



IMPROVING THE EFFICIENCY OF OOCYTE CRYOPRESERVATION IN VETERINARY MEDICINE

Objective : This study aimed to evaluate the effect of overnight holding (H) and naloxone (Nx) on vitrified equine immature cumulusoocyte complexes (COCs).

<u>Materials and Methods</u> : COCs were collected from abattoir ovaries from February to March 2023. Combining the addition of Nx (10⁻⁸ mMol) and H, COCs were split into six groups: fresh control (F), holding control (H), vitrified (VIT), vitrified+Nx (VIT-Nx), holding+vitrified (H-VIT), holding+vitrified+Nx (H-VIT-Nx). After warming, intact oocytes were stained with H2DCFDA, Celltraker Blue CMF2HC, and JC1, to evaluate the reactive oxygen species (ROS), the glutathione levels (GSH), and the mitochondrial potential (MP) respectively.

<u>**Results</u></u> : The rate of intact oocytes was lower (P<0.05) in VIT-Nx (49/66, 74.2%) than in F (58/64, 90.6%) and H-VIT 47/52, 90.4%) but not different from H (56/68, 82.4%), VIT (43/54, 79.6%) and H-VIT-Nx (50/61, 82.0%). ROS were lower (P<0.05) in H-VIT-Nx (1.9\pm0.4) than F (39.4\pm2.2) but not different from the other groups (H 29.3\pm2.3; VIT 30.0\pm4.4; 9.9\pm2.3; H-VIT 10.2\pm1.4). No differences were observed for GSH levels among groups, while MP was higher (P<0.05) for H-VIT-Nx (100.6\pm4.7) than the others (F 39.8\pm2.5; H 19.4\pm1.1; VIT 33.4\pm2.7; VIT-Nx 38.4\pm3.0; HVIT 44.6\pm3.9).</u>**

Conclusions : These preliminary results confirm a good survival rate after vitrification of both fresh and hold equine immature COCs.

Future Proposal: Further analyses are needed to interpret the data regarding the effects of H and Nx on ROS and MP, and to evaluate the maturation rate of the oocytes and the the likelihood of producing an embryo.

References : Effect of holding and naloxone on vitrification of immature horse oocytes: preliminary results Gugole P., Iacono E, Merlo B.