A181 Physiology of Reproduction in Male and Semen Technology

Effects of serine proteases inhibitors in bovine sperm cryopreservation

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Cryopreservation is partially harmful to bovine semen fertility and induces capacitation-like changes in sperm. Seminal plasma contains serine proteases and serine proteases inhibitors, which are involved in mammalian fertilization. Due toits function, serine proteases inhibitors can be applied to prevent cold-induced sperm capacitation. We analysed the effect of differentconcentrations of two serine proteases inhibitors, Plasminogen activator inhibitors 1 - PAI-1 (70 ηg, 140 ηg and 210 ηg) and Antipain (10 µg, 50 µg and 100 µg) in supplementation to the extender of bovine semen cryopreservation. Thirty-six ejaculates from four Curraleiro Pé-Duro bulls were collected by electroejaculation and evaluated for macroscopic characteristics (volume and aspect) and microscopic characteristics (motility, vigor, concentration and sperm pathologies). Posteriorly, semen was diluted in Tris-Egg yolk culture medium according treatments and then placed on 0.5mL straws, frozen in a TK 3000®machine (TK Ltda, Uberaba, Brazil), and stored in a cryopreservation tank (-196ºC). The effect of the inhibitors on the sperm parameters (sperm kinetics - CASA, acrosome integrity, plasma membrane integrity, mitochondrial membrane potential, sperm defects and acrosome reaction rate) were evaluated in the post-thaw semen. Sperm cryopreservation with Antipain (10 µg, 50 µg e 100 µg) decreased post-thaw kinetics parameters of progressive motility (MP - µm/s), straight-line speed (VSL - µm/s), linearity (LIN - %), straightness (SRT - %) and the percentage of hyper-activated spermatozoa in comparison to the control ($P < 0.05$). PAI-1 (210 ηg) decreased VSL and LIN in comparison to the control ($P < 0.05$). Antipain and PAI-1 had no effect on integrity parameters of the plasma membrane, mitochondrial membrane potential and sperm defects. Sperm cryopreserved in the presence of Antipain (10 µg, 50 µg e 100 µg) and PAI-1 (70 and 140 ηg) had acrosome integrity values higher than the control, demonstrating the Antipain inhibitors and PAI -1 ability to preserve the acrosome integrity ($P < 0.05$). There was no statistical difference among the studied treatments for variable rate of acrosome reaction, demonstrating that cryopreserved spermatozoa with Antipain and PAI-1 were able to complete the in vitro acrosome reaction. In conclusion, the serine proteases inhibitors, Antipain and PAI-1 (70 and 140ηg) are able to preserve the acrosome integrity of cryopreserved bovine sperm.