producing the vital spermatozoa by eels for a longer period of time.

At the same time, an influence of testosterone and desoxycorticosterone on eel males was considered.

Material and method

The material consisted of eels taken and transported from some Polish lakes in October 1974. The fish were divided into seven groups, each of ten specimens. Five groups were kept separately in aquariums of 30 l, with the flowing fresh water of 20°C. The remaining two groups were put into aquariums of 60 l, with non-flowing artificial sea water of 20°C which was changed twice weekly. Composition of the artificial sea water was as follows:

NaCl	-	27.213 g/l
MgCl ₂	-	3.807 g/l
MgSO ₄	-	1.658 g/l
CaSO ₄	-	1.260 g/l

K_2SO_4	-	0.863 g/l
$CaCO_3$	-	0.123 g/l
$MgBr_2$	-	0.076 g/l

Every week each group of fish received different kinds of injections that are listed below:

- 1st group, fresh water - HCG of French make /Russel/, 100 i.u. per fish;
- 2nd group, fresh water - HCG of Polish make /Biogonadyl/, 100 i.u. per fish;
- 3rd group, fresh water - Testosterone proprionate, 5 mg per fish;
- 4th group, fresh water - Desoxycorticosterone, 5 mg per fish;
- 5th group, fresh water - control physiological salt solution, 0.2 ml per fish;
- 6th group, "sea water" - HCG of Polish make /Biogonadyl/, 100 i.u. per fish;
- 7th group, "sea water" - control physiological salt solution, 0.2 ml per fish.

HCG, i.e., human chorionic gonadotropin, and physiological salt solution were given intramuscularly. Whereas, testosterone proprionate and desoxycorticosterone being oil substances were injected into the body cavity. The first injections took place on December 17th, 1974. The last gonadotropin injection was made on January 28th, and the last testosterone and desoxycorticosterone injection on March 25th, 1975. The experiments carried out in fresh and artificial sea water were terminated on February 11th and April 1st, 1975, respectively.

A total of 14 eels died during the experiments, and 9 eels proved to be females.

Results

Neither testosterone nor desoxycorticosterone injections had influence on the testes which, with respect to the development stage, did not differ from the control fish gonads at the end of the experiment. The eels receiving the HCG injections reached full maturity, on the other hand, and produced vital spermatozoa /Table 1, Fig.1/. All the males being kept in fresh or artificial sea water and injected six times with HCG /both Russel and Biogonadyl/ have reached

the maturity simultaneously. For the first time sperm was collected from all males on January 29th, 1975, i.e., six weeks upon beginning the experiments.

Relative weight of gonads of males kept in the fresh water was lower in comparison with the weight of gonads developing in the artificial sea water /7.3% to 7.5% and 9.6% respectively, as in Table 1/. The volume of semen obtained from male at a time was smallest in the 2nd group of fish /fresh water, Biogonadyl injections/ and amounted to 0.57 cu.cm on an average. The largest average volume equal to 1.12 cu.cm has been found in the 1st group /fresh water, Russel injections/. In the group 6 /artificial sea water, Biogonadyl injections/ the average sperm volume equalled 0.81 cu.cm /Table 2/.

The shortest average time of spermatozoon motility was observed in the 1st group of eels /3.32 min./; a slightly longer average time was found in the 2nd group /3.45 min/. The time of motility of spermatozoa in the group 6 /from artificial sea water/ was considerably longer amounting to 4.93 min. on the average /Table 2/.

The lowest spermatozoon concentration, namely 5,673,000 and 4,363,000 spermatozoa per cu.mm of sperm on the average, could be made out in the eel groups 1 and 2 kept in the fresh water. Spermatozoon concentration in the sperm of eels from the group 6, kept in the artificial sea water, was considerably higher and amounted to 8,036,000 spermatozoa per cubic mm on the average /Table 2/.

Due to an invasion of ichthyophthirius it was necessary to interrupt the experiments with the eels in the fresh water on February 11th, that is two weeks after obtaining their sperm. When the eels were kept in the artificial sea water, their sperm producing was taking place from January 29th to March 25th, 1975, i.e., throughout the period of eight weeks.

Discussion

The investigations showed that it is possible to accelerate

maturity of European eel males /*Anguilla anguilla* L./ keeping them in fresh or artificial sea water and applying the intramuscular injections of HCG either of Polish or French make. However, sperm of the males kept in the artificial sea water was of higher quality.

A possibility of an inhibitory action of prolactin /abundantly produced by prolactin cells in the pituitary of the eels kept in fresh water/ on the gonadotropic activity of pituitary was not excluded. As it is common with fishes, prolactin plays a role in retention of Na ions, and is more intensively secreted in fresh water.

Characteristic external changes like those described, among others, by Meske /1973/ were observed during the process of eel maturation. They consisted in enlargement of eye diameter /Fig.2/, as well as, in widening and flattening of the skull on which a narrowing could be stated behind the eyes. The bases of pectoral fins grew more colored, and sexual papillae stained more intensively /Fig.3/. Similar changes appeared in the eels receiving testosterone injections, but they were less visible in comparison with the eels upon injecting HCG, and no development of testes could be noticed.

The results obtained are consistent with the hitherto known information on the activity of sexual hormones which are responsible for producing the changes in spawner appearance /so called "wedding garment"/ and, on the other hand, cause an inhibitory action affecting the secretion of gonadotropic hormones in consequence of a feed-back mechanism. Since the "wedding garment" of eels injected with testosterone was not distinct, it can be inferred that the hormone used differed from the sexual hormones of the European eel males. Simultaneously, the results do not exclude a possibility of a stimulating activity of other sexual hormones which might be applied in different, probably much lower doses to influence the development of testes.

Recent scientific literature records data on a stimulating action of corticosterone hormones accelerating sexual maturity

and ovulation /Gosvami et al., 1971/. Although our results did not show the influence of desoxycorticosterone on the development of eel testes, they do not preclude a possibility of such effect after applying other pituitary hormones and other dosages. It must be admitted, however, that the results presented by Gosvami et al. can hardly be explained from the physiological point of view.

Conclusions

1. Full maturity of eel males kept in fresh or artificial sea water at the temperature 20°C was obtained upon the injections of the Polish or French HCG applied in the amount of 100 i.u. per fish weekly for the period of six weeks.
2. Between 0.1 and 2.8 cu.cm of semen was obtained from one male. The concentration of spermatozoa in millions per cubic mm of semen ranged from 3.680 to 13.380; the motility of them measured in minutes ranged from 2.02 to 9.40.
3. Taking into account the concentration and period of motility of spermatozoa, the semen of eel males from the artificial sea water displayed a higher quality.
4. In the artificial sea water eel males produced semen by a slight pressure exercised on the abdominal body part during the period of eight weeks.
5. No effect of testosterone propionate or desoxycorticosterone on the development of eel male gonads was stated when the hormones were given into the body cavity in the amount of 5 mg per fish.
6. Changes of the skull structure and eye size were found in the immature eel males receiving testosterone injections.

Literature cited

- Boëtius I. and Boëtius J., 1967. Studies on the European eel *Anguilla anguilla* /L./. Experimental induction of the male sexual cycle, its relation to temperature and other factors. *Meddelelser fra Danmarks Fiskeriog Havundersøgelser*, 4, no.11, København. 18, 4: 339-405.
- Fontaine M., 1936. Sur la maturation complète des organes génitaux de l'*Anguilla* mâle et l'émission spontanée de ses produits sexuels. *C. R. Acad. Sci.*, 202. I: 313-314.
- Gosvami S. V., Sundarraraj B. I., 1971. Temporal effects of ovine luteinizing hormone and desoxycorticosterone acetate on maturation and ovulation of oocytes of the catfish *Heteropneustes fossilis* /Bloch/ on in vivo and in vitro study. *Jour. Exp. Zool.* 178, 4: 457-465.
- Meske Ch., 1973. Experimentally induced sexual maturity in artificially reared male eels */Anguilla anguilla* L./. *Genetics and Mutagenesis of Fish.* Springer-Verlag Berlin Heidelberg New York: 161-170.
- Olivereau M., 1961. Maturation sexuelle de l'anguille mâle en eau douce. *C. R. Acad. Sci.*, Paris. 252/23/: 3660-3662.
- Yamamoto K., Hirano T., Morioka T., 1971. Artificial maturation of cultivated male Japanese eels by syrahonin injection. *Bull. Jap. Soc. Sci. Fish.* 38: 1083-1090.

Table 1

Characteristic features of eels used for investigations

Group No	Injection	Medium	Number of fish				Average length cm	Average weight of fish g		Average weight of gonads g %		Eye diameter, mm
			initial	dead	females	final						
1	HCG Russel	fresh water	10	1	5	4	40.5	100	/82/	6.09	7.3	9.2
2	HCG Biogonadyl		10	1	4	5	39.8	101	/83/	6.19	7.5	8.5
3	Testosteron proprionate		10	0	0	10	41.6	106	/95/	1.27	1.0	7.9
4	Desoxycorticosteron		10	0	0	10	40.4	94	/83/	0.60	0.7	6.6
5	Physiological solution		10	6	0	4	41.9	109	/99/	0.87	0.6	5.2
6	HCG Biogonadyl	artificial sea	10	4	0	6	38.0	61	/51/	6.44	9.6	9.3
7	Physiological solution	water	10	2	0	8	38.2	74	/67/	0.52	0.2	8.0

HCG - Human chorionic gonadotropin,

g in brackets - weight of fish after gutting,

% - weight of gonads in relation to the fish weight after gutting.

Table 2

Characteristic features of sperm obtained and spermatozoa

	Date	Group No	Number of fish	Sperm features	Spermatozoa features	
				Average volume in cubic cm	Average motility in minutes	Average concentration in millions per 1 cu.mm
Fresh water	29 Jan.	1	4	2.10 /1.5-2.8/	3.17 /2.55-3.52/	6.470 /6.450-6.480/
	29 Jan.	2	5	0.30	3.51 /2.45-4.59/	4.040 /3.680-4.400/
	4 Feb.	1	4	0.65 /0.1-0.9/	4.57 /4.37-5.29/	5.950 /5.700-6.200/
	4 Feb.	2	5	1.20 /0.5-2.0/	4.45 /4.37-4.54/	4.475 /4.250-4.700/
	11 Feb.	1	2	0.60	2.21 /2.12-2.30/	4.600 /4.400-4.800/
	11 Feb.	2	4	0.20 /0.1-0.3/	2.39 /2.02-3.01/	4.575 /4.350-5.100/
Artificial sea water	29 Jan.	6	6	0.25 /0.1-0.4/	0.00	6.320 /5.100-7.150/
	4 Feb.	6	6	0.92 /0.2-2.0/	4.01 /3.42-4.11/	5.700 /5.305-6.100/
	11 Feb.	6	6	1.55 /0.5-2.8/	5.49 /4.40-6.47/	6.215 /4.800-7.300/
	18 Feb.	6	6	1.20 /0.5-2.0/	5.58 /4.12-7.45/	9.315 /5.895-11.430/
	25 Feb.	6	6	1.01 /0.4-2.2/	6.35 /5.00-8.45/	9.506 /6.945-13.380/
	4 Mar.	6	6	0.83 /0.2-1.3/	4.47 /4.02-6.45/	8.226 /6.025-10.345/
	11 Mar.	6	6	0.50 /0.1-0.9/	7.36 /4.15-9.40/	9.066 /6.700-11.700/
	18 Mar.	6	6	0.23 /0.1-0.5/	6.18 /2.37-8.48/	9.938 /8.905-10.625/
	25 Mar.	6	6	traces	vibrations	not examined

Ranges of variation in brackets.

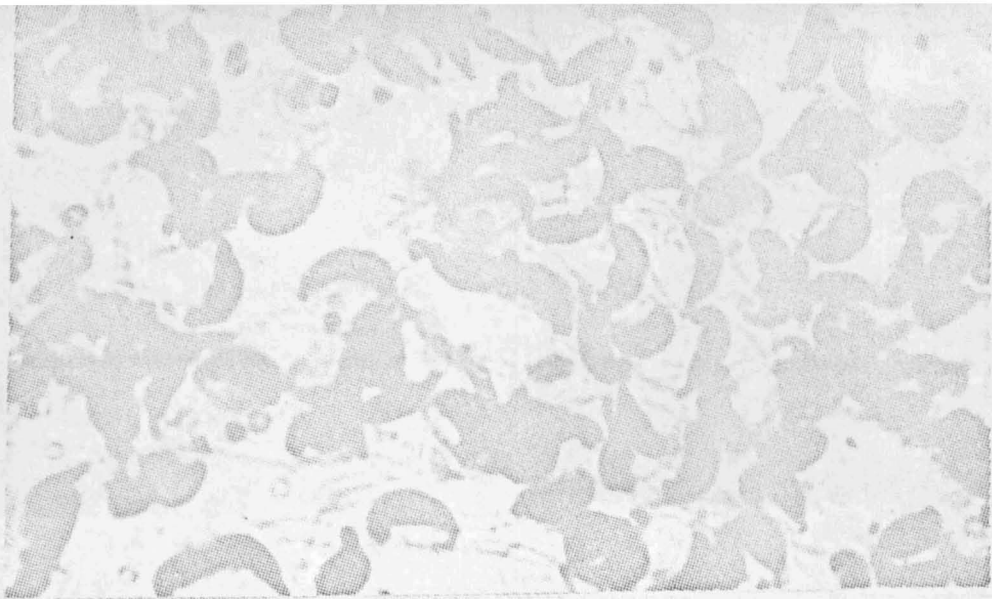


Fig. 1 Spermatozoa of eels



Fig. 2 Differences in the exterior build between mature and immature eel males

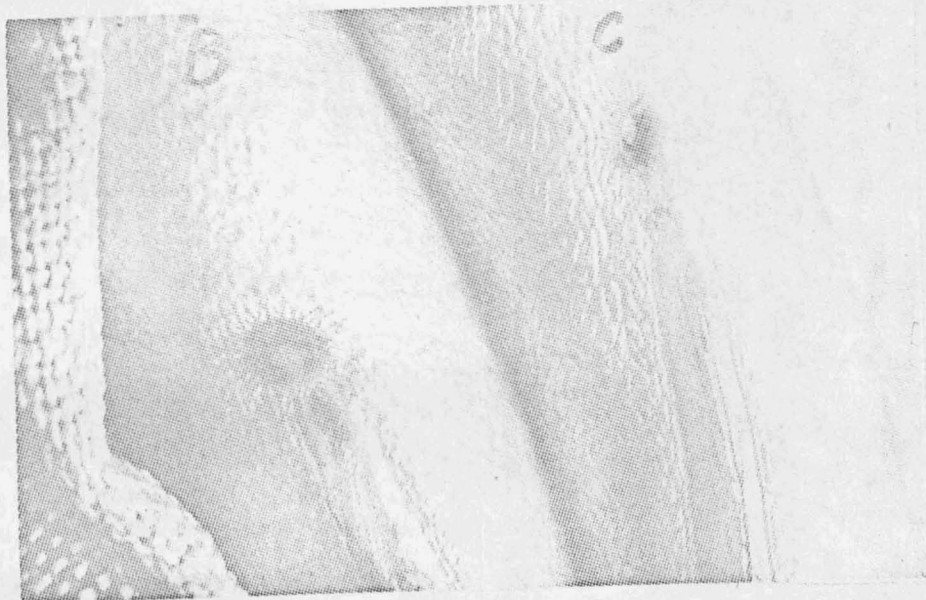


Fig. 3 Sexual papillae of mature and immature eel male