

## Effects of Sperm Separation Techniques on Removal of Cryo-damaged Canine Sperm

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**Purpose:** The present study was designed to evaluate damage induced by freeze-thawing procedure and to compare the effects of sperm separation techniques on removal of cryo-damage in canine semen.

**Materials and Methods:** Five healthy and sexually mature dogs were used in this study, and semen was obtained by digital manipulation and cryopreserved. After thawing, sperm conventional parameters, membrane integrity, apoptosis, oxidative stress and DNA integrity were evaluated for comparison with fresh semen. And thawed semen was treated by sperm separation techniques using glass wool and Percoll to identify the effect of them on removal of cryo-damaged sperm, and treated samples was also evaluated.

**Results:** Freeze-thawing process decreased motility, viability, normal morphology and membrane integrity in canine sperm as previously reported ( $p < 0.05$ ). And this process also decreased alive and non-apoptotic sperm, and increased apoptotic index, intracellular hydrogen peroxide level and DNA fragmentation ( $p < 0.05$ ). The sperm samples treated by glass wool filtration after thawing showed improved motility, viability, normal morphology, membrane integrity ( $p < 0.05$ ). And the glass wool filtration increased the percentage of alive and non-apoptotic sperm and decreased sperm with high level of intracellular hydrogen peroxide compared with control (not treated) ( $p < 0.05$ ) but DNA fragmentation was not significantly different between the glass wool filtration and control. The sperm samples treated by Percoll showed decrease in level of intracellular hydrogen peroxide evaluated by DCF MFI ( $p < 0.05$ ) but were not significantly different from control in the other sperm parameters.

**Conclusions:** The freeze-thawing procedure significantly affects membrane integrity, apoptosis, oxidative stress and DNA integrity as well as conventional parameters in canine semen. These cryo-damages can be improved by treatment of glass wool filtration.

**Key words:** cryo-damage, glass wool filtration, Percoll, semen cryopreservation

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