UNIVERSITI PUTRA MALAYSIA

EFFECTS OF LHRHa IMPLANTATION ON OOCYTE MATURATION, HORMONAL PROFILE AND BREEDING OF IRANIAN STURGEON (Acipenser persicus BORODIN)

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By

KOUROSH AMINI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia In Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2012

DEDICATION

This work is especially dedicated to my decendent father, my beloved family: mother, brothers, wife and daughter (Sogand) Thank you for the never ending loves and support.

Also to my friends who have directly or indirectly help me with my study.

Thanks for the encouragement, motivation and patience that you gave all these years.

Thank you,
KOUROSH AMINI
UNIVERSITI PUTRA MALAYSIA
September, 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairperson: Professor Siti Shapor Siraj, PhD

Faculty: Agriculture

The Persian sturgeon (*Acipenser persicus*) is one of the most commercially important sturgeon species. It is considered as an endemic sturgeon in the south part of the Caspian Sea and provides the highest Iranian caviar production. Due to overfishing, degradation of the rivers conditions for natural reproductive on the fish stocks is continuously decreased. This leads Iran to embark in a program to release millions of *Acipenser persicus* fingerlings into the rivers for conservation in Iran. The sturgeon industry is having problems due to a high cost of artificial propagation for fingerlings production, decreasing of the natural stocks and also low quality and immature caught broodstocks.

The immature breeders do not response to hormonal therapy at sturgeon hatcheries as most having polarization Index (more than 10) and large numbers of breeders caught and transported to the hatcheries were unable to reproduce. This study was attempted to determine the effects of luteinizing hormone releasing hormone analogue

implantation on oocytes maturation and spawning of Persian sturgeons. Field studies were carried out at Shahid Marjani, a sturgeon hatchery located at Gorgan city in Golestan province. Broodstocks were caught from the southeast region of the Caspian Sea. The selected female broodstocks (PI>10) ranged from 24 to 37.5 kg were implanted with luteinizing hormone releasing hormone analogue cholesterol pellets in different treatments at concentrations of 0 (control), 10 (treatment 1), 15 (treatment 2), 20 (treatment 3) in three replicates.

The luteinizing hormone releasing hormone analogue cholesterol pellets were prepared and blood was collected from the caudal vessels before and within 24 hours after implantation. The collected blood samples were centrifuged for 8 minutes at 8000 rpm to obtain the plasma for analysis. The following hormones: 17-β-Estradiol (ng/ml), Esteriol Unconjugated (ng/ml), 17-α-Hydroxy Progesterone (ng/ml), 17-β-Hydroxy-4-Androsten-3-one (Testosterone) (ng/ml), 4-Pregnene-3, 20-Dione (Progesterone) (ng/ml) and Cortisol (ng/ml) were measured by using radioimmunoassay.

Artificial propagation and eggs incubation were carried out according to the current method in Iranian and also Russian sturgeon hatcheries. The results from this study indicated that females treated with luteinizing hormone releasing hormone analog implantation at 10, 15, 20 µg per kg body weight reached final maturation. These results were observed for all fish from treatment 2 (15 µg/ kg) and treatment 3 (20 µg/kg), however only one fish reached final maturation in treatment 1 (10 µg/ kg). luteinizing hormone releasing hormone analogue implantation showed different effects and variation on steroid hormones before and after the implantation. The results also demonstrated that estrogens (17-β-estradiol, Esteriol Unconjugated) were increased

then decreased when progesterone showed a rapid increased in the fish serum. The results suggested that final maturation can be achieved and lead to high fertilization rate (78.33% ±13.87, 68.33% ±4.16 in treatment 2 and 3, respectively) and hatching rate (85.3% ±9.07, 68.33% ±7.64 in treatment 2 and 3, respectively). Larval growth performance study was conducted for 80 days in rearing tanks of 2× 2× 0.5 m^3 in volume. The average weight, total length, survival rate and specific growth rate showed that the best result was obtained in treatment 3 (larvae produced via fish implanted with luteinizing hormone releasing hormone analog at 20 ug/kg Body Weight) with values of 3.93±0.8g, 92.0±1 mm, 71 ±2.00, 5.3±0.1 respectively in comparison with the other treatments: treatment 1 (larvae produced via fish implanted with luteinizing hormone releasing hormone analogue at 10 ug/kg Body Weight), treatment 2 (larvae produced via fish implanted with luteinizing hormone releasing hormone analogue at (15 ug/kg Body Weight) and control group (larvae produced via fish propagated in Shahid Marjani Centre). Thus, the information from this study will be very useful for artificial propagation of not-fully-matured females of Persian sturgeon at sturgeon hatcheries especially in Iran. In future this new method can be suggested to be used for other fishes having the same maturation problem.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

KESAN IMPLANTASI LHRHa TERHADAP KEMATANGAN OOSIT, PROFIL HORMON DAN PEMBIAKAN STURGEON IRAN (*Acipencer percicus* BORODIN)

Oleh

KUOROSH AMINI

Julai 2012

Pengerusi: Profesor Siti Shapor Siraj, PhD

Fakulti: Pertanian

Sturgeon Parsi (*Acipenser persicus*) merupakan salah satu spesies sturgeon komersil penting di Laut Caspian yang dianggap sturgeon endemik di bahagian selatan Laut Caspian dan membekalkan pengeluaran "caviar" Iran terbesar. Memandangkan berlaku tangkapan berlebihan dan keadaan sungai yang tercemar, habitat pembiakan semulajadi ikan ini telah berkurang. Justeru, program pemuliharaan di Iran dijalankan dengan pelepasan jutaan benih *Acipenser persicus* dalam sungai. Industri pengeluaran besar-besaran sturgeon mengalami pelbagai masalah disebabkan oleh belanja pembiakan aruhan yang meningkat, berkurangnya bekalan induk di alami dan kualiti induk yang ditangkap adalah rendah dan belum matang.

Kebanyakan induk yang tidak matang tidak memberi respons terhadap proses aruhan hormon di hatcheri sturgeon (kebanyakan mempunyai PI<10). Bilangan besar induk yang ditangkap dan dibawa ke hatcheri tidak menghasilkan fri. Kajian ini dijalankan bagi mengetahui kesan implantasi LHRHa terhadap kematangan dan pembiakan sturgeon Parsi. Kajian lapangan dijalankan di hatcheri sturgeon Shahid Marjani yang

terletak di Bandar Gorgan. Induk ditangkap di kawasan timur laut Laut Caspian. Induk betina terpilih yang mempunyai PI>10 dengan saiz berjulat daripada 24 hingga 37.5 kg diimplant dengan pelet kolesterol LHRHa dalam kepekatan 0 μg/kg (kawalan), 10 μg/kg (rawatan 1), 15 μg/kg (rawatan 2) dan 20 μg/kg berat badan (rawatan 4) secara 3 replikasi.

Pelet LHRHa kolesterol disediakan dan sampel darah diperolehi menerusi "caudal vasculature" sebelum dan dalam masa 24 jam implantasi. Sampel darah diempar selama 8 minit pada 8000 rpm bagi mendapatkan hormon plasma untuk dianalisis. Hormon berikut: 17-β-Eestradiol (ng/ml), Esteriol Unconjugated (ng/ml), 17-α-Hydroxy Progesterone (ng/ml), 17-β-Hydroxy-4-Androsten-3-one (Testosterone) (ng/ml), 4-Pregnene-3, 20-Dione (Progesterone) (ng/ml), dan Cortisol (ng/ml) dianalisis menggunakan "radioimmunoassay" (RIA).

Pembiakan tiruan dan pengeraman telur dijalankan mengikut kaedah terkini di hatchery Iran dan Rusia. Hasil kajian menunjukkan bahawa induk betina yang menerima rawatan implantasi hormon LHRHa pada kepekatan 10, 15 dan 20 μg/kg mencapai kematangan akhir. Keputusan ini terbukti untuk semua ikan pada semua rawatan 2 (15 μg/kg) dan rawatan 3 (20 μg/kg), walau bagaimanapun cuma satu induk betina mencapai matang akhir dalam rawatan 1 (10 μg/kg). Hormon LHRHa menunjukkan kesan yang berbeza dan pelbagai terhadap hormon steroid sebelum dan selepas implantasi. Keputusan juga menunjukkan estrogens (E1, E2) adalah meningkat dan seterusnya menurun di mana progesteron melihatkan peningkatan mendadak dalam serum. Keputusan ini mencadangkan bahawa kematangan akhir boleh dicapai dengan peningkatan kadar persenyawaan (78.33% ±13.87, 68.33% ±4.16 dalam

rawatan 2 dan 3, masing-masing) dan kadar penetasan (85.3% ±9.07, 68.33% ±7.64 dalam rawatan 2 dan 3, masing-masing). Kajian prestasi pertumbuhan larva dijalankan selama 80 hari dalam tangki yang berukuran 2 x 2 x 0.5 m^3. Purata berat, panjang penuh, kadar kemandirian dan kadar pertumbuhan spesifik menunjukkan keputusan yang terbaik diperolehi dalam rawatan 3 (larva dihasilkan oleh induk yang diimplan dengan LHRHa pada 20µg/kg BW) dengan nilai 3.93±0.8g, 92.0±1 mm, 71±2.0, 5.3±0.1, masing-masing; berbanding dengan lain-lain rawatan: rawatan 1 (larva dihasilkan oleh induk yang diimplan dengan LHRHa pada 10µg/kg BW), rawatan 2 (larva dihasilkan oleh induk yang diimplan dengan LHRHa pada 15µg/kg BW) dan rawatan kawalan. Justeru, maklumat kajian ini sangat berguna untuk propagasi aruhan bagi induk betina sturgeon Parsi yang belum cukup matang di hatcheri khususnya di Iran. Pada masa akan datang kaedah baru ini dicadangkan diguna terhadap ikan lain yang mengalami masalah yang sama.

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The Most Merciful

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To All- The Best Wishes and Thanks and May God Bless You.

I certify that a Thesis Examination Committee has met on 25 July 2012 to conduct the final examination of Kourosh Amini on his thesis entitled "Effects of LHRHa Implantation on Oocyte Maturation, Hormonal Profile and Breeding of Iranian Sturgeon (*Acipenser Persicus* Borodin)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U**.** **(**A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follow:

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DECLARATION

I declare that the thesis is based on 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 other institutions.

KOUROSH AMINI

Date: 25 July 2012

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