



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF SYNTHETIC HORMONES ON BREEDING PERFORMANCE
OF MALAYSIAN MAHSEER (TOR TAMBROIDES BLEEKER) IN
CAPTIVITY**

NIK MD. AZUADI B. NIK DAUD

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BERILMU BERBAKTI

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By

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Thesis submitted to the School of Graduate Studies Universiti Putra Malaysia, in
fulfillment of the requirements for the degree of Master of Science

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January 2013

Chair: Professor Siti Shapor Siraj, PhD

Faculty: Agriculture

Plasma sex steroids and breeding performances of *Tor tambroides* were studied. Filial generation one (F₁) broodstocks (range body weight of 1.4 to 4.4 kg and range total length 43.9 to 73.0 cm) were used for artificial propagation. All females were given pretreatment with Ovaplant (23.4-44.9 µg kg⁻¹) for 6 weeks prior to induction. Selected synthetic hormones such as Ovaprim, Ovatide, Luteinizing hormone releasing hormone analogue (LHRHa), human chorionic gonadotropin (HCG), salmon gonadotropin releasing hormone analogue (sGnRH_a/OvaRH), and carp pituitary extract (CPE) were used for the induction and the effect of hormones on breeding performance and larval quality of *Tor tambroides* were then evaluated. The best hormone from this induction was chosen to test its effectiveness at various dose levels in inducing ovulation of *T. tambroides*. In order to investigate what factors improve breeding performance of *T. tambroides*, an experiment was conducted to test the effect of dopamine antagonist domperidone (DOM) to accelerate the maturation and ovulation. Blood were collected from each treated female breeders at

0 h (prior to administration), 6, 12 and 24 h post injection. Plasma samples were analyzed for Testosterone (T) and 17 β -estradiol (E₂) concentrations using Enzyme Link Immunosorbent assay (ELISA). Ovulatory response, fertilization rate, hatching rate, and survival rate were determined and data for plasma steroid hormones were analyzed using analysis of variance (ANOVA).

The results showed that Ovatide (0.5 mL/kg BW) was the most reliable in inducing ovulation in female *T. tambroides* with highest ($p < 0.05$) breeding performance (egg and larval qualities) overshadowing the other tested hormones. Ovaprim (0.5 mL/kg BW) was next best followed by OvaRH (10 μ g/kg BW). No ovulation occurred in the group treated with LHRHa (10 μ g/kg BW), HCG (1000 I.U/kg BW) and CPE (10 mg/kg BW). Ovatide was further chosen to test its effectiveness at various dose levels in inducing ovulation of *T. tambroides*. The result indicated that the total stripped eggs and fecundity were significantly highest ($p < 0.05$) when females were treated with Ovatide at a dose of 0.5 mL/kg BW compared to those administered at other levels, with values of percent fertilization, hatching, survival and normal larvae of 88, 84, 81 and 74%, respectively. Dose of higher or lower than 0.5 mL/kg BW Ovatide resulted in over ripening of ova and partial ovulation leading to production of higher deformed larvae.

Sex steroids plasma concentration of T and E₂ increased significantly in association with ovulation. The levels fluctuated and reached a peak at 12 h, then dropped dramatically at 24 post injection (PI). In contrast in the non-ovulated groups; LHRHa (10 μ g/kg BW), CPE (10 mg/kg BW), HCG (1000 I.U /kg BW) and DOM (5 mg/kg BW), the sex steroids hormone concentration showed little increment after injection

and rose at 12 h PI, then slowly dropped at 24 h PI. Changes in sex steroids hormone level after hormone induction lead to vitellogenesis, oocyte development and ovulation in female breeders.

This study will provide an insight into sex hormone steroids variations during oocyte development and these results provide important basic information and possible use of plasma parameters as predictive factors of the success of hormonally induced ovulation in *T. tambroides* in captivity.



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sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN HORMON-HORMON SINTETIK KE ATAS PRESTASI
PEMBIAKAN KELAH MALAYSIA (TOR TAMBROIDES BLEEKER)
DALAM KURUNGAN**

Oleh

NIK MD. AZUADI B. NIK DAUD

Januari 2013

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Steroid seks plasma dan prestasi pembiakan *Tor tambroides* betina dikaji. Baka generasi pertama (F₁) (julat berat badan daripada 1.4 kepada 4.39 kg dan julat jumlah panjang daripada 43.9 kepada 73 cm) digunakan untuk pembiakan tiruan. Kesemua betina telah diberi prarawatan dengan Ovaplant (23.4-44.9 $\mu\text{g kg}^{-1}$) selama 5-6 minggu sebelum induksi. Hormon-hormon terpilih seperti Ovaprim, Ovatide, analog hormon perembesan hormon luteinizing (LHRHa), gonadotropin korion manusia (HCG), analog hormon perembesan Gonadotropin salmon (sGnRH_a/OvaRH), dan ekstrak pituitari kap (CPE) digunakan untuk aruhan dan kesan hormon ke atas prestasi pembiakan dan kualiti larva *Tor tambroides* dinilai. Hormon yang paling berpotensi dari aruhan ini dipilih untuk menguji keberkesanan pada pelbagai tahap dos dalam mendorong ovulasi *T. tambroides*. Dalam usaha untuk menyiasat apakah faktor meningkatkan prestasi pembiakan *T. tambroides*, satu eksperimen telah

dijalankan untuk menentukan kesan antagonis dopamin domperidone (DOM) bagi mempercepatkan kematangan dan ovulasi. Darah dikumpulkan dari setiap induk betina yang dirawat pada 0 h (sebelum suntikan), 6, 12 dan 24 jam selepas suntikan. Sampel plasma dianalisis untuk mengetahui kepekatan Testosteron (T) dan 17β -estradiol (E2) dengan menggunakan teknik *Enzyme Link Immunosorbent assay* (ELISA). Tindak balas ovulasi, kadar persenyawaan, kadar penetasan dan kadar kemandirian dikira dan data bagi kadar plasma hormon steroid dianalisis menggunakan *analysis of variance* (ANOVA).

Hasil kajian menunjukkan bahawa Ovatide (0.5 mL / kg BT) adalah yang paling berpotensi dalam mendorong ovulasi dalam *T. tambroides* betina dengan prestasi pembiakan (kualiti telur dan larva) tertinggi ($p < 0.05$) membayangi hormon-hormon lain yang diuji. Ovaprim (0.5 mL / kg BT) adalah hormon yang seterusnya terbaik diikuti oleh OvaRH (10 μ g / kg BT). Ovulasi tidak berlaku dalam kumpulan yang dirawat dengan LHRHa (10 μ g / kg BT), HCG (1000 iu / kg BT) dan CPE (10 mg / kg BT). Ovatide kemudian dipilih untuk diuji keberkesanan pada pelbagai tahap dos dalam mendorong ovulasi *T. tambroides*. Hasil menunjukkan bahawa jumlah telur dilucutkan dan kadar kesuburan ketara tertinggi ($p < 0.05$) apabila betina dirawat dengan Ovatide pada kadar dose 0.5 mL / kg BT berbanding dengan yang disuntik di peringkat lain, dengan nilai peratus persenyawaan, penetasan, kemandirian dan kenormalan larva 88, 84, 81 dan 74%, masing-masing. Apabila dos yang lebih tinggi dan lebih rendah daripada 0.5 ml / kg BT Ovatide disuntik ia menyebabkan ova terlebih matang dan ovulasi pertengahan membawa kepada penghasilan larva cacat yang lebih tinggi. Oleh itu, kepekatan 0.5 mL / kg BT Ovatide didapati optimum untuk mendorong ovulasi *T. tambroides* dalam kurungan.

Kepekatan plasma steroid seks T dan E₂ meningkat dengan ketara setara dengan ovulasi. Tahapnya naik turun dan mencapai kemuncaknya pada 12 jam, kemudian jatuh secara mendadak pada 24 jam selepas suntikan (SS). Berbeza dalam kumpulan yang tiada ovulasi; LHRHa (10 µg / kg BW), CPE (10 mg / kg BT), HCG (1000 iu kg BT /) dan DOM (5 mg / kg BT), kepekatan hormon seks steroid menunjukkan peningkatan sedikit selepas suntikan dan meningkat pada 12 jam SS, kemudian perlahan-lahan jatuh pada 24 jam selepas suntikan. Perubahan dalam tahap hormon steroid seks selepas induksi hormon akan membawa kepada vitellogenesis, pembangunan oosit dan ovulasi dalam induk betina.

Kajian ini akan memberi penjelasan kepada variasi steroid hormon seks semasa pembangunan oosit dan keputusan ini menyediakan maklumat asas yang penting dan kemungkinan penggunaan parameter plasma sebagai faktor ramalan kejayaan ovulasi diaruhkan hormon *T. tambroides* dalam kurungan.

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I certify that an Examination Committee met on 17 February 2013 to conduct the final examination of Nik Md. Azuadi B. Nik Daud on her Master of Science thesis entitled “Evaluation of synthetic hormones on breeding performance of Malaysian mahseer, *tor tambroides* (Bleekers 1854) kept in captivity” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree.

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DECLARATION

I hereby declare that the thesis is on my original work for quotation and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at University Putra Malaysia or other institutions.

NIK MD. AZUADI B. NIK DAUD

Date: 17 January 2013

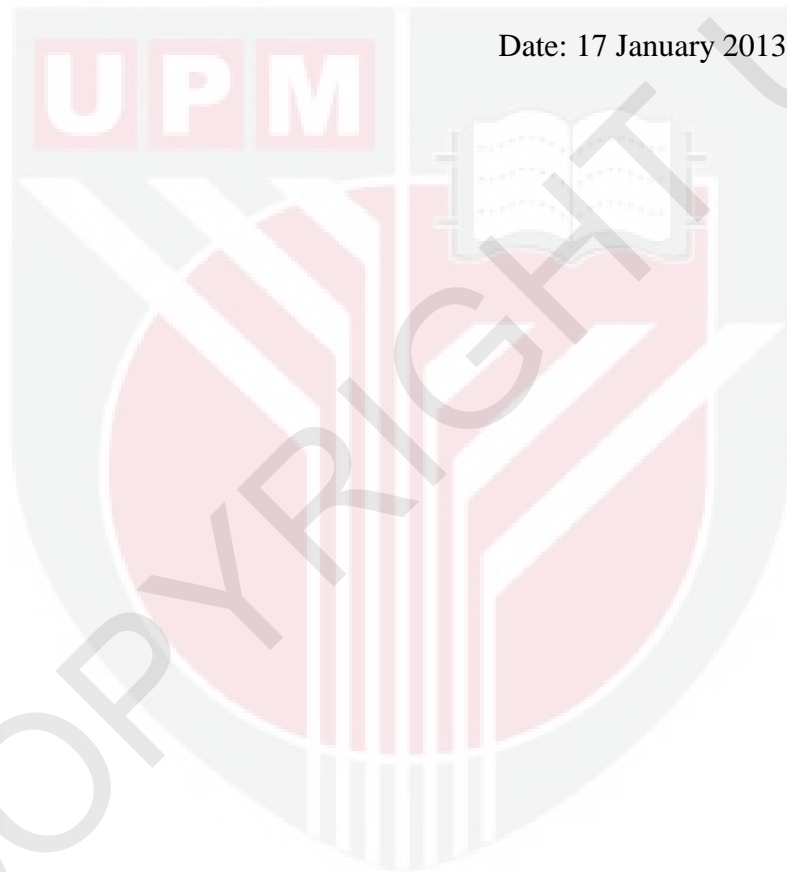


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