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Artificial induction of sexual maturation in the European eel males (*Anguilla anguilla* L.) (Preliminary results)

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ABSTRACT

The aim of our present study was to induce sexual maturation and sperm release by hormone treatment in European eel males. Fish were kept in fresh water (temperature of 24±1ºC) without feeding. Motile sperm was obtained from the 5th hCG injection. The volume of sperms stripped on the 7th week ranged between 0.1-1.9 cm³. Cell counts in Bürker chambers resulted 1 to 7 million spermia per mm³. The motility of sperms varied between 10 to 70%. This motility is lower than what can be found in other publications, however our results suggest that a simultaneous egg stripping and sperm release may contribute to solve the artificial propagation of European eel.
(Keywords: European eel, sexual maturation, hCG)

ÖSSZEFOGLALÁS

Az európai angolna (*Anguilla anguilla* L.) hímeinek ivarérés indukálása (Előzetes eredmények)

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Célunk hím angolnák hormonális ivarérés indukálása, illetve spermiációja volt. A kísérleti állatokat édesvízben tartottuk (vízhőmérséklet 24±1ºC) táplálás nélkül. A hCG kezelések hatására az 5. héttől mozgó spermát fejtünk. A 7. héten lefejt sperma mennyisége halanként 0,1-1,9 cm³ volt. A Bürker kamrás számlálás adatai a spermiumok koncentrációja 1-7 millió/mm³ közötti értékeket mutatott. Az angolnasperma motilitása 10-70% között alakult. Ez ugyan kisebb, mint az irodalmakban eddig közölt adatok, azonban ez az eredmény is biztató arra nézve, hogy egy azonos időben végrehajtott ikra és spermafejéssel sikerülni fog az angolna mesterséges szaporítása is.
(Kulcsszavak: angolna, ivarérés, hCG)

INTRODUCTION

An important element of fish farming is the artificial induction of reproduction. While Hungary has been in the forefront in developing technologies for the efficient propagation of several important farmed fish, such as carp and wels. The propagation of European eel has not been solved yet. Eel farms in Europe base their annual production on the capture of glass eels entering into river mouths in autumn and winter (*Perez et al.,*

2000). The artificial reproduction and nursing larvae to glass eels would be a great improvement in this industry.

There are several reports about the induction of sexual maturation of males in different eel species: in European eel *Anguilla anguilla* (*Meske,* 1973*; Bieniarz & Epler,* 1977*; Boetius & Boetius,* 1985*; Dollerup & Graver,* 1985*; Perez et al.,* 2000) investigating the ultra-structures of spermatozoa (*Billard & Ginsburg,* 1973*)*, describing of histology changes of brain during the maturation (*Sokolowska et al., 1978),* in Japanese eel *A. japonica* (*Yamamoto & Yamauchi,* 1974*; Yamauchi et al.,* 1976*; Ohta & Izawa,* 1996*; Ohta & Tanaka,* 1997*; Ohta et al.,* 1997*; Okumara et al.,* 2000), in American eel *A. rostrata* (*Sorensen & Winn,* 1985) and in Austral eel species *A. diffenbachi* and *A. australis (Lokman & Young,* 2000*).*

Our long term goal is the artificial propagation, but the aim of the present study was simply to obtain motile sperm of eel males.

MATERIAL AND METHODS

Fourteen male eels (body weight 123.5±23.9 g) were transported from Köröm eel farm in July 2001. They were selected from a 3-year-old group. The fish were kept in 400L volume tanks in circulated fresh water. The temperature was held at 24±1ºC. Fish were not fed during the experimental period. The experimental stock was separated into two groups. 7 fishes were given 250 IU hCG and three weeks later 1000 IU hCG per fish. The other 7 fishes received 250 IU hCG every week per fish. The human chorion gonadotropoin was injected in the abdomen. Before the hCG injections fish were anaesthetized by clove oil. The first injection took place on Aug/1, 2001. The last (7^{th}) hCG injection was made on Sept/12, 2001. Sperm collections followed injections 24 hours later by a gentle pressure of the abdomen. The collected sperms were diluted 1:500 with seawater (salinity 35 gl^{-1}) as activating solution for motility and were examined under microscope. Categories of motilities were used after *Perez et al.* (2000) where 0 represented no motile sperm, I.<25%, II.~25-50%, III.~50-75%, IV.~75-90%, and V.~90-100% of the species were vigorously motile. We counted the spermatozoa number (dilute 1:500 freshwater) with Bürker chamber.

RESULTS

Spermiation of males and milt production

At the 5^{th} injections 2 of 10 males produced milt (some drops). At the 6^{th} treatment 6 males released sperm of 8 (Two of them died). We examined the quantities and qualities of the sperm at the 7^{th} injections (*Table 1*). 7 males of 8 ones gave sperm. Stripping of milt and fixed sperm can be seen in *Figures 1* and *2*.

Milt concentration

The concentration of sperm cells in the two experimental groups were as follows:

Group one 3.109±1.75*10^6 spermatozoa mm^{-3} and Group two 4.069±3,187*10^6 mm^{-3}.

Milt volume

There were big individual differences between the volumes of collected sperm. 4 individuals released ≤0,4 cm^3 and the other three animals gave 1,1-1,9 cm^3. The results were 0,51±0,46 ml sperm per 100 g body weight in group one and 0,7±0,56 ml sperm per 100 g body weight in group two.

Sperm motility
The motility of sperms varied between 10 to 70%. In our experiment only one individual produced sperm motility of stage III. (*Table 1*).

Table 1

Summary results of treated males

Number of fish (1)	Treatment (2)	Body weight (g) (3)	Volume (cm^3) (4)	Concentration (millions per mm^3) (5)	Motility (6)
Group 1	weakly hCG (7)	121	1,4	1.159	II
		152	0.2	4.185	II
		89	0.5	4.935	I
		126	0.3	2.16	II
Group 2	2 X hCG (8)	142	1.1	1.44	I
		154	1.9	3.1525	II
		98	0.1	7.615	III
		130	0	0	0

1. táblázat: Összesítő táblázat a kezelt hímekről

Halak sorszáma(1), Kezelés(2), Testtömeg(3), Mennyiség(4), Koncentráció(5), Mozgóképesség(6), Heti hCG(7), 2 hCG(8)

Mortality
Six fish died during the experiment all together due to an outbreak of infection of *dactylogyrosis.* Four and two died at the 5^{th} and 6^{th} treatment respectively.

DISCUSSION

We obtained motilable sperm of hCG treated male eels after the 5^{th} treatment. The main body weight of the males by the time of the 7^{th} week were 126,5±23,6 g, what is close to those ones used in the experiments of *Meske,* (1973); *Bieniarz & Epler,* (1976); *Perez et al.* (2000).

We examined the main parameters of the stripped milt. The concentration of spermatozoa ranged (1.44-7.615)*10^6 cell per mm^3. This is between the values found by *Bieniarz & Epler* (1977) (3.68–11.7)*10^6 and *Perez et al.* (2000) (1.0-1.4)*10^6 cell mm^{-3}. *Bieniarz & Epler* (1977) used experimental fish from wild catch, while *Perez et al.* (2000) experimented on farmed fish similarly as we did.

Regarding the volumes of sperm stripped on the seventh week we got great individual variation. The milt volumes varied between 0.1-1.9 cm^3. This is also in correspondence with the data of *Bieniarz & Epler* (1977).

Perez et al. (2000) reported a gradually increasing motility from the beginning of spermation until it reached the best motility category. It peaked 5 weeks later (ninth week of treatment) when 97.1% of the male showed motile sperm. At this time 47.1% showed classes of motility ≥II., and 29.4% showed classes of motility ≥IV. We got only one individual having sperm of motility stage III. on the 7^{th} week (second stripping).

Although these results can be considered as preliminary ones, they are of great importance for there is hope that the males of European eel can be induced successfully in fresh water.

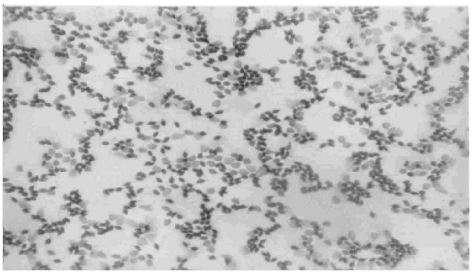
Figure 1

Sperm stripping

1. ábra: Sperma fejés

Figure 2

Fixed eel spermatozoa



2. ábra: Fixált angolnaspermium sejtek

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