The effect of extender and freezing rate on the quality of frozen-thawed ram spermatozoa

Yana Kuptsova, Sydney University

2025

Ram spermatozoa are uniquely susceptible to cryoinjury, making extender formulation and freezing rate critical for post-thaw function and artificial insemination (AI) outcomes.

In Experiment 1, semen was collected from three mature Merino rams (n = 12) and each ejaculate split and extended in Triladyl, OptiXcell, AndroMed or Salamon's (bespoke tris-citrate-glucose-egg yolk-glycerol diluent) freezing media, packaged in 0.25 mL straws and frozen in liquid nitrogen (LN) vapour at -9°C/min.

In Experiment 2, semen was collected from three mature Merino rams (n = 12), extended in Triladyl, packaged in 0.25 mL straws and frozen in LN vapour at freezing rates of -26, -13, -9, or -6 °C/min. Sperm motility and kinematics (HT CASA IVOS II) as well as viability and acrosome integrity (H33342/PI/FITC-PNA) were assessed at 0, 2, 4 and 6h post-thaw (37°C for 30 s).

In Experiment 1, diluent, time, and their interaction significantly affected most post-thaw metrics ($P < 0.05$). Sperm frozen with Triladyl displayed the highest total motility (TM) and velocity than all other diluents at 0, 2, 4, and 6h post-thaw ($P < 0.05$). Samples extended using OptiXcell retained the highest proportion of viable, acrosome-intact sperm, while AndroMed consistently produced the lowest motility and kinematic performance.

In Experiment 2, freezing rate significantly affected TM, straight-line velocity (VSL), average path velocity (VAP), and viability ($P < 0.05$). When frozen at a rate of -9 °C/min, sperm displayed higher TM, progressive velocities and viability/acrosome integrity 0-6h post-thaw when compared to samples frozen at slower or faster rates.

These, findings highlight the importance of optimising freezing protocols to species-specific sperm characteristics, as both extender composition and freezing rate strongly determine post-thaw viability. This study establishes the methods of processing that best preserve the structure and function of ram spermatozoa during freezing, providing a framework for refining cryopreservation protocols to improve AI outcomes.