TESTING RECOMBINANT GONADOTROPINS FOR THE PROPAGATION OF EUROPEAN EEL (*ANGUILLA ANGUILLA*), PRETREATED BY FEMINIZATION, SIMULATED MIGRATION AND STEROID IMPLANTS

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Introduction

The current protocol for the induced maturation of female European eel consists of long-term weekly injections with carp or salmon pituitary extract to stimulate sexual maturation and with 17α,20β-dihydroxy-4-pregnen-3-one (DHP) to induce ovulation (Palstra et al., 2005). However, egg quality is often poor and when larvae are produced they die before functional exogenous feeding.
As these problems may well originate from the used protocol and broodstock background, we commence the conditioning of female broodstock from the glass eels stage by feminization, simulated migration and steroid implants. This study is the second in a series of ongoing experiments in our lab aiming to replace treatment with pituitary extract by highly stable eel-specific recombinant gonadotropins in order to improve gamete quality and reproductive success. In a preliminary trial with wild eels we managed to mature one female with recombinant FSH up to a gonadosomatic index (GSI) of 38.

Materials and methods

Single chain recombinant gonadotropins (recFSH and recLH) were obtained by fusing the respective β and α European eel specific subunits with a linker peptide. These sequences were expressed in a mammalian cell line (CHO) and subsequently the gonadotropins were semi-purified by ion exchange chromatography.
Experimental eels had been subjected to a simulated lifecycle approach: A batch of wild glass eels was feminised by feeding them with 17β-estradiol (E2) coated pellets over a 6 month-period. After an additional 6 months of feeding them with a custom-made broodstock diet, eels of ~400 g were selected, transferred to seawater and fed no longer. For 2 months, eels were then subjected to simulated migration: constant swimming in the dark at daily alternating temperatures between 10 and 15 °C to make them silver (Mes et al., 2016).
Sixteen of these eels were then randomly selected, PIT-tagged, weighed and measured for body girth, body length and eye diameters to calculate the Pankhurst eye index. For an additional 2 months, eels were treated with a steroid implant (Thomson-Laing et al., 2019) containing 17-methyltestosterone (5 mg) and E2 (0.5 mg). Again, eels were weighed and measured, and GSI was non-invasively determined by applying ultrasound. Eels were then divided between two groups for hormonal stimulation of sexual maturation by weekly intramuscular injection of either carp pituitary extract (CPE at a dose of 20 mg kg^{-1}; CPE group; N=8) or recFSH (12 µg; REC group; N=8).
Starting in week 7, two days after each injection, eels were weighed to determine whether oocyte hydration had commenced as indicated by an increased body weight index (BWI).

At BWI increase and at recFSH injection 16, final oocyte maturation (FOM) was induced by injecting CPE in the CPE group, or by injecting recFSH in combination with recLH (8 µg) in the REC group. Ovulation for eels in both groups was induced by injecting DHP (2 mg kg^{-1}). Eels were stripped for eggs which were fertilised and reared.

Results and discussion

At the end of migration and start of injecting implants, eels were 59 ± 3 cm long, weighed 402 ± 33 g, body girth was 11.5 ± 0.4 cm and eye index was 7.19 ± 1.46. At the end of this period when dividing both treatment groups, eels increased significantly in weight up to 418 ± 33 g, in body girth up to 12.7 ± 0.5 cm and in eye index up to 10.64 ± 1.09, indicating initiation and progression of vitellogenesis. Indeed, the GSI values were between 3.1 and 7.3, well above values of 2-2.5 which mark the initiation of vitellogenesis (Jéhannet et al., 2019).

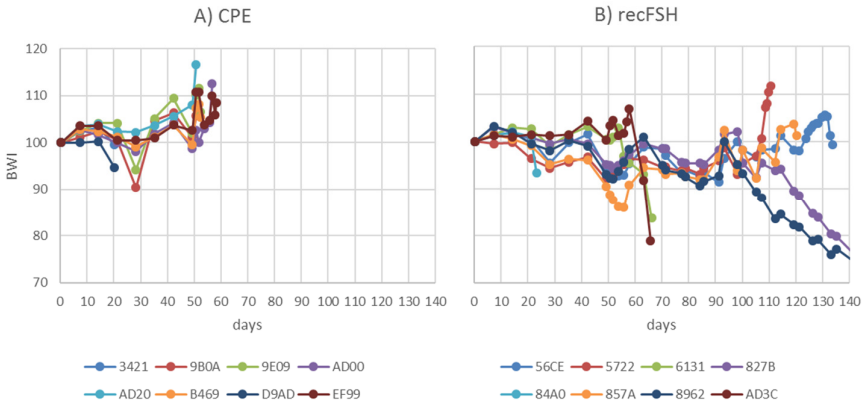

Fig. 1. Body weight index (BWI) during the period of weekly hormone injections with (A) CPE and (B) recFSH.

Eels from the CPE group matured much faster than eels from the recFSH group: 8 ± 1 vs. 19 ± 3 injections (i.e., after combining recFSH with recLH). Six eels (75%) in the CPE group matured of which two yielded batches of larvae surviving for 4 and 9 days post hatching. Three eels (38%) in the REC group matured and from one eel (AD3C) eggs could be stripped (80% floated). However, no larvae were obtained from eels from this group. Four other eels in the REC group increased in BWI just before dropping weight and dying. Recombinant FSH appeared to work very well but the FOM protocol using recLH needs further improvement and is under current investigation.

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References

Jéhannet, P., Kruijt, L., Damsteegt, E.L., Swinkels, W., Heinsbroek, L.T.N., Lokman, P.M., Palstra, A.P., 2019. A mechanistic model for studying the initiation of anguillid vitellogenesis by comparing the European eel (*Anguilla anguilla*) and the shortfinned eel (*A. australis*). Gen Comp Endocrinol, in press.

Mes, D., Dirks, R.P., Palstra, A.P., 2016. Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*). Aquaculture 452, 367–372.

Palstra, A.P., Cohen, E., Niemantsverdriet, P., van Ginneken, V., van den Thillart, G.E.E.J.M., 2005. Artificial maturation and reproduction of European silver eel: Development of oocytes during final maturation. Aquaculture 249: 533-547

Thomson-Laing, G., Damsteegt, E.L., Nagata, J., Ijiri, S., Adachi, S., Todo, T., Hiramatsu, N., Lokman, P.M. 2019. Synergistic effects of estradiol and 11-ketotestosterone on vitellogenin physiology in the shortfinned eel (*Anguilla australis*). Biology of Reproduction, in press.