The Addition of a Xenobiotic Prior to Cryopreservation of Bovine Semen Increases Its Resilience Post-Thaw

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Extremolytes, a distinct class of compatible solutes, play a pivotal role in protecting macromolecules and cell structures from extreme environmental stress. The objectives of this study were to assess the biocompatibility of a bacterial derived extremolyte xenobiotic with bull sperm during the cryopreservation process and to assess the potential of the xenobiotic to shield sperm under challenging conditions such as osmotic and heat stress. Semen was collected from bulls (n=8) in a commercial stud and diluted in OptiXcell2 containing either 0 mM (control), 0.5, 5 and 50 mM of the xenobiotic. The semen was packaged into artificial insemination straws and held at 4 °C overnight following which it was cryopreserved as per routine procedures. Subsequently, the semen was thawed and the motility (computer assisted sperm analysis) as well as the viability, membrane integrity and acrosomal status (flow cytometry) were assessed. Following this, the thawed bovine sperm was exposed to heat (42 °C for 4 h) and osmotic (150 mOsm for 15 min) stress. All results are reported as mean ± s.e.m. Sperm cryopreserved in the presence of the xenobiotic at concentrations ranging from 0.05 to 5 mM had similar motility, viability, membrane fluidity, and acrosomal integrity compared to the control post thaw ($P > 0.05$). At a concentration of 50 mM, there was a decline in total motility compared to the control (49.5 ± 4.11 % and 65.5 ± 4.05 %, respectively; $P < 0.05$). Incubation of the control sperm in media of low osmolarity reduced total motility to 21.4 ± 4.16% and addition of the xenobiotic did not affect this. Similarly, there was no effect of the xenobiotic on sperm viability across all treatment groups ($P > 0.05$). However, when sperm were subjected to heat stress at 42 °C for 4 h, a significant interaction between time and the treatment was observed on sperm motility ($P < 0.01$). This was manifested by no difference between treatments at 0 h but after a 2-h incubation, there was a 36% decrease in motility in the control group ($P < 0.001$) while the xenobiotic treatment groups maintained their motility. These results indicate that the xenobiotic assessed is not cytotoxic to sperm in the range 0 to 5 mM. and while no protective effects were observed when the sperm was subjected to osmotic stress at 150 mOsm, promising results were observed with improvement in sperm motility under heat stress conditions. This shows the potential application of the xenobiotic to shield sperm under challenging conditions, with further studies needed to assess additional protective effects of the xenobiotic on sperm.