## Dottorato di ricerca in Scienze Veterinarie [XXXIII] CICLO - Anno di corso: [1${ }^{\circ}$ ] Dott: Penelope Maria Gugole Curriculum: Scienze Cliniche Veterinarie Supervisor: Barbara Merlo Cosupervisor(s): Eleonora Iacono, Daniele Zambelli

## IMPROVING THE EFFICIENCY OF OOCYTE CRYOPRESERVATION IN VETERINARY MEDICINE

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

Materials and Methods : COCs were collected from abattoir ovaries from February to March 2023. Combining the addition of $\mathrm{Nx}\left(10^{-8}\right.$ 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.

Results : The rate of intact oocytes was lower ( $\mathrm{P}<0.05$ ) in VIT-Nx (49/66, 74.2\%) than in $\mathrm{F}(58 / 64,90.6 \%)$ 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 $\pm 0.4$ ) than F (39.4 $\pm 2.2$ ) but not different from the other groups (H 29.3 $\pm 2.3$; VIT 30.0 $\pm 4.4 ; 9.9 \pm 2.3$; H-VIT 10.2 $\pm 1.4$ ). No differences were observed for GSH levels among groups, while MP was higher ( $\mathrm{P}<0.05$ ) for H-VIT-Nx (100.6 $\pm 4.7$ ) than the others ( $\mathrm{F} 39.8 \pm 2.5 ; \mathrm{H} 19.4 \pm 1.1$; VIT 33.4 $\pm 2.7$; VIT-Nx 38.4 $\pm 3.0$; HVIT $44.6 \pm 3.9$ ).

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.

