UNIVERSITI PUTRA MALAYSIA

IN VIVO ACTIVITY OF GONADOTROPIN- RELEASING HORMONE (cGnRH-II, sGnRHa and LHRHa) IN AFRICAN CATFISH, (Clarias gariepinus B.) AND JAVANESE BARB, (Barbodes gonionotus B.)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Masters of Science

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Chair: Assoc. Prof. Sharr Azni Bin Harmin, PhD

Faculty: Agriculture

Gonadotropin hormone releasing hormone (GnRH) is a peptide hormone that is responsible for stimulating the release of gonadotropins from pituitary and consequently influence the steroid hormone production level in the ovary. Six experiments have been conducted to determine the effectiveness of cGnRH-II as an inducing agent for maturation and ovulation in *Clarias gariepinus* and *Barbodes gonionotus.* The first experiment determined the effectiveness of native cGnRH-II when compared with analog LHRHa, and sGnRHa with saline used as control. The concentration of all the hormones used in this experiment was 20µg/kg. For the second experiment, three different concentrations were given; 2 µg/kg, 20 µg/kg and 200 µg/kg with saline as a control. Finally, the third experiment examined the pimozide (PIM) as dopamine inhibitor with the combination of cGnRH-II to accelerate the maturation and ovulation. The hormones involved were cGnRH-II 200 µg/kg; 5mg/kg PIM; cGnRH-II 200 µg/kg + 5mg/kg PIM and saline. All the hormones

were administered as a single injection. The experimental parameters observed were hormonal changes in the fishes and Germinal Vesicle (GV) movement during the experiment. Each group consisted of six sexually mature females ranging from 450-550 g Body Weight (BW). The GV was examined prior to injection at 0h, 6h, 12h and 24h post injection and ranked from 1 (GV at central location) to 4 (at ovulation stage). Blood were sampled at the same time as the GV examination. Plasma sample were analyzed for Testosterone (T) and 17ß-Estradiol (E2) concentrations using Enzyme Link Immunosorbent assay (ELISA). Hatching and fertilization rates were determined and data for plasma steroid hormones and the GV were analyzed using analysis of variance (ANOVA).

For African catfish, sGnRHa showed the most outstanding effect compared to the two treatments (LHRHa and cGnRH-II) since it is more resistance to degradation when compared with native peptides. The 200µg/kg cGnRH–II proved to be effective in stimulating maturation and ovulation. Saline injected fish remained unchanged where the initial GV was at stage 1.00±0.00. The cGnRH-II alone was enough to induce maturation and ovulation in African catfish. All fish ovulated in both groups (cGnRH-II alone and cGnRH-II + PIM) while the GV showed significant migration throughout the experiment ($P<0.05$). Fertilization rate constituted 73% and 80% while hatching rates were 58% and 67% for cGnRH-II alone and cGnRH-II + PIM treatments, respectively.

For Javanese barb, sGnRHa was more potent compared to the cGnRH-II and LHRHa. In the graded dosage experiment, 200 µg/kg cGnRH-II seemed to induce for oocyte maturation but no ovulation occurred. However, addition of Pimozide increase the plasma steroid level compared to cGnRH-II alone. The plasma steroid hormone for the saline treated group in all the experiment remained the same throughout the study ($P>0.05$). Although no ovulation was observed, the plasma steroid hormones and GV of cGnRH-II treated group showed significant effect compared to saline group ($P<0.05$).

The cGnRH-II was proven to effectively induced maturation and ovulation in both the Javanese barb and African catfish although large number of dosage was required. The use of a potent cGnRH-II analog for the improvement of spawning induction therapies is strongly suggested to increase the performance of cGnRH-II.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

AKTIVITI HORMON PEREMBESAN GONADOTROPIN (cGnRH-II, sGNRHa DAN LHRHa) PADA KELI AFRIKA, (*Clarias gariepinus* B.) DAN LAMPAM JAWA, (*Barbodes gonionotus* B.)

Oleh

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Hormon perembesan Gonadotropin (GnRH) ialah sejenis hormone peptida yang bertanggungjawab untuk merangsang pengeluaran gonadotropin dari kelenjar pituitari dan seterusnya mempengaruhi pengeluaran hormon steroid dalam ovari. Enam eksperimen telah dijalankan untuk melihat keberkesanan cGnRH-II sebagai agen yang mempercepatkan kematangan dan peneluran *Clarias gariepinus* dan *Barbodes gonionotus.* Eksperimen pertama melihat potensi cGnRH-II *native* apabila dibandingkan dengan LHRHa dan sGnRHa analog dan salin sebagai kawalan. Semua hormon menggunakan kepekatan yang sama iaitu 20 µg/kg. Bagi eksperimen kedua pula, tiga kepekatan yang berbeza telah diberikan kepada tiga kumpulan (2 µg/kg, 20 µg/kg and 200 µg/kg) dan salin. Eksperimen ketiga pula melihat keberkesanan pimozid sebagai penghalang dopamin dengan kombinasi cGnRH-II untuk mempercepatkan kematangan dan peneluran. Hormon yang terbabit adalah cGnRH-II 200 µg/kg; 5mg/kg PIM; cGnRH-II 200 µg/kg + 5mg/kg PIM dan salin. Kesemua ikan hanya diberi sekali suntikan hormon sahaja. Parameter

bagi eksperimen di atas adalah perubahan hormon pada ikan dan pergerakan Germinal Vesicle (GV) semasa eksperimen. Setiap kumpulan mengandungi enam ekor ikan betina yang telah matang dengan berat badan 450-550 g setiap seekor. GV telah diperiksa sebelum suntikan iaitu jam 0, 6 jam, 12 jam dan 24 jam selepas suntikan. Germinal Vesicle telah diklasifikasikan mengikut tahap yang bermula pada 1 (GV berada di tengah oosit) sehingga tahap 4 (semasa peneluran). Darah diambil pada masa yang sama dengan pemerhatian GV. Plasma daripada sampel Testosterone (T) dan 17β-Estradiol (E2) dianalisis kepekatannya menggunakan teknik *Enzyme Link Immunosorbent assay 'ELISA*'. Kadar penetasan dan persenyawaan dikira dan data bagi kadar plasma hormon steroid dan GV dianalisis menggunakan *analysis of variance (ANOVA)*.

Bagi keli Afrika, sGnRHa menunjukkan kesan yang paling efektif berbanding LHRHa dan cGnRH-II kerana ia lebih resistan berbanding peptida yang *native*. 200µg/kg cGnRH–II telah terbukti efektif untuk merangsang kematangan dan peneluran. Ikan yang disuntik saline tidak menunjukkan perubahan dimana GV berada pada tahap 1.00±0.00 sepanjang eksperimen. Hormon cGnRH-II yang disuntik bersendirian sudah mencukupi untuk merangsang kematangan dan peneluran bagi keli afrika. Kedua-dua kumpulan (cGnRH-II sahaja dan cGnRH-II + PIM) menunjukkan 100% peneluran dan GV menunjukkan perubahan signifikan sepanjang eksperimen ($P<0.05$). Kadar persenyawaan adalah 73% (cGnRH-II sahaja) dan 80% (cGnRH-II + PIM) manakala kadar penetasan pula menunjukkan 58% (cGnRH-II sahaja) and 67% (cGnRH-II + PIM)

Bagi lampam Jawa, sGnRHa menunjukkan kesan lebih efektif berbanding LHRHa dan cGnRH-II. Bagi eksperimen untuk menentukan kepekatan optimum, 200 µg/kg cGnRH-II terbukti berkesan untuk merangsang kematangan oosit walaupun tiada peneluran berlaku. Namun begitu, penambahan pimozid dapat meningkatkan kadar hormon steroid berbanding hanya menggunakan cGnRH-II sahaja. Kadar plasma hormon steroid bagi kumpulan yang disuntik salin tidak mengalami perubahan signifikan sepanjang eksperimen ($P>0.05$). Walaupun tiada peneluran berlaku, kadar plasma hormon steroid dan GV bagi kumpulan yang disuntik dengan cGnRH-II menujukkan perubahan signifikan berbanding salin ($P<0.05$).

cGnRH-II telah menunjukkan kesan efektif untuk merangsang kematangan dan peneluran walaupun memerlukan dos yang tinggi. Penggunaan cGnRH-II analog adalah digalakkan untuk menghasilkan keputusan yang lebih baik untuk proses pembiakan dan meningkatkan keberkesanan cGnRH-II

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I certify that an Examination Committee has met on date of viva voce to conduct final examination of Norhidayah Bt Mohd Taufek on her degree thesis entitled "*In vivo* activity of Gonadotropin Releasing Hormone (cGnRH-II, sGnRHa and LHRHa) in African catfish, *Clarias gariepinus* and Javanese barb, *Barbodes gonionotus*" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the (Master of Science)

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory committee were as follows:

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Date: December 2010

DECLARATION FORM

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NORHIDAYAH BT MOHD TAUFEK

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