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Cryopreservation of spermatophores in whiteleg shrimp, Litopenaeus vannamei – A case study

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Preliminary studies were done to develop a freezing protocol for cryopreservation of spermatophores of whiteleg shrimp, *Litopenaeus vannamei.* Spermatophores were collected by electro – ejaculation method. The toxicity effects were studied using cryoprotectants such as Dimethyl sulfoxide (DMSO), Dimethyl acetamide (DMA) and Methanol (MeOH) at various concentrations (5, 10, and 15% v/v) with Sterile Seawater (SSW) as an extender. The general sperm characteristics such as sperm morphology and viability were assessed using staining method. Based on the toxicity assay, cryoprotectants such as DMSO, DMA and MeOH at different concentrations with extender were used for cryopreservation studies. Freezing protocol of -1°C/min until -80°C was achieved by a one-step cooling rate using programmable freezer (Cryo planner, Kryo-360 – 1.7). The samples were then maintained for 2 min above the liquid nitrogen surface and directly immersed in liquid nitrogen (LN2, -196 °C). Thawing was performed in a water bath at 30°C for 2 min; this yielded live sperm after storage for 30 days in liquid nitrogen. Results indicated the maximum viability of sperm 87.47 ± 3.1% with 5% DMSO for 30 minutes equilibration time. However, the MeOH (15%) showed low percentage viability (24.93±4.2%). In control, average sperm viability of cells was obtained from fresh spermatophores (94.13±3.1%). Post – thaw sperm showed a viability of 52.93±3.5% with DMSO (5%) held for 30 days and lowest sperm viability of 16.04±3.0% with MeOH (15%). Further studies are underway to optimize the suitable freezing protocol for successful cryopreservation of spermatophore in *Litopenaeus vannamei.*

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Usable nucleic acids from problematic sources

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The rapid advances made in personalized medicine are compelling the scientific and medical communities to gather an increasing volume of genetic/genomic information from an ever broadening diversity of sources. One of the major issues with this endeavor is the quality of the nucleic acids samples which can be obtained from the source material. Another one has to deal with how the purified nucleic acids are being conserved for future reference, which in itself would be the topic of another presentation. This presentation will focus on two particular cases, whose handling requires some precaution in order to safely obtain usable nucleic acids. The first case deals with the purification of sequence-able nucleic acids from paraffin-embedded tissues (FFPE). An automated protocol will be described, from starting sample to DNA ready to be sequenced. The second case addresses more specifically the purification of DNA samples from large volume, complex and heterogeneous samples, such as in the cases of microbiome and metagenome analysis. The automated protocols which are being developed for these applications will be described as well.

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