Acta Biologica Hungarica 54 (3–4), pp. 313–322 (2003)

EFFECTS OF VARIOUS HORMONES ON THE SEXUAL MATURITY OF EUROPEAN EEL (*ANGUILLA ANGUILLA* L.) FEMALES FROM FARM AND LAKES

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(Received: May 25, 2003; accepted: July 31, 2003)

Previously described and alternative methods of the induction of sexual maturation in the European eel were investigated. Weekly administrations of a gonadoliberin agonist (GnRH-A=D-Phe^6-GnRH-Ea) did not induce statistically significant effect on the gonads of treated eels in none of the dosages used (0.1 µg and 10 µg/fish). Carp pituitary extract and carp pituitary extract together with a dopamine antagonist caused considerable external changes (increase in eye size) and significant gonadal development in two treatment groups: wild and cultivated stocks. The induction of the ovulation by double amount of CP and gonadoliberin agonist with dopamine antagonist mixture was not successful in a wild stock. Fertilisation of stripped eggs of farm eel was attempted unsuccessfully in, due to low egg quality. An advanced phase of the sexual maturation process could be induced in specimen infected by *Anguillicola crassus* indicating, that nematode infection is not a limiting factor in the artificial propagation of the European eel.

Keywords: Eel – artificial maturation – *Anguillicola crassus* – hormonal treatment

INTRODUCTION

Breeding of European eel is still an unresolved problem as no reports have been published about the rearing of eel larva up to glass eel size in captivity. All of the eels found in waters of Europe originate from natural spawning. At the beginning of the migration of freshwater eels their gonads are immature, and sexual maturation will never occur in animals kept in captivity [3]. The maturation process can be induced by different kind of exogenous hormonal treatments. There are several reports about the artificial propagation of Japanese eel *A. japonica* [16, 17, 18, 20, 25, 29, 30, 31, 32], American eel *A. rostrata* [26], New Zealand freshwater eel *A. australis* and *A. dieffenbachii* [12], but reports about artificial induction of sexual maturation in European eel are rare [1, 2, 5]. Successful fertilisation was achieved in only a few occasions [21, 22], while the longest larval rearing lasted only for four days.

In the last decades a new swim-bladder parasite *A. crassus*, – a parasite of the Japanese eel – has appeared in Europe infecting the European eel. It caused severe fish mortality in Lake Balaton in 1991 [13]. This infection can lead to a serious hand-

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icap for the infected fish reducing their swimming speed [27]. It may decrease the reproduction fitness in natural environment. Our investigation suggests that the infection of *A. crassus* does not hinder the artificially induced sexual maturation of female European eels [14]. The goal of the present study was to investigate the possible ways of artificial induction of sexual maturation of female European eels.

MATERIALS AND METHODS

Four experiments were carried out in the fish reproduction laboratory of the Georgikon Faculty of Agriculture, University of Veszprém in Keszthely. The eels used in the experiments were collected from Lake Balaton (Hungary), Lake Ioannina (Greece) and an eel farm, in Köröm (Hungary). Before starting the hormone treatments experimental fish stocks were disinfected by malachite green-formaldehyde bath and were adopted to seawater conditions during a five day period (reaching 30‰ salinity). In order to prevent *pseudodactylogyrosis* the experimental stocks were treated by mebendazole (Vermox®, Richter Gedeon Co.) [28]. In the first and second experiments females were kept in a 1000 l tank connected with a 50 l filtertank while in the third and fourth experiments a 400 l fish tank was applied also with the same filtertank. No feeding was applied during the experiments. Females were treated with abdominal injections after anaesthesia by clove oil *(Syzygium aromaticum)*. The animals were kept in a photoperiod close to natural seasonal rhythm. Experimental arrangement is summarised in Table 1.

First experiment

Eels were selected from a catch by commercial electric fishing in Lake Balaton in June 2001. Three experimental groups were established:

– a non-injected control group, 3 individuals, $w = 383.7 \pm 75.5$ g (Control)

– a group injected with 0.1 µg ovurelin/fish (D-Phe^6-GnRH-Ea, Reanal, Co.) twice a week, 3 individuals, $w = 402.3 \pm 32.4$ g (GnRH-A-0.1)

– a group injected with 10 µg ovurelin/fish twice a week, 4 individuals, $\hat{w} = 393.6 \pm 97.7$ g (GnRH-A-10).

The injections were carried out in an 81 day period. Water temperature was maintained at 21 ± 0.5 °C.

Second experiment

Five females, selected from a 3-year-old stock $w = 487.4 \pm 39.6$ g, were transported from the eel farm in Köröm (Hungary) in November 2001. Fish were injected once a week with 15 mg powdered carp pituitary in 0.65% NaCl solution/kg body weight, (CP-Farm). Water temperature was maintained at 20.3 ± 1.2 °C. For induction of

ovulation 20 mg carp pituitary/kg body weight + half pellet OVOPEL per fish (10 µg D-Ala^6,Pro^{9}NE GnRH/a and 10 µg metoclopramid as dopamine antagonist, Interfish Ltd. Hungary) were used. In order to obtain sperm 5 males were prepared for spermiation by weekly injections of 250 IU human chorion gonadotropin/fish (Richter Gedeon Co.). In a fertilisation test a mixture of sperm from all five males was added to stripped eel eggs.

Third experiment

Five female eels ($w = 1626.6 \pm 217.9$) were imported from Ioannina (Greece) in March 2002. They were injected by 15 mg carp pituitary/kg body weight twice a week (CP-Greece). Two individuals died on the 34th and the 45th days due to unidentified reasons. For inducing ovulation the same hormones were used as in the second experiment. Water temperature ranged between 21.5–24 °C.

Fourth experiment

Five females ($w = 488.4 \pm 144.9$) were selected from a catch from Lake Balaton in August 2002. Each female was treated by 15 mg carp pituitary/kg body weight and 2 mg motilium (dopamine antagonist Janssen Pharmaceutical Co.) twice a week (CP-Bal). Water temperature was maintained at 17.3 ± 0.8 °C.

At the end of the experimental cycles eels were killed by decapitation and several external and internal signs of sexual maturity were examined: I index [19]: $\{(A+B/4)^2 \pi/L\}$ where A: horizontal eye diameter, B: vertical eye diameter, L: body length. GSI was measured (gonado-somatic index) = gonad weight/body weight including the gonads ×100. Pieces of gonads were fixed in 8% formaline, and 5–7 µm thick histological slices were stained with haematotoxylin-eosin. The oocytes, showing clear nucleoli were characterised according to Hibiya [6] and Nagahama [15]. Classification of the oocytes from the oogonium stage (I) through to migratory nucleolus stage (VII) was carried out as described by Lokman and Young [11]. The histological observations were carried out by using an Olympus B061 microscope and images were taken by a Fuji FinePix 2800 digital camera.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Relationship between the GSI and eye indexes was investigated by correlation and regression analysis. Eye indexes of control group was compared to the eye indexes of treated eels using a two-sample *t*-test. Differences were considered significant for $p < 0.05$.

RESULTS

Eye index

Eye indexes of the two groups injected with gonadoliberin agonist (GnRH-A-0.1 7.83 ± 2.24; GnRH-A-10 7.68 ± 3.26) were lower than that of the control group (11.27 ± 2.40), but the difference was not significant. The eye index values of the CP treated females were higher (CP-Farm 13.1 ± 1.70; CP-Greece 15.4 ± 2.09; CP-Bal 13.89 ± 1.08) than in the control group, but the difference was not significant.

Table 1
Individual data of the experimental fish

No.	Treatment	Number of treatments	Length of life in the experiment (day)	GSI (%)	Oocyte developmental stages	Number of *A. crassus*
1	control	–	81	0.79	II, II/III	3
2		–	81	0.86	II, II/III	2
3		–	81	0.86	II, III	0
4	GnRH-A-0.1	23	81	0.9	II, II/III	5
5		23	81	0.92	II, II/III	3
6		23	81	0.83	II	2
7	GnRH-A-10	23	81	1.1	II, III	1
8		23	81	1.22	II, III	0
9		23	81	0.33	II	0
10		23	81	0.86	II, III	5
11		23	81	0.41	II, III	6
12	CP-Farm	17	119	12.20	IV, V	
13		17	119	17.80	IV, V, VI	
14		18	129	4.4	III, IV	
15		19	136	NA*	stripped egg	
16		22	157	21.30	IV, V, VI	
17	CP-Greece	21	67	32.60	IV, V, VI, VII, preovulated egg	
18		23	75	22.30	IV, V, VI, VII, preovulated egg	
19		23	75	23.60	IV, V, VI, VII, preovulated egg	
20	CP-Bal	28	101	11.78	IV, V	5
21		30	109	15.99	IV, V, VI	0
22		32	113	16.29	IV, V, VI	9
23		33	115	4.24	III, IV	2
24		33	116	12.46	IV, V	3

* NA = not assayed

Induced gonadal development

The two different dosages of gonadotropin analogues were not able to induce remarkable increase of ovaries in the treated groups as indicated by GSI values (control 0.84 ± 0.04; GnRH-A-0.1 0.88 ± 0.05; GnRH-A-10 0.78 ± 0.40). The hormonal treatment with carp pituitary proved to be effective (CP-Farm 13.93 ± 7.37; CP-Greece 26.17 ± 5.61; CP-Bal 12.15 ± 4.87). All individuals of the CP-Greece group reached the final maturational phase but the induction of the ovulation was not successful. The treated females died within 12 hours after the final treatment. At this time small pieces of their ovaries were extruded from the genital hole.

In only one occasion a female of CP-Farm group successfully ovulated. 12 hours after the last injection, eggs were stripped by a gentle pressure on the abdomen. The stripped eggs were placed in seawater (37‰ salinity) where only some eggs (cca. 5%) showed the signs of ripening (perivitelline space and normal size of animal pole appearing). Fertilisation tests were not successful.

Histological study

All typical stages of the oogenesis from previtellogenic oocytes to mature eggs were found in the ovaries of treated groups (Table 1). Ovaries of the untreated females contained predominantly previtellogenic oocytes (81±16.97 μm) with basophilic cytoplasm and no or only a few oil droplets (Fig. 1/a). Less amount of oocytes belonged to the categories between the perivitellogenic and cortical alveolus stages (108 ± 16.41 μm) where the oil drops start to fill the cytoplasm. The histological picture of GnRH-A-0.1 injected ovaries were similar to the control while the ovaries of GnRH-A-10 injected females consisted of oocytes predominantly from the cortical alveolus stage (154 ± 27.88 μm) (Fig. 1/b). In the case of CP groups, there were all stages of oocytes found from the early vitellogenic phase to maturated eggs. The rate of the various stages depended on the GSI value of the fish. The early vitellogenic oocytes are characterised by large and small peripherial yolk granules (349 ± 54.72 μm) (Fig. 1/c). In the oocytes in midvitellogenic phase (436 ± 47.37 μm) (Figs 1/d, e) the granules filled out the cytoplasm The following stage detected was the last stage of vitellogenesis (526 ± 54.66 μm), where the yolk granules fused with each other to form a single mass of yolk. There were some large lipid droplets in the ooplasm (Fig. 1/g). There were many cells found in the migratory nucleolus stage (567.5 ± 45.72 μm) (Fig. 1/f). Some pre-ovulated eggs were also found that contained some smaller lipid droplets (559 ± 42.16 μm). All fish from the CP-Greece group reached full sexual maturity indicated by the lipid fusion in oocytes (Fig. 1/h, Fig. 2).

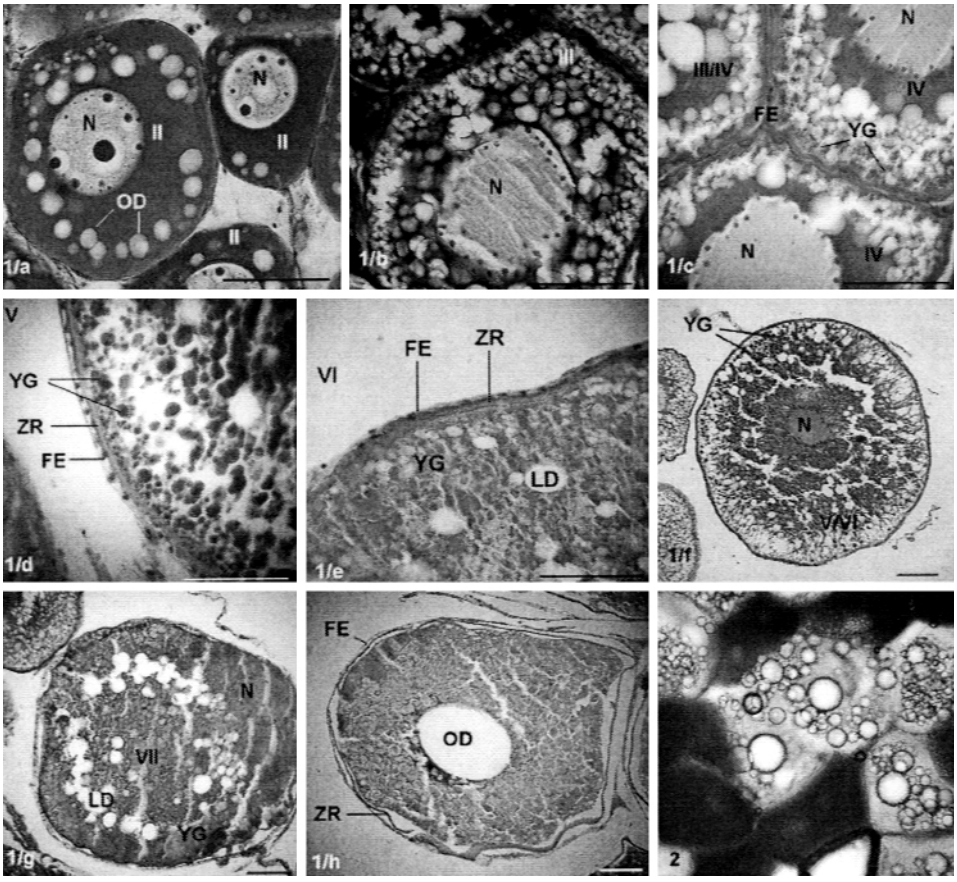

Fig. 1. Histological pictures of oocyte developmental phases. Scale bar: Figs 1/a–e 50 µm, 1/f–h 100 µm. Fig. 1/a. 2nd control (GSI = 0.79%), Fig. 1/b. 8th GnRH-A-10 (GSI = 1.22%), Fig. 1/c. 14th CP-Farm (GSI = 4.4%), Fig. 1/d. 20th CP-Bal (GSI = 11.78%), Fig. 1/e. 13rd CP-Farm (GSI = 17.8%), Fig. 1/f. 16th CP-Bal (GSI = 32.6%), Fig. 1/g. 17th CP-Greece (GSI = 21.3%), Fig. 1/h. 18th CP-Greece (GSI = 22.3%)

Fig 2. Native photo of piece of ovary from 19th CP-Greece fish between two glass-slides

Abbreviations: FE follicular epithelium, G granulosa cell, LD lipid droplet, N nucleus, OD oildrop, YG yolk granule, ZR zona radiata, II previtellogenic oocyte, III stages III oocyte, IV stages IV oocyte, V stages V oocyte, VI stages VI oocyte, VII stages VII oocyte

Correlation between the GSI and the eye size

There was no significant relationship between the GSI and eye size neither in the GnRH-A injected nor in the control group. There was a weak correlation between the GSI and the eye size index in the CP groups ($r^2 = 0.51$, $P < 0.05$).

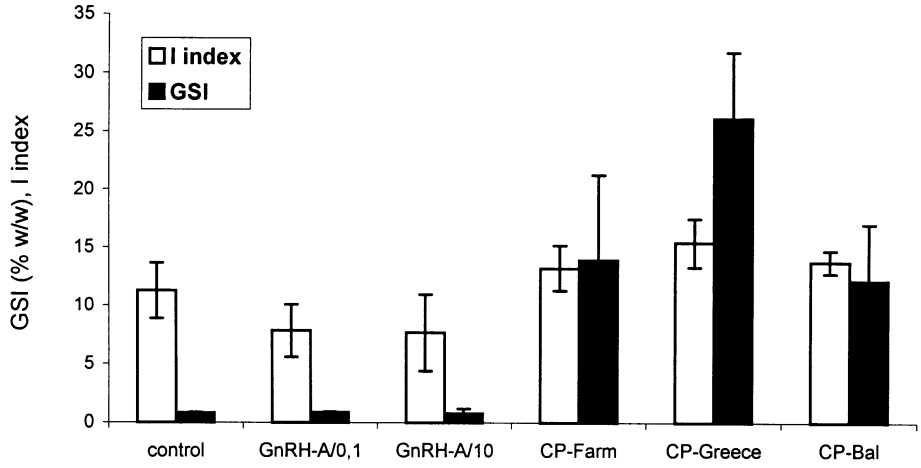

Fig. 3. Summarised diagram of the GSI and eye data among the trials

Anguillicola crassus infection

The swim-bladder walls of fish coming from Balaton lake were found thicker then the normal state, showing the effect of *Anguillicola infection* in all individuals, while eels from the Köröm farm and from Greece (Ioannina lake) showed intact and normal swim bladders. In the swim-bladders of Balaton eels there were nematodes from larval to adult stage. The number of nematodes found in one fish ranged between 1 and 9 (see Table 1). Characteristically, infected fish displayed swim-bladders, dotted with tiny blood drops.

DISCUSSION

Different kinds of gonadoliberin agonists are used efficiently to induce ovulation and sperm release in artificial propagation of farmed fish species. In contrast, in the present work long-term treatment (81 days) of two doses of OVURELIN did not induce sexual maturation in European eel females. The oocytes in the OVURELIN treated group remained in the previtellogenic and cortical alveoli phase, similarly to the control. Dufour and co-workers [3, 4] found, that estradiol-17β (E_2)-pretreated females injected by an other form of GnRH-A (des-Gly^{10},(D-Ala^6)-LHRH ethylamide, SIGMA), or dopamine antagonists (pimozide or domperidome) alone were unable to increase GTH concentration in blood, or to increase GSI. GnRH-A and pimozide or domperidome together caused a significant increase in plasma GTH level and the fish reached max. 4.56% GSI by the end of the 78 days experiment. The histological analysis of the oocytes indicated that the vitellogenesis process started.

In an earlier paper we reported ovulation and egg stripping by using carp-pituitary and OVOPEL mixture [14]. In the present study four females reached the preovulation stage (preovulated eggs with oil drop fusion), but we could strip eggs only from one of them. Although freshly stripped sperm was mixed to the eggs, no signs of embryonal development could be observed.

The body weights in the CP-Greece group were bigger than those in the CP-farm and CP-Bal groups, but this did not influence the efficiency of artificial maturation. In our previous experiments we managed to strip fertilisable eggs from individuals of body weights ranging between 377–734 g [14].

Pituitary extracts or commercial gonadotropins, pituitary extract, LHRH in turn may be an applicable method to obtain ripe eel eggs, however, the efficiency of the method is far from the practical needs as reported by several authors (*Anguilla anguilla* – [2, 5]; *A. japonica* – [25, 31, 32]). Prokhorchik and co-workers [21, 22] used eel and eel pituitary extract for the induction of sexual maturation and ovulation but the percentage of ovulated females and hatching rate of eggs were not reported. Best results to induce ovulation and obtain fertilizable eggs, were obtained by using 17α-20β-dihydroxi-pregnen-3-one or 17α-hydroxyprogesterone (*A. rostrata* – [26]; *A. dieffenbachii* and *A. australis* [*12*]; *A. japonica* [7, 9, 10, 16, 17, 18, 20, 23, 24, 30].

The GSI values of fully maturated *A. anguilla* females, ranged from 26.17 ± 5.61% (present study), 31.5% [5], over 40% [2], 42.7 ± 7.56% [14] to 68.4% [1].

Our observations that farmed European eels are suitable for the artificial maturation process are in accordance with results on Japanese eel [7, 8, 9, 10, 16, 17, 24]. The speed of induced ovary maturation was highest in silver eels, on average after 10 weeks of treatment, while feminised and cultivated eels developed slower, reaching sexual maturity only in about 17 to 19 weeks respectively. There were considerable differences in silver, feminised and cultivated eels regarding the percentage of fish reaching the maturation phase (100% silver, 64% feminised and 29% cultivated eels) [8].

In this study, sexual maturation was induced in *Anguillicola crassus* infected European eel females. With our preliminary result [14] we provide support to the theory that though the nematode infections can cause problems in the migration of eels to the Sargasso Sea, but it may not limit hormonally induced artificial propagation.

ACKNOWLEDGEMENTS

We would like to thank to Mr. Ferenc Németh, Mr. István Szabó, Mr. István Báthory for catching trial eels from Lake Balaton, Mr. Ákos Lukács Köröm eel farm and Mr. Lazaros Natzis DELI, Greece for providing the experimental fish. We are very grateful to the Pathology Department of the Keszthely Hospital for the histological slices. We also thank to Dr. Ferenc Müller (Institute of Toxicology and Genetics, Karlsruhe), who reviewed and corrected the manuscript. This work was supported by the Ministry of Education (BIO-169/2001 and OM-00440/2001).

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