CRYOPRESERVATION OF CLIMBING PERCH, ANABAS TESTUDINEUS (BLOCH,1792) SPERMATOZOA

AYAD GUMA AYAD SAMMUD

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DEDICATION

This thesis is dedicated to my lovely parents and family for their support and time they gave to me in entire my life.

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Chairperson : **Prof. Anuar Hassan, Ph.D**
Member : **Mithun Sukumaran, Ph.D**
Faculty : **Faculty of Agrotechnology and Food Science**

Anabas testudineus is considered endangered and vulnerable species because frequent expose to combination of overexploitation such as "pesticide and aquatic pollution, spread of disease, uncontrolled introduction of exotic fishes, and habitat modification due to industrialization. Cryopreservation is the method to preserve the milt. Cryopreservation has several benefits such as stock protection, a stable supply of sperm for optimal utilization in hatchery production and laboratory experiments, easy stock transportation, improvement in selective breeding and gene transfer.

A study on cryopreservation of climbing perch (*A. testudineus*) spermatozoa was carried out in hatchery and laboratory of Faculty of Agrotechnology and Food Science of Universiti Malaysia Terengganu. The objectives of the studies are to characterize the spermatozoa of *A. testudineus* and to determine the best extender and combination of cryoprotectants of the

spermatozoa of *A. testudineus*. The spermatozoa were taken from 30 number of male bloodstock of *A. testudineus*, after injected with 0.5ml/kg ovaprim. After Collected the milt by abdominal massage (stripping) and collected with a syringe (without needle), Spermatozoa were stored at four different temperatures (0, -4, -20, -196° C) with and without extender. Sperm were cryopreserved with three different cryoprotectants (Methanol, Glycerol, and DMSO) then stored *A. testudinues* spermatozoa in 0, -4, -20, -196° C. spermatozoa were used to fertilize the eggs of *A. testudinues* after one and three month storage. The quality of the preserved sperm was evaluated by duration of sperm motility, sperm survival and fertilization rate. All the data were analyzed by using SAS program as two way ANOVA and the average were compared by Duncan multiple range 0.5. The best storage temperature of *A. testudinesus* spermatozoa with and without extender was -4° C. The addition of extender increased the sperm survival of *A. testudineus* from 7 hours until 58 hours. In 3rd experiment, the best cryoprotectant for cryopreservation of *A. testudineus* spermatozoa was 20% DMSO. After 30 days storage, the sperm survival at 20% DMSO as cryoprotectant was 30.547%. The highest fertilization rate after 1 and 3 month storage was 20% DMSO (78.98% and 76.22% respectively).

As a conlussion, 20% DMSO was found to be the best cryoprotectant for cryopreservation of *A. testudineus* spermatozoa. These results imply the potential commercial application of the cryopreservation technique in climbing perch fish hatcheries.

Abstrak ini telah dibentangkan kepada Senat Universiti Malaysia Terengganu untuk memenuhi keperluan untuk Sarjana Sains.

KRYOPRISERVASI PADA IKAN PUYU, *Anabas testudineus* (Bloch, 1792) SPERMATOZOA

AYAD GIUMA AYAD SAMMUD

2011

Pengerusi : **Prof. Anuar Hassan, Ph.D**
Ahli : **Mithun Sukumaran, Ph.D**
Fakulti : **Faculty of Agrotechnologi dan sains makanan**

Anabas testudineus telah dikenalpasti sebagai spesies terancam dan berharga kerana seringkali terdedah kepada pelbagai eksploitasi yang tinggi seperti "pencemaran racun serangga dan pencemaran akuatik, penyebaran penyakit, pendedahan kepada spesies ikan eksotik, dan perubahan habitat akibat aktiviti perindustrian. Kriopreservasi adalah kaedah untuk mengawet sperma. Kriopreservasi mempunyai beberapa kebaikan seperti melindungi stok, membekalkan sperma yang stabil untuk pengunaan optima didalam produktiviti hatcheri dan kajian makmal, memudahkan pengangkutan stok, peningkatan terhadap pembenihan terpilih dan perpindahan gen.

Satu kajian kepada kriopreservasi terhadap ikan puyu (*A. testudineus*) spermatozoa telah dijalankan di hatcheri dan makmal Fakulti Agroteknologi dan Sains Makanan, Universiti Malaysia Terengganu. Objektif kepada kajian ini adalah i) untuk mencirikan spermatozoa *A. testudineus* ii) untuk menentukan extender dan kombinasi cryoprotectants yang terbaik terhadap spermatozoa *A. testudineus*. Spermatozoa tersebut telah diambil dari 30

ekor induk jantan *A. testudineus*, selepas disuntik dengan 0.5ml/kg ovaprim. Selepas sperma tersebut dikumpul dengan urutan abdominal (stripping) lalu diambil dengan menggunakan pincagari (tanpa jarum), Spermatozoa telah disimpan didalam empat suhu yang berbeza (0, -4, -20, -196° C) dengan dan tanpa extender. Sperma telah dikriopreservasikan dengan tiga cryoprotectant yang berbeza (Methanol, Glycerol, dan DMSO) kemudian spermatozoa *A. testudinues* disimpan di dalam suhu 0, -4, -20, -196° C. Spermatozoa kemudiannya digunakan untuk mensenyawakan telur-telur *A. testudinues* setelah sebulan dan tiga bulan tempoh penyimpanan. Kualiti sperma yang telah disimpan dinilai melalui kadar kematian sperma, kemandirian sperma dan kadar persenyawaan. Kesemua data telah di analisa dengan menggunakan program SAS sebagai ANOVA dua hala dan purata dibandingkan dengan kadar 0.5 Duncan multiple. Suhu penyimpanan yang terbaik untuk spermatozoa *A. testudinesus* dengan dan tanpa extender adalah -4° C. Penambahan extender meningkatkan kadar kemandirian sperma *A. testudineus* dari 7 jam sehingga 58 jam. Dalam kajian ketiga, cryoprotectant terbaik untuk kriopreservasi terhadap spermatozoa *A. testudineus* adalah 20% DMSO. Selepas 30 hari penyimpanan, kemandirian sperma pada 20% DMSO sebagai cryoprotectant adalah 30.547%. Kadar persenyawaan tertinggi selepas 1 dan 3 bulan penyimpanan adalah didalam 20% DMSO (78.98% dan 76.22% masing-masing).

Kesimpulannya, 20% DMSO telah dikenalpasti sebagai cryoprotectant terbaik untuk kriopreservasi spermatozoa *A. testudineus*. Keputusan ini boleh digunakan dan diaplikasi untuk kegunaan komersial sebagai kaedah kriopreservasi di dalam hatceri ikan puyu.