

## 学 位 論 文 の 要 旨

論文題目 Study on negative effects of ROS on fresh and frozen bull sperm with antioxidants treatment  
(新鮮および凍結ウシ精子における活性酸素種 (ROS) の影響と抗酸化因子の役割に関する研究)

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Mammalian sperm travels through the uterus to the oviduct, maintaining a linear motility pattern during in vivo fertilization. This migration requires significant energy, primarily derived from mitochondrial ATP production. However, during cryopreservation, the quality of sperm deteriorates due to the excessive production of reactive oxygen species (ROS). Elevated ROS levels negatively impact sperm membrane integrity, acrosomal integrity, and mitochondrial function. Oxidative stress, stemming from both internal and external factors, impairs sperm function by damaging essential components and decreasing metabolic activity, ultimately reducing fertility.

This study aimed to examine the effects of oxidative stress on bull sperm and assess the effectiveness of targeted antioxidants in counteracting these harmful effects. Fresh bull semen samples were exposed to hydrogen peroxide ( $H_2O_2$ ) and antimycin to induce oxidative stress, while the antioxidants PQQ, ergothioneine, and vitamin C were used to mitigate the stress. Sperm motility, viability, and ROS levels in the cytoplasm and mitochondria were measured using computer-assisted sperm analysis (CASA) and flow cytometry.

Results indicated that  $H_2O_2$  rapidly decreased sperm viability, while antimycin-induced mitochondrial ROS primarily reduced sperm motility. PQQ and vitamin C were effective in lowering mitochondrial ROS, whereas ergothioneine and vitamin C reduced cytosolic ROS. In freeze-thawed sperm, oxidative stress was elevated in both the cytoplasm and mitochondria, and all three antioxidants improved sperm motility by inhibiting ROS

production. Immunofluorescence detection of oxidized lipids (4-hydroxynonenal) in sperm showed that oxidative stress affects the head and mid-piece of sperm.

These findings underscore the potential of targeted antioxidants to mitigate the adverse effects of oxidative stress on bull sperm, offering valuable insights to enhance semen quality and optimize antioxidant use in artificial insemination. Incorporating antioxidants into insemination media could reduce the negative impact of oxidative stress and boost sperm motility in the female reproductive tract, aiding sperm in reaching the fertilization site. This novel approach to thawing frozen sperm from the present study could increase conception rates, improve livestock production, reduce poverty, increase income, and help to achieve the United Nations' Sustainable Development Goals (SDGs).