

The effect of cryoprotectant concentration and equilibration time on the post-thaw quality of ram spermatozoa

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This study aimed to investigate the effect of cryoprotectant composition and equilibration times on the post-thaw quality of cryopreserved ram spermatozoa.

Experiment 1 was conducted to assess the interaction between dimethylformamide (DMF; 1, 1.5, 2, and 3%) and glycerol (G; 2.5, 3.5, and 4.5%) concentrations on post-thaw semen quality, alongside the current industry standard treatment (0% DMF + 5% G). Semen (4 ejaculates from 3 merino rams; n=12) was diluted in tris-based cryodiluent to 300×10^6 spermatozoa/mL, cryopreserved in straws, and thawed using industry standard techniques.

Experiment 2 examined equilibration time (2, 4 and 24 hours) with the current industry standard 0% DMF + 5% G extender (3 ejaculates from 3 merino rams; n=9). Post-thaw motility and acrosome and membrane integrity were assessed in both experiments using computer-assisted sperm analysis (CASA) and flow cytometry (PI/FITC-PNA).

Experiment 1 found 5% glycerol alone produced higher post-thaw quality outcomes than any of the DMF and glycerol combinations. Higher concentrations of DMF negatively impacted performance.

Experiment 2 showed no significant differences ($P < 0.05$) in post-thaw quality between the different equilibration times. In conclusion, the significant association between increasing concentrations of DMF and low post-thaw quality indicates a lack of synergism between these cryoprotectants.

Ram sperm should be frozen with 5% glycerol and equilibrated up to 24h without compromising post-thaw quality, a finding that has significant practical implications. This approach would allow collection of semen in larger volumes without the necessity of same day freezing, thereby reducing logistical constraints and facilitating more efficient processing.