FEMINIZATION OF FALSE CLOWNFISH (*Amphiprion ocellaris*) TO PRODUCE FUNCTIONAL BROODSTOCK USING 17 β–ESTRADIOL

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Amphiprion ocellaris, commonly referred to as the false clownfish, is a popular marine aquarium fish with high market value in the ornamental fish trade due to its unique and beautiful body coloration. The social structure of the false clownfish typically consists of a pair of functional brooders within a population of individuals which remain as protandrous hermaphrodites usually incapable of breeding. This situation often hampers the commercial scale production of large numbers of clownfish offspring and broodstock. To enhance breeding outputs, protandrous hermaphrodites must be converted to functional brooders with a distinct sex prior to pairing and breeding. This study is the first trial to solve this situation. In this study, 17β-estradiol (E2) was used to feminize juveniles of *Amphiprion ocellaris* by immersion and oral administration. Mating between the induced females and the ordinary male was observed for 63 days to evaluate pairing possibility and breeding.

Twenty *Amphiprion ocellaris*, measuring 3.5-5.5 cm in total length, were immersed separately in 0, 0.1, 0.2 and 0.4 mg L^{-1} of E2 for 15 days, with each tank containing 50 L of seawater. Fish not subjected with E2 served as controls. After the treatment period, fish were maintained for 2 months for post-treatment. Gonad samples were taken from 3 fishes per each group immediately at the end of the hormone treatment period, 1 month post-treatment, and 2 months post-treatment for histological studies. All the fish receiving E2 employed induced feminization, as shown by histology profiles revealing degenerate testicular tissues and several developed ovarian cells at different stages (oogonia, previtellogenic and vitellogenic) 30 days after E2 incubation. Conversely, gonads of non-treated fish were not differentiated throughout experiment. The gonads possessed both ovarian and testicular tissues, a typical characteristic indicating that the fish are still non-breeding and male phase with ambisexual gonad. The highest survival rate (95%) was collected from dosage of 0.1 mg L^{-1} after hormone treatment period compared to 80% and 75% in 0.2 and 0.4 mg L^{-1}, respectively.

For oral administration of E2, twenty fish were randomly assigned to each of four 100-L tanks to accommodate the control group and the 30, 60, 120 mg kg^{-1} E2 treatments. The E2 diets were given to fish for 2 months and then fish were reared another 2 months for post-treatment analysis. For the histological study, three fish from each group were dissected and gonadal tissue was collected for histological observation at the end of E2 oral administration, again 1 and 2

months after hormone treatment. All treated fish became functional females, as shown by the complete transition of the gonad from a non-breeding stage to a female stage after treatment duration of two months. At this stage, gonad profiles of treated fish were filled fully with previtellogenic stage oocytes while spermatogenic germ cells were absent. The presence of vitellogenic oocytes was observed in the treated fish gonads during post-treatment. Similar to E2-immersion study controls, gonadal histology indicated that non-treated fish are non-breeding and at a male phase throughout experiment. The survival rate for the oral administration treatments were lower compared to the immersion method. The treatment groups had survival rate of 75%, 80% and 55% for the E2 diets of 30 mg kg^{-1}, 60 mg kg^{-1} and 120 mg kg^{-1}, respectively.

For a comparison between the two hormonal treatment pathways, it can be concluded that 0.1 mg L^{-1} E2 may be the optimal dosage for feminization in false clownfish due to safer method, ease in manipulation, and shorter treatment duration.

Pairing behaviors in the treated and non-treated clownfish were observed to evaluate pairing possibility and breeding. Five males (6.7±0.57 cm in total length) individuals collected form wild were randomly chosen for mating with five E2-treated females (7.84±0.57 cm in total length) from the feminization experiments. Each pair was removed to 100-L tanks (n=5) and kept for 63 days for mating behavior observation. A polyvinyl clorua (PVC) pipe (10 cm diameter

and 20 cm length) was placed in center of each experimental tank as substitute for a host sea anemone. E2-induced females could pair with the ordinary males 63 days after introduction. Females expressed dominant behavior throughout observation period including occupying the PVC pipe substrate and attacking the males. The pairing process was completed when induced female shared a territory with the male and they cleaned substrate together. These behaviors predicted future breeding.

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FEMINISASI IKAN FALSE CLOWNFISH (*Amphiprion ocellaris*) UNTUK MENGHASILKAN INDUK BETINA MENGGUNAKAN 17β-ESTRADIOL

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Amphiprion ocellaris, adalah ikan akuarium marin yang popular dengan nilai yang tinggi dalam dagangan ikan hiasan di sebabkan warnanya yang unik dan menarik. Struktur sosial ikan ini biasanya terdiri daripada sepasang induk dan ikan yang lebih kecil yang tidak boleh membiak. Keadaan ini sering menghalang penghasilan ikan dan induk spesies ini secara meluas. Untuk meningkatkan hasil pembiakan, ikan ini perlu di tukar menjadi induk berlainan seks yang boleh membiak sebelum proses pemasangan induk untuk membiak. Kajian ini merupakan ujian pertama untuk mengatasi masalah ini. Untuk itu, 17β-estradiol (E2) digunakan untuk memfemininkan juvenil *Amphiprion ocellaris* dengan menggunakan kaedah rendaman dan pemberian makanan. Ikan betina yang di rawat akan dipasangkan dengan ikan jantan normal telah diamati selama 63 hari untuk menilai kemungkinan pembiakan.

Dua puluh ekor ikan *Amphiprion ocellaris*, berukuran 3.5-5.5 cm, di rendam secara berasingan di dalam 0.1, 0.2 dan 0.4 mg L^{-1} E2, dan satu kumpulan kawalan dengan 0 mg L^{-1}, selama 15 hari, dengan setiap tangki mengandungi 50 L air. Selepas tamat rawatan, ikan terus dijaga selama 2 bulan. Sampel gonad di ambil dari 3 ikan daripada setiap rawatan pada akhir rawatan, 1 bulan selepas rawatan dan 2 bulan selepas rawatan untuk kajian histologi. Semua rawatan yang dilakukan menggalakkan feminisasi, seperti yang ditunjukkan melalui profil histologi di mana tisu testicular semakin berkurangan dan sel ovary ınula berkembang dalam pelbagai peringkat (oogonia, previtellogenik dan vitellogenik) 30 hari selepas rawatan E2. Sebaliknya, gonad ikan di dalam kumpulan kawalan tidak menunjukkan sebarang perubahan. Ia masih mempunyai kedua-dua tisu ovari dan testicular, menunjukkan ia ikan jantan ataupun ikan 'non-breeding' dengan gonad ambiseksual. Kadar kemandirian tertinggi (95%) dicatatkan dari rawatan 0.1 mg L^{-1} selepas rawatan hormon berbanding 80% dan 75% dari rawatan 0.2 and 0.4 mg L^{-1}.

Bagi rawatan pemberian makanan mengandugi E2 pula, 20 ekor ikan di letakkan di dalam 4 tangki berkapasiti 100 L secara berasingan dirawat dengan 3 kepekatan E2 iaitu 30, 60 dan 120 mg kg^{-1} dengan satu kumpulan kawalan. Diet mengandungi E2 diberikan selama 2 bulan dan terus dijaga selama 2 bulan. Untuk kajian histologi, 3 ekor ikan daripada setiap kumpulan akan dibedah dan tisu gonad akan di ambil untuk pemerhatian histology pada akhir rawatan, 1 bulan selepas rawatan dan 2 bulan selepas rawatan. Semua ikan yang

di rawat menjadi ikan betina, yang mana perubahan gonad daripada peringkat 'non-breeding' ke peringkat betina dapat di lihat selepas 2 bulan tempoh rawatan. Pada peringkat ini, gonad ikan di penuhi dengan sel peringkat previtellogenik dan vitellogenik manakala sel testicular tidak di temui. Sama seperti kaedah rendaman, ikan kawalan dalam eksperimen ini juga tidak menunjukkan perubahan yang ketara dalam gonad masing-masing. Kadar kemandirian dalam rawatan pemberian makanan adalah lebih rendah berbanding kaedah rendaman. Kumpulan rawatan mempunyai kadar kemandirian sebanyak 75%, 80% dan 55% untuk kumpulan rawatan E2, iaitu 30 mg kg^{-1}, 60 mg kg^{-1} dan 120 mg kg^{-1}. Apabila membandingkan antara kedua-dua rawatan, boleh di simpulkan bahawa dos optimal untuk feminisasi ikan clownfish adalah 0.1 mg L^{-1} E2 kerana kaedah yang lebih selamat, senang untuk di manipulasi and masa rawatan yang lebih singkat.

Tabiat pemasangan ikan yang di rawat dengan ikan yang tidak di rawat di perhatikan untuk menilai kemungkinan untuk membiak. Lima ekor ikan jantan (6.7±0.57 cm panjang) yang ditangkap daripada liar dipilih secara rawak untuk dipasangkan dengan lima ekor ikan betina yang dirawat menggunakan E2 (7.84±0.57 cm panjang) daripada eksperimen feminisasi. Setiap pasangan dipindahkan ke dalam akuarium 100-L (n=5) dan dijaga selama 63 hari untuk memerhati tabiat pembiakan. Satu paip polyvinyl clorua (PVC) (10 cm diameter dan 20 cm panjang) diletakkan di dalam setiap akuarium untuk menggantikan anemone.Ikan yang dirawat dengan E2 boleh di pasangkan dengan ikan jantan

biasa dalam masa 63 hari. Ikan betina menunjukkan sifat dominan seperti menghuni substrat PVC dan menyerang ikan jantan. Proses pemasangan ini selesai selepas ikan betina yang di rawat berkongsi kawasan dan membersihkan substrat bersama-sama ikan jantan. Proses ini menunjukkan kemungkinan ikan ini untuk membiak.