EFFECT OF DIFFERENT CYROPROTECTANTS FOR SPERMATOZOA CYROPRESERVATION OF ORANGE MUD CRAB, *Scylla olivacea* (HERBST, 1796)

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By

Teng Phei Yin

Research Report submitted in partial fulfillment of the requirement for the degree of Bachelor of Science (Marine Biology)

Department of Marine Science
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DEPARTMENT OF MARINE SCIENCE
FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
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DECLARATION AND VERIFICATION REPORT

FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:
Effect of Different Cryoprotectants for Spermatozoa Cryopreservation of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) by Teng Phei Yin, Matric No. UK16706 have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree Bachelor of Science Marine Biology, Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu.

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BW	-	Body weight
Ca-F saline	-	Calcium-free saline
CW	-	Carapace width
cm	-	Centimeter
Cells/ml	-	Cells per mililiter
°C	-	Degree celcius
°C min^{-1}	-	Degree celcius per minute
DMSO	-	Dimethyl sulfoxide
g	-	Gram
kg	-	Kilogram
LN	-	Liquid nitrogen
L	-	Liter
mL	-	Mililiter
µL	-	Micro liter
M	-	Molarity
min	-	Minute
M	-	Molarity
%	-	Percentage
rpm	-	Revolutions per minute
v/v	-	Volume to volume
NaCl	-	Sodium Chloride
KCl	-	Potassium Chloride
H_3BO_3	-	Boric Acid

NaOH	-	Sodium Hydroxide
$MgSO_4.7H_2O$	-	Magnesium Sulphate
HCl	-	Hydrochloric Acid
1N	-	1 Normality
Ppt	-	Part per thousand

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ABSTRACT

The objectives of this study were to determine the effect of cryoprotectants and sperm density for a long-term storage of *S. olivacea* spermatozoa. Spermatozoa were obtained by homogenized the spermatophores by a glass homogenizer in an ice-bath and centrifugation at 4 ° C. Spermatozoa were suspended in calcium-free saline (Ca-F saline) containing the cryoprotectants glycerol, dimethyl sulfoxide (DMSO) and methanol at concentration of 5%. Sperm which were vibrated and rotated was counted as live in sperm viability assessment. Samples of spermatozoa were cooled to -196 ° C by two-step freezing, first to -80 ° C and then by plunging in liquid nitrogen (LN). Gradual cooling (1 ° C min^{-1}) was done by cooling the spermatozoa. Thawing was done at 30 ° C water bath for 2 min. This yielded live sperm after storage in LN for 30 days. The best sperm viability was obtained from density of 10^8 cells per ml in DMSO. There is no significant different ($P > 0.05$) between cryoprotectant toward sperm viability. However, there is a significant different ($P < 0.05$) between density toward the sperm viability.

KESAN ANTARA KRIOPROTEKTAN YANG BERBEZA DI DALAM KRYOAWETAN SPERMA KETAM NIPAH, *Scylla olivacea* (HERBST, 1796)

ABSTRAK

Kajian ini bertujuan untuk mengetahui pengaruh kryoprotektan dan kepadatan sperma untuk simpanan jangka panjang sperma *S. olivacea.* Sperma diperolehi dengan menghomogenize spermatophore dengan menggunakan homogenizer kaca di dalam 'ice-bath' dan sentrifugasi pada 4 ° C. Sperma disimpan di dalam saline yang tidak mempunyai calcium (Ca- F saline) dan mengandunig kryoprotektan gliserol, dimetil sulfoksida (DMSO) dan metanol pada kepekatan 5%. Sperma yang bergetar and berputar dicatat sebagai sperma hidup untuk penilaian viabilitas sperma. Sampel sperma didinginkan sehingga -196 ° C dengan pembekuan dua langkah, pertama didinginkan sehingga -80 ° C dan kemudian dicelupkan ke dalam cecair nitrogen. Pendinginan sperma secara berperingkat (1 ° C min^{-1}) telah dilakukan. Pencairan dilakukan pada 30 ° C dengan mengunakan 'water bath' selama 2 minit. Ini membolehkan perolehan sperma hidup setelah disimpan selama 30 hari dalam cecair nitrogen. Viabilitas sperma yang terbaik diperoleh daripada kepadatan 10^8 sel/ml di dalam DMSO. Tiada perbezaan yang nyata ($P > 0.05$) di antara kryoprotektan terhadap viability sperma. Walaubagaimanapun, terdapat perbezaan yang nyata ($P < 0.05$) antara kepadatan sperma terhadap viabilitas sperma.