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# Hypotaurine protects viability and mitochondrial membrane potential during cryopreservation of Sheep spermatogonial stem cells

Spermatogonial stem cells (SSCs) are germline stem cells that form the foundation of spermatogenesis and are essential for maintaining male fertility throughout life. For their effective application in animal genetics and reproductive biotechnology, it is crucial to preserve SSCs over long periods while ensuring maximum viability and cryosurvival. Since cryopreservation induces oxidative stress, the present study was undertaken to optimize cryopreservation media by supplementing antioxidants. Specifically, the objective was to evaluate the effect of different concentrations of hypotaurine on reducing cryoinjury during SSC cryopreservation.
Sheep SSCs (n = 6) were isolated, purified, cultured, and cryopreserved using a basal cryopreservation medium composed of 10% DMSO, 40% FBS, and 200 mM trehalose. The antioxidant hypotaurine was supplemented at concentrations of 1, 5, 10, 15, 20 and 50 mM, while the control group was maintained without hypotaurine. Post-thaw cell viability was assessed using the trypan blue exclusion test, reactive oxygen species (ROS) production was measured via H2DCFDA staining, and mitochondrial membrane potential (MMP) was evaluated using JC-1 staining. Quantification of ROS and MMP-positive cells were performed by flow cytometry
The post-thaw viability (%) was significantly higher ($P<0.05$) in cryomedia containing hypotaurine at 50 mM (69.00 ± 1.66) when compared to the control group (56.12 ± 6.08). Moreover, SSCs cryopreserved with hypotaurine at 1 mM (28.61 ± 1.95), 5 mM (27.17 ± 2.21), 10 mM (22.92 ± 2.07), 15 mM (24.25 ± 2.34), 20 mM (25.86 ± 1.23), and 50 mM (27.84 ± 1.37) showed a significantly lower ($P < 0.01$) proportion of ROS-positive cells compared to the control group (41.17 ± 2.72). In addition, the JC-1 polymer-to-monomer ratio, an indicator of MMP, was significantly higher in 15 mM (1.18 ± 0.02; $P < 0.05$), 20 mM (1.21 ± 0.07; $P < 0.05$), and 50 mM (1.39 ± 0.08; $P < 0.01$) hypotaurine supplemented as compared to control group (1.00 ± 0.07). These findings indicate that the inclusion of 50 mM hypotaurine in the cryopreservation medium is effective in protecting the viability and quality of SSCs during cryopreservation.

**Author:** Mr SADIKH, Muhammed (DST SERB Junior Research Fellow)

**Co-authors:** Dr BINSILA, Balakrishnan (Senior Scientist); Ms TOMCY, Anjilikal Tomy (DBT JRF); Ms ATHIRA, Thomas (Young Professional II); Dr KRISHNAPPA, Balaganur (Senior Scientist); Ms SHIYAMALA, Elango (ICAR SRF); Dr RAMACHANDRAN, Natesan (Principal scientist); Dr SELVARAJU, Sellappan (ICAR National fellow and Principal Scientist)

**Presenter:** Mr SADIKH, Muhammed (DST SERB Junior Research Fellow)

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