

Summary of the DVM thesis No 26183, Faculty of Veterinary Medicine, Urmia University.

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Thesis Title: The effect of hydroxyapatite along with raffinose and glutathione on sperm quality after sperm freezing and thawing: Evidence on the relationship between antioxidant capacity, DNA integrity, and lipid and protein peroxidation.

Abstract:

This study examines the effects of Zn and Cu-doped hydroxyapatite combined with glutathione (GSH) and raffinose on sperm motility, viability, DNA integrity, and oxidative stress markers in mouse sperm, evaluated both before freezing and after thawing. Sperm samples were collected from 10 albino mice and divided into six groups, including a control group with a commercial cryopreservation medium and experimental groups using hydroxyapatite (H), GSH, and raffinose (R), alone or in combination, as cryopreservation media. After freezing at -196°C for 72 hours, sperm were thawed and assessed for motility, viability, DNA fragmentation, total antioxidant capacity (TAC), total oxidant status (TOS), lipid peroxidation, protein oxidation, and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR). The results showed significantly higher motility in the H+GSH+R medium compared to the commercial control medium, with individual use of GSH and raffinose resulting in lower motility ($p<0.05$). DNA damage was markedly reduced in the H+GSH+R group compared to the control ($p=0.0001$), alongside a decrease in sperm mortality ($p<0.01$). The H+GSH+R medium showed increased TAC pre-freezing ($p=0.002$), though this difference was not significant post-thaw. TOS levels were significantly lower in the H+GSH+R group at both time points ($p<0.0001$), suggesting enhanced oxidative stress protection. Additionally, lipid peroxidation (MDA content) was significantly lower in the GSH-only medium ($p=0.003$), while protein oxidation (carbonyl content) was significantly reduced in the H-alone and H+GSH+R media ($p=0.001$ and $p=0.006$, respectively). The redox enzyme activities (SOD, CAT, GR, and GPX) were also significantly higher in the H+GSH+R medium compared to the commercial control. In conclusion, the combined use of Zn and Cu-doped hydroxyapatite, GSH, and raffinose in cryopreservation media offers significant protection against oxidative damage and enhances sperm quality, both pre-freezing and post-thaw.

Keywords: Cryopreservation media, Zn and Cu-doped hydroxyapatite Sperm, Oxidative stress, DNA integrity.