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The study was designed to evaluate the effect of dimehtlyformamide (DMF) usage as a permeable cryoprotectant for Indian red jungle fowl spermatozoa on post thaw semen quality and fertility. A total of 40 ejaculates were collected on alternate days from eight Indian red jungle fowl cocks (5 replicates), pooled, diluted in red fowl extender and divided into five aliquots containing different concentrations of DMF (0% (control), 4%, 6%, 8% and 10%). Diluted semen cooled from 37°C to 4 °C @ -0.275 min^{-1}, 20% glycerol added to control and equilibrated for 10 minutes. After equilibration, semen was filled in 0.5 mL French straws, kept over liquid nitrogen vapor (5cm above the level of LN_2) for 10 minutes and plunged into liquid nitrogen. Semen samples were thawed at 37°C for 30sec. The data on the effect of different stages of cryopreservation and the cryoprotectant concentration were analyzed by ANOVA using two factor factorial design and post-hoc comparisons were made, when F-ratio was found significant (P>0.05). After thawing, sperm motility, plasma membrane integrity, viability and acrosomal integrity were determined. Cryosurvival of Indian red jungle fowl was affected by cryopreservation stages and different concentrations of cryoprotectant used. Highest sperm motility, plasma membrane integrity, viability and acrosomal integrity were recorded in a diluent containing 8%DMF at post-dilution, cooling, equilibration and freeze-thawing. Highest fertility results were obtained after artificial insemination with 8% DMF compared to 20% glycerol. It is concluded that 8% DMF as a permeable cryoprotectant improves the post thaw semen quality and fertility in Indian red jungle fowl and can be used effectively to avoid the contraceptive effects of glycerol.
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MODEL PROTOCOL FOR CRYOPRESERVATION OF LIZARD SPERM USING THE PHOSPHODIESTERASE INHIBITOR CAFFEINE

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We investigated sperm cryopreservation in the yellow-spotted monitor (*Varanus panoptes*), a lizard from northern Australia severely impacted by an invasive toad. The objective of the study was to create an optimised cryopreservation protocol for the spermatozoa of *V. panoptes* testing concentrations of two cryoprotectants (Me_2SO and glycerol) at concentrations of 0.68, 1.35 and 2.7M in PBS. A phosphodiesterase inhibitor (caffeine) was added post-thaw to stimulate motility. We also tested for cold-shock by rapidly cooling unfrozen sperm. Cryoprotectant toxicity was tested by incubating sperm with cryoprotectants at 25°C for five hours. For cryopreservation, sperm were cooled in straws suspended in liquid nitrogen vapour at approximately -32.1°C / minute, before plunging into liquid nitrogen, and later thawing in a water bath at 35°C. Samples were incubated post-thaw for 10 minutes in the presence or absence of 10 mM caffeine. Both cryoprotectant type ($P < 0.001$) and concentration ($P < 0.001$) significantly affected percent sperm motility pre-freezing, with Me_2SO being less cytotoxic than glycerol and motility decreasing at higher concentrations. Cold shock did not significantly affect spermatozoa motility. Both cryoprotectant type ($P = 0.009$) and concentration ($P < 0.001$) significantly affected the motility of post-thawed spermatozoa, with mid-range concentrations (1.35 M) yielding the greatest post-thaw motility for both cryoprotectants, and Me_2SO yielding greater post-thaw motility than glycerol. The best protocols, involving 1.35 M and 2.7 M DMSO with the addition of caffeine post-thaw, resulted in a motility of 48% and 42.3% respectively, comparable to percent motility at similar cryoprotectant concentrations pre-freeze. Addition of caffeine to samples post-thaw significantly increased motility of sperm for both cryoprotectants ($P < 0.001$), with higher stimulation associated with higher cryoprotectant concentrations ($P < 0.003$). Our study established a successful sperm cryopreservation protocol for *V. panoptes*, with increased post-thaw motility by caffeine, indicating that metabolic cryo-injury can be reversed.
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BIOCHEMICAL CHANGES DURING POTATO SHOOT TIP CRYOPRESERVATION

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Conservation of *Solanum tuberosum* L. genetic resources is essential for breeding. Cryopreservation is used as an effective method for long-term storage of meristematic plant tissue at IPK in Gatersleben, Germany. However, different protocols to cryopreserve potato shoot tips are published. Therefore, the aim of this study was to compare the regeneration of 30 potato genotypes after cryopreservation by using two approaches: a droplet freezing protocol using Me_2SO and a droplet vitrification method using PVS3. Furthermore, an adapted version including a shortened preculture step (PVS3A) was applied to four genotypes and biochemical changes alongside the protocols analysed. Significant different regeneration results were found between Me_2SO Droplet Freezing (49.5 ±7.9 %), PVS3 (57.3 ± 6.8 %) and PVS3A Droplet Vitrification (9.2 ± 2.6 %). Over all genotypes and protocols, soluble sugar content increased during preculture and remained at high levels until rewarming. Differences in mono- and disaccharide uptake from preculture solutions and cryoprotectant agents were found after regrowth between the three protocols. Energetic status, measured as adenosine triphosphate (ATP) content, was reduced after preculture and increased after rewarming during regeneration indicating a complete disruption of energy metabolism during cryopreservation. Differential scanning calorimetry revealed that PVS3 exposure led to typical glass transition, while during Me_2SO treatment ice crystallization appears. The thermodynamic behaviour of remaining water in the shoot tips did not correlate to regeneration ability. To compare regeneration after PVS3 Droplet Vitrification and Me_2SO Droplet Freezing, routine genebank cryopreservation was conducted using 26 potato accessions. Significant higher plant regeneration results and less visible contaminations were observed using PVS3 vitrification. Concluding, physiological changes during explant cryopreservation are strongly depend on the cryogenic procedure and the stress management of relevant accession. Overall, PVS3 influenced positively regeneration but there was no direct correlation between explant regeneration, water, sugar and ATP content.
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TRANSCRIPTOMIC, BIOCHEMICAL, AND ULTRASTRUCTURAL ANALYSIS FOR UNDERSTANDING CRYO-STRESS IN GARLIC

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Garlic (*Allium sativum* L.) is a medicinal crop, consumed as a popular condiment and green vegetable. Garlic can only be propagated vegetatively and genetic resources are maintained in vivo or via cryopreservation. The major challenge for cryopreservation is exposing the explant to cryoprotectant-mediated dehydration and ultra-low temperature (−196