

CIÊNCIAS AGRÁRIAS

VETERINÁRIA

Inseminação Artificial e Biotécnicas da Reprodução

EVALUATION OF PRESERVED RAM SPERMATOZOA BY FLOW CYTOMETRY FOLLOWING FREEZING AND COOLING

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The process of sperm preservation damages spermatozoa due to induction of structural and functional changes in the membrane and reflects the reduction in fertility after artificial insemination. Many semen preservation protocols including freezing and cooling rates coupled with different diluents, have been studied in order to reduce sperm cryo-damage, increase the fertilizing capacity and increase the time of storage. To improve freezing and cooling processes, the objectives of this study were to determine the influence of two diluents on the viability of frozen-thawed semen and liquid storage semen. Semen samples were collected from two mature rams, pooled, and diluted in Botubov® and Bovimix®. Semen samples were frozen on liquid nitrogen. Straws were thawed at 37°C for 20 sec. For cooling studies, pooled ram semen was diluted in Botubov® and Bovimix®, stored in a refrigerator at 5°C and evaluated after 72 hours of cooling. For viability assessment, semen samples were stained using Live/Dead Sperm Viability Kit (Molecular Probes). Spermatozoa viability was analyzed by using FACScalibur BD. Fresh semen showed viability 66%. Samples diluted in Botubov® and Bovimix® showed viability 2% after thawing, and 26 and 60% after 72h of cooling, respectively. In conclusion, Botubov® and Bovimix® were not efficient to preserve sperm viability after freezing/thawing. Cooling protocol reduced sperm viability when compared to fresh semen. Freezing/thawing harmed more spermatozoa function than cooling. Our results suggest that the diluent Bovimix® caused the least cellular damage on cooling, and is more efficient to preserve sperm viability than Botubov®.