EFFECTS OF DIFFERENT EXTENDERS, CRYOPROTECTANTS, EQUILIBRATION AND VAPOUR EXPOSURE ON FREEZABILITY OF AFRICAN CATFISH (*Clarias gariepinus*) SPERM

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ABBREVIATIONS

ABEL	Animal Biotechnology-Embryo Laboratory
ALH	Amplitude of lateral head displacement
ANOVA	Analysis of variance
BCF	Beat-cross frequency
CPA	Cryoprotectant agent
DMRT	Duncan's Multiple Range Test
DMSO	Dimethyl-sulfoxide
FRE	Fish-Ringer Extender
ISB	Institute Biological Sciences
LIN	Linearity
LN_2	Liquid nitrogen
SEM	Standard error mean
SPSS	Statistical Package for Social Science
TCAYE	Tris-Citric Acid Yolk Extender
VAP	Average path velocity
VCL	Curvilinear velocity
VSL	Straight line velocity
STR	Straightness

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ORIGINAL LITERARY WORK DECLARATIONS

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ABSTRACT

The aim of this study was to develop an optimal freezing protocol for African catfish (*Clarias gariepinus*) sperm with special reference to type of extender and cryoprotectant, molarity, equilibration duration, vapour temperature and vapour exposure duration. Using Tris-Citric Acid Yolk Extender (TCAYE), a 3x3x3x3 factorial experiment was carried out consisting of 3 molarities of glycerol (0.5, 1.0 and 2.0 M), 3 equilibration durations (120, 140 and 160 minutes), 3 vapour temperatures (-80, -90 and -100°C) and 3 vapour exposure durations (5, 10 and 15 minutes). In addition, using Fish-Ringer Extender (FRE), a 3x3x3 factorial experiment was also conducted involving 3 equilibration durations (120, 140 and 160 minutes), 3 vapour temperatures (-80, -90 and -100°C) and 3 vapour exposure durations (5, 10 and 15 minutes). The molarity of cryoprotectant in FRE extender was fixed at 10% DMSO. Briefly, the straws containing the sperm were placed in refrigerator at 4°C with the fixed equilibration duration after which exposed to liquid nitrogen vapour at the fixed vapour temperature with the fixed vapour exposure duration. Subsequently, the straws were directly plunged into liquid nitrogen. The frozen sperm were thawed at 30°C for 30 seconds to evaluate the sperm motility characteristics using the automated semen analyzer (IVOS; Hamilton Thorne, USA). The effects of factors and parameters measured were analysed using Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). In Experiment 1, large body weight (BW) of African catfish gave the highest fresh sperm total motility (82.40±4.59%) followed by medium BW (51.64±9.82%) and small BW (40.40±12.16%), whereby small BW fish were significantly different in total motility compared with the other two groups studied. In Experiment 2, glycerol with molarity of 0.5 M showed significantly the highest value of frozen-thawed sperm total motility (32.27±2.05%) as compared to 1.0 M (24.50±1.81%) and 2.0 M (2.63±0.29%). At 140 minutes equilibration duration, the value of total motility (31.69±2.19%) was significantly

higher as compared to 120 minutes (25.26±1.76%). There were no significant differences (P>0.05) in value of total motility for -80, -90 and -100°C which were ranged from 25.95±2.34% to 29.41±1.69%. The value of total motility did not show any significant differences (P>0.05) among the three vapour exposure durations (5, 10 and 15 minutes), which were ranged from 27.63±2.02% to 28.45±2.14%. In Experiment 3, there were no significant differences (P>0.05) in values of total motility at 120 minutes (76.65±2.27%) and 160 minutes equilibrations (76.01±2.04%), but these durations gave comparatively higher values of total motility than 140 minutes (66.90±2.60%). The values of total motility for vapour temperatures of -90°C (74.07±2.02%) and -100°C (74.95±1.88%) did not show any significant differences (P>0.05), but they were significantly different with -80°C, which gave comparatively lower values (64.59±5.08%). There were no significant differences (P>0.05) in values of total motility for 5, 10 and 15 minutes which were ranged from 72.67±2.27% to 73.99±2.34%. In Experiment 4, there were no significant differences (P>0.05) for values of total motility between 1.0 M (24.50±1.81%) and 2.0 M of glycerol in TCAYE (26.74±2.14%), but they were comparatively lower than 0.5 M of glycerol that showed higher significant value (32.27±2.05%). On the other hand, combination of DMSO (10%) in FRE extender showed the highest significant value of total motility (73.52±1.35%) as compared to the three molarities of glycerol in TCAYE extender. In summary, the best combination to obtain the highest frozen-thawed sperm motility characteristics for TCAYE extender was 0.5 M of glycerol, 140 minutes equilibration duration, -90°C vapour temperature and 5 to 15 minutes vapour exposure duration, whereas for FRE extender was 120 minutes equilibration duration, -100°C vapour temperature and 5 to 15 minutes vapour exposure duration. In conclusion, results obtained in this study showed that 10% DMSO with FRE extender produced higher frozen-thawed sperm total motility than TCAYE extender. Future studies are needed through refinement in factors

involved during freezing process that influence sperm survival before it can be used routinely in the reproduction of African catfish (*Clarias gariepinus*).

ABSTRAK

Matlamat kajian ini adalah untuk membangunkan protokol penyejukbekuan sperma yang optimum bagi keli Afrika (*Clarias gariepinus*) dengan merujuk khusus kepada jenis ekstender dan krioprotektan, molariti, tempoh pengimbangan, suhu pengewapan serta tempoh pendedahan kepada wap nitrogen cecair. Dengan menggunakan ekstender TCAYE, eksperimen berbentuk faktorial 3x3x3x3 dijalankan yang terdiri daripada 3 molariti gliserol (0.5, 1.0 dan 2.0 M), 3 tempoh pengimbangan (120, 140 dan 160 minit), 3 suhu pengewapan nitrogen (-80, -90 dan -100°C) dan 3 tempoh pendedahan kepada wap nitrogen cecair (5, 10 dan 15 minit). Di samping itu, eksperimen berbentuk faktorial juga dijalankan ke atas ekstender FRE yang melibatkan 3x3x3, terdiri daripada 3 tempoh pengimbangan (120, 140 dan 160 minit), 3 suhu pengewapan nitrogen (-80, -90 dan -100°C) dan 3 tempoh pendedahan kepada wap nitrogen cecair (5, 10 dan 15 minit). Molariti krioprotektan dalam ekstender FRE ditetapkan pada 10% DMSO. Secara ringkas, straw yang mengandungi sperma diletakkan ke dalam peti sejuk pada suhu 4°C dalam tempoh pengimbangan yang telah ditetapkan dan seterusnya didedahkan kepada wap nitrogen cecair pada suhu pengewapan dan tempoh pendedahan yang telah ditetapkan. Berikutnya, straw dijunamkan secara langsung ke dalam nitrogen cecair. Sperma yang telah mengalami proses penyejukbekuan dinyahsejukbekukan pada suhu 30°C selama 30 saat untuk menganalisis ciri-ciri motiliti sperma menggunakan penganalisis semen automatik (IVOS; Hamilton Thorne, USA). Kesan faktor-faktor dan parameter-parameter yang diukur dianalisis dengan menggunakan Analisis Varians (ANOVA), diikuti dengan "*Duncan Multiple Range Test*" (DMRT). Dalam Eksperimen 1, didapati bahawa berat badan ikan keli Afrika yang besar menunjukkan peratusan kadar motiliti sperma segar yang paling tinggi (82.40±4.59%), ini diikuti oleh ikan yang memiliki berat badan yang sederhana (51.64±9.82%) dan berat badan ikan yang kecil (40.40±12.16%), yang mana ikan berberat

badan yang kecil menunjukkan perbezaan yang signifikan dalam peratusan kadar motiliti berbanding kedua-dua kumpulan yang dikaji. Dalam Eksperimen 2, gliserol dengan molariti 0.5 M menunjukkan peratusan kadar motiliti sperma sejukbeku-nyahsejukbeku signifikan yang paling tinggi (32.27±2.05%) berbanding 1.0 M (24.50±1.81%) dan 2.0 M (2.63±0.29%). Dalam tempoh 140 minit pengimbangan, peratusan kadar motiliti menunjukkan nilai signifikan yang tinggi (31.69±2.19%) berbanding 120 minit (25.26±1.76%). Tiada perbezaan yang signifikan ($P>0.05$) didapati bagi peratusan kadar motiliti pada suhu -80, -90, -100°C yang berjulat daripada 25.95±2.34% sehingga 29.41±1.69%. Peratusan kadar motiliti bagi masa pendedahan kepada wap nitrogen cecair (5, 10 dan 15 minit) juga tidak memberikan perbezaan yang signifikan ($P>0.05$) yang berjulat daripada 27.63±2.02% sehingga 28.45±2.14%. Dalam Eksperimen 3, tiada perbezaan yang signifikan ($P>0.05$) bagi peratusan kadar motiliti pada 120 minit (76.65±2.27%) dan 160 minit (76.01±2.04%) tempoh pengimbangan, akan tetapi kedua-duanya menunjukkan peratusan kadar motiliti yang tinggi berbanding 140 minit (66.90±2.60%). Peratusan motiliti sperma bagi suhu pengewapan -90°C (74.07±2.02%) dan -100°C (74.95±1.88%) tidak menunjukkan perbezaan yang signifikan ($P>0.05$), akan tetapi kedua-duanya adalah berbeza dengan signifikan pada suhu -80°C, yang menunjukkan nilai yang paling rendah (64.59±5.08%). Tiada perbezaan yang signifikan ($P>0.05$) bagi peratusan kadar motiliti sperma dalam masa 5, 10 dan 15 minit pendedahan ke atas wap nitrogen cecair yang berjulat 72.67±2.27% sehingga 73.99±2.34%. Dalam Eksperimen 4, tiada perbezaan yang siginifikan ($P>0.05$) antara 1.0 M (24.50±1.81%) dengan 2.0 M gliserol (26.74±2.14%) yang terkandung di dalam ekstender TCAYE, akan tetapi kedua-duanya menunjukkan peratusan kadar motiliti sperma yang rendah berbanding 0.5 M gliserol yang mencatatkan peratusan yang tinggi (32.27±2.05%). Selain itu, kombinasi 10% DMSO bersama ekstender FRE memberikan peratusan kadar motiliti yang paling tinggi

(73.52±1.35%) berbanding ketiga-tiga molariti gliserol di dalam ekstender TCAYE. Secara rumusannya, kombinasi yang terbaik bagi menghasilkan ciri-ciri motiliti sperma yang paling tinggi untuk ekstender TCAYE adalah 0.5 M of gliserol, 140 minit tempoh pengimbangan, -90°C suhu pengewapan dan selama 5 hingga 15 minit tempoh pendedahan ke atas wap nitrogen cecair, manakala bagi ekstender FRE adalah 120 minit tempoh pengimbangan, -100°C suhu pengewapan dan tempoh 5 hingga 15 minit tempoh pendedahan ke atas wap nitrogen cecair. Kesimpulannya, keputusan yang diperoleh dalam kajian ini menunjukkan bahawa 10% DMSO bersama ekstender FRE pada amnya mengekalkan ciri-ciri sperma sejukbeku-nyahsejukbeku yang normal berbanding ekstender TCAYE. Kajian selanjutnya perlu diteruskan pada masa akan datang melalui penelitian yang lebih mendalam dalam faktor-faktor yang terlibat semasa proses penyejukbekuan yang mempengaruhi keterushidupan sperma sebelum ianya dapat digunakan secara rutin dalam pembiakan keli Afrika (*Clarias gariepinus*).