INTISARI

Uji Efektivitas Ekstrak Alga Merah *(Acantophora spicifera)* Terhadap *Cryotolerance* Spermatozoa Sapi Simmental

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Kriopreservasi spermatozoa penting dalam program inseminasi. Proses kriopreservasi dapat menurunkan kualitas spermatozoa akibat *stress oksidatif* dan kerusakan DNA. Penambahan antioksidan dalam ekstender sperma, termasuk dari sumber alami seperti alga merah (*Acantophora spicifera*) berpotensi meningkatkan *cryotolerance* spermatozoa, namun belum banyak diteliti. Penelitian ini bertujuan untuk mengevaluasi efektivitas ekstrak alga merah dalam mempertahankan kualitas spermatozoa sapi setelah kriopreservasi. Alga merah dikeringkan dengan *freeze dryer* diikuti dengan penggilingan dan maserasi menggunakan methanol selama 48 jam. Aktivitas antioksidan ekstrak alga merah dievaluasi menggunakan metode *2,2-difenil-1-pikrilhidrazil* (DPPH). Ejakulat sperma dari 4 ekor sapi Simmental dibagi menjadi 5 alikuot yang diencerkan dalam kuning telur dan susu *skim.* Ekstrak kental alga merah dilarutkan ke ekstender sperma berisi *buffer* susu skim, antibiotik, kuning telur, fruktosa, dan gliserol untuk mencapai konsentrasi 250,500,1000, dan 2000 ppm. Sperma dicampurkan ke dalam ekstender sesuai konsentrasi perlakuan ekstrak alga merah dan dilakukan kriopreservasi. *Straw* hasil kriopreservasi selanjutnya dimasukkan dalam waterbath bersuhu 37°C dan diamati parameter motilitas, viabilitas, integritas membran, dan akrosom dengan mikroskop cahaya. Penentuan kadar *reactive oxygen species* (ROS), Indeks DNA terfragmentasi (DFI), dan membran potensi mitokondria diamati menggunakan mikroskop *fluorescence* sesuai protokol kit. Pengujian kadar enzim katalase dan protein *heat shock protein-70* (HSP70) dilakukan dengan *Enzyme-Linked Immunosorbent Assay*. Data motilitas, viabilitas, integritas membran, integritas akrosom, kadar ROS, DFI, katalase, dan protein HSP70 dianalisis dengan *Analysis of Variance* (ANOVA) menggunakan aplikasi R studio versi 4.5 untuk menguji perbedaan antar perlakuan. *Heatmap* korelasi Pearson dan *biplot PCA* digunakan untuk visualisasi hubungan antarvariabel dan struktur data. Perlakuan 250-1000 ppm ekstrak alga merah secara signifikan ($P<0,05$) meningkatkan motilitas (44-45%), viabilitas (80-82%), integritas akrosom (77-80%), aktivitas mitokondria (0,7-1), menstimulasi katalase (0,8-1ng/ml) dan HSP70 (18-19 ng/ml), menurunkan jumlah produksi ROS dan DFI spermatozoa. Perlakuan 2000 ppm berpotensi menjadi pro-oksidan dengan produksi ROS tinggi pada spermatozoa. Dapat disimpulkan pemberian ekstrak alga merah meningkatkan kualitas spermatozoa *post-thawing.*

Kata kunci: Ekstrak alga merah, *cryotolerance*, ROS, kualitas spermatozoa *post-thawing*, antioksidan

ABSTRACT

Effectivity Test of Red Algae *(Acantophora Spicifera)* Extract on Bull Spermatozoa Cryotolerance

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Spermatozoa cryopreservation is essential in bovine artificial insemination programs. The cryopreservation process reduce spermatozoa quality due to oxidative stress and DNA damage. The addition of antioxidants to sperm extenders, including natural sources such as red algae (*Acantophora spicifera*), has the potential to enhance spermatozoa cryotolerance, although it has not been widely studied. This study aims to evaluate the effectiveness of red algae extract in maintaining the quality of bovine spermatozoa after cryopreservation. Red algae were freeze-dried, ground, and macerated in methanol for 48 hours. The antioxidant activity of the extract was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Sperm ejaculates from four Simmental bulls were divided into five aliquots and diluted with an egg yolk and skim milk-based extender. The concentrated red algae extract was dissolved in the sperm extender containing skim milk *buffer*, antibiotics, egg yolk, fructose, and glycerol to achieve final concentrations of 250, 500, 1000, and 2000 ppm. Sperms was then mixed with the extender according to the treatment concentrations and cryopreserved. The frozen *straw*s were thawed in a 37°C waterbath. Post-thaw evaluations included sperm motility, viability, membrane integrity, and acrosome integrity using light microscopy. Reactive oxygen species (ROS), DNA fragmentation index (DFI), and mitochondrial membrane potential were assessed by fluorescence microscopy following kit protocols. Catalase enzyme and heat shock protein-70 (HSP70) levels were quantified using Enzyme-Linked Immunosorbent Assay (ELISA). Data were analyzed using Analysis of Variance (ANOVA) in R Studio version 4.5, while Pearson correlation heatmaps and Principal Component Analysis (PCA) biplots were employed to visualize variable relationships and data structure. Treatments with 250–1000 ppm red algae extract significantly ($P<0.05$) enhanced motility (44–45%), viability (80–82%), acrosome integrity (77–80%), and mitochondrial activity (0.7–1), while also increasing catalase (0.8–1 ng/mL) and HSP70 (18–19 ng/mL) levels and reducing ROS and DFI values. In contrast, the 2000 ppm concentration exhibited potential pro-oxidant effects, with elevated ROS production. These findings suggest that red algae extract improves post-thaw spermatozoa quality and may serve as a natural antioxidant supplement in cryopreservation protocols.

Keywords: Red algae extract, cryotolerance, ROS, post-thawing spermatozoa quality, antioxidant