Studi Ultrastruktur, Fisiologi, dan Molekuler Spermatozoa Ikan Patin Albino (Pangasianodon hypophthalmus) Pasca Kriopreservasi = Study of Ultrastructure, Physiology, and Molecular of Albino Pangasius Catfish Spermatozoa Post Cryopreservation

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Abstrak

Penelitian kriopreservasi spermatozoa ikan patin albino bertujuan untuk menganalisis ultrastruktur, fisiologi, dan molekuler spermatozoa ikan patin albino pasca kriopreservasi. Kriopreservasi dilakukan pada suhu -80°C selama 14 hari menggunakan kombinasi krioprotektan intraseluler yaitu metanol 10% dan krioprotektan ekstraseluler yaitu susu skim. Hasil ultrastruktur spermatozoa menunjukkan bahwa pada spermatozoa segar bagian membran sel kepala, mid piece, dan bagian flagel masih dalam kondisi utuh dan baik. Ultrastruktur spermatozoa pasca ekuilibrasi nampak ada perbesaran lebar dan panjang kepala spermatozoa dibandingkan spermatozoa segar, walaupun secara struktur masih tampak utuh. Ultrastruktur spermatozoa pasca pencairan tampak terjadi kerusakan membran bagian kepala dan flagel. Hasil pengukuran morfometri spermatozoa menunjukkan adanya peningkatan lebar kepala spermatozoa yaitu 1,59 µm pada spermatozoa segar menjadi 1,97 µm pada spermatozoa pasca ekuilibrasi dan 2,40 µm pada spermatozoa pasca pencairan. Demikian pula, terdapat perubahan panjang kepala spermatozoa yaitu 3,70 µm pada spermatozoa segar menjadi 3,81 µm pada spermatozoa pasca ekuilibrasi, dan 3,90 µm pada spermatozoa pasca pencairan. Analisis viabilitas spermatozoa didapatkan penurunan viabilitas spermatozoa pasca pencairan (61±2,30%) dibandingkan spermatozoa segar (92±0,58%) dan spermatozoa pasca ekuilibrasi (80±3,51%). Analisis fisiologi spermatozoa didapatkan penurunan fungsi mitokondria pada spermatozoa pasca ekuilibrasi (57±7%) dan spermatozoa pasca pencairan (42±3,2%) dibandingkan spermatozoa segar (98±2%). Analisis motilitas spermatozoa menunjukkan penurunan motilitas spermatozoa pasca ekuilibrasi (79±4,5%) dan spermatozoa pasca pencairan (30±3,2%) dibandingkan spermatozoa segar (87±1,5%). Penetasan telur pasca 24 jam fertilisasi pada perlakukan spermatozoa pasca ekuilibrasi didapatkan hasil lebih tinggi (64±17%) dibandingkan spermatozoa segar (38±4%), sedangkan spermatozoa pasca pencairan tidak ditemukan ada penetasan telur. Analisis molekular spermatozoa pada gen CO1 dan SOD2 didapatkan jumlah lesi gen SOD2 spermatozoa pasca ekuilibrasi yaitu 15,83 lesi / 10 kb dan spermatozoa pasca pencairan yaitu 17,14 lesi / 10 kb. Lesi gen CO1 pada spermatozoa pasca ekuilibrasi yaitu 9,24 lesi / 10 kb dan spermatozoa pasca pencairan yaitu 10,26 lesi / 10 kb. Sehingga disimpulkan kriopreservasi spermatozoa berpengaruh terhadap ultrastruktur, fisiologi, dan molekuler spermatozoa ikan patin albino.

.....Research of cryopreservation on albino Pangasius catfish spermatozoa aims to analyze about ultrastructure, physiology, and molecular spermatozoa of albino Pangasius catfish post cryopreservation. Cryopreservation was carried out at -80°C for 14 days using a combination of intracellular cryoprotectants which is 10% methanol and extracellular cryoprotectant which is skim milk. The results of the spermatozoa ultrastructure showed that the cell membrane of the spermatozoa head, the midpiece, and the flagellum of fresh spermatozoa were still intact and good. The spermatozoa ultrastructure after post equilibration, shown enlargement of the head width and length compared to the fresh spermatozoa, although structurally were still intact. The ultrastructure of frozen-thawed spermatozoa, appeared a membrane damage at the head and

flagellum. The results of spermatozoa morphometric measurements showed an increase at the head width of spermatozoa from 1.59 µm in fresh spermatozoa to 1.97 µm in post-equilibration spermatozoa and 2.40 µm in frozen-thawed spermatozoa. Similarly, there was an increase in the head length of spermatozoa, from 3.70 µm in fresh spermatozoa, to 3.81 µm in post-equilibration spermatozoa, and 3.90 µm in frozen-thawed spermatozoa. The viability analysis showed a decrease of frozen-thawed spermatozoa viability (61±2.30%) compared to fresh spermatozoa (92±0.58%) and post-equilibration spermatozoa (80±3.51%). The analysis physiology of spermatozoa showed a decrease in mitochondrial function in post equilibration spermatozoa (57±7%) and frozen-thawed spermatozoa (42±3.2%) compared to fresh spermatozoa (98±2%). The analysis of motility of spermatozoa showed a decrease in post equilibration spermatozoa (79±4.5%) and frozen-thawed spermatozoa (30±3.2%) compared to fresh spermatozoa (87±1.5%). Egg hatching after 24 hours of fertilization for the post-equilibration spermatozoa was higher (64±17%) than fresh spermatozoa (38±4%), whereas frozen-thawed spermatozoa were not hatched. The analysis of molecular on CO1 and SOD2 genes obtained the number of gene lesions in the spermatozoa SOD2 gene after equilibration were 15.83 lesions/10 kb and frozen-thawed were 17.14 lesions/10 kb. The CO1 gene lesions in post-equilibration spermatozoa were 9.24 lesions/10 kb, while the CO1 gene lesions in frozen-thawed spermatozoa were 10.26 lesions/10 kb. It can be concluded that there is an effect of cryopreservation on ultrastructure, physiology, and molecular in spermatozoa of albino Pangasius catfish.