ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
ULTRASTRUCTURAL EVALUATION OF IN VITRO MATURERED CANINE OOCYTES

Christine Viaris de Lesegno, Karine Reynaud, Christine Pechoux*, Martine Chebrout, Sylvie Chastant-Maillard
UMR 1198 INRA/ENVA/CNRS FRE 2857, Biologie du Développement et Reproduction, Ecole Nationale Vétérinaire d’Alfort, 7 avenue du général de Gaulle, F-94704 Maisons-Alfort, France. Email: schastant@vet-alfort.fr
* INRA, UR 1196; Génomique et Physiologie de la lactation, Plateforme de microscopie électronique, 78350 Jouy-en-Josas Cedex, France

Introduction - Meiosis resumption rates obtained to date with anoestrus canine oocytes are desesperately low. The bad quality of the collected oocytes, originating from very small follicles, may be one cause of the inefficiency of the In Vitro Maturation (IVM) process. Moreover, for the few oocytes reaching the metaphase II stage, fertilization rates are also very limited with a high frequency of polyspermy (Luvoni et al, 2005). Both phenomenon could be related to an insufficient or abnormal cytoplasmic maturation.

Objectives - This study was thus designed to evaluate first the quality of oocytes used for IVM and secondly to compare the cytoplasmic maturation of in vitro matured oocytes to their in vivo matured counterparts. We also aimed to examine whether the oocytes that did not reach metaphase II stage after IVM are at erratic stages or intermediate stages with incomplete but normal maturation.

Materials and methods - Cumulus-oocyte complexes were recovered from ovaries of bitches at anestrus stage. Ultrastructural organization was determined before or after 72 hr IVM by Transmission Electron Microscopy on 60 oocytes examined under ultra-thin sections. Ultrastructural studies performed on ewe (Cran et al. 1980), mare (Grondhal et al, 1995) and cow (Hyttel et al., 1986) allowed to define as signs of cytoplasmic immaturity a poor quantity of mitochondria heterogeneously distributed, scarce cortical granules and association of Golgi apparatus with TZP endings.

Results - Before IVM, two populations of canine anestrus oocytes were present: some had a centrally located germinal vesicle, a transcriptionnally active (reticulated) aspect and an immature cytoplasm while others had an eccentric nucleus, a transcriptionnally inactive aspect and a more mature cytoplasm. After the 72hr in vitro period, 75% of the oocytes were still at the GV (Germinval Vescicle) stage, with three different patterns: centrally located nucleus, peripheral nucleus with non surrounded nucleolus, peripheral nucleus with surrounded nucleolus. All these configurations showed a good synchronization between nuclear and cytoplasmic maturation. In vitro matured metaphase II oocytes had a very poor cytoplasmic maturation compared to the one observed after the in vivo process (Viaris de Lesegno et al, 2008), especially when spontaneously denuded during the culture period. Metaphase I oocytes had a similar cytoplasmic maturation than in in vivo maturation. This ultrastructural analysis demonstrates that anestrus canine oocytes are very immature. Even when they achieve nuclear maturation, significant cytoplasmic maturation may not be obtained in vitro in parallel. All in vitro maturation systems should thus be evaluated both on nuclear and cytoplasmic criteria.
References