

P-17 Comparative Functional analysis of Malate Dehydrogenase (Mor2) During in vitro Maturation of the Mouse and Porcine Oocytes by RNA Interference (RNAi)

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Objectives: There are some species, such as porcine, where its in vitro oocyte maturation rates are still very low. This comparative study was conducted to investigate the role of Mor2 during oocyte maturation in vitro by doing microinjection of mouse and porcine Mor2 (mMor2 and pMor2) into immature mouse and porcine oocytes, respectively.

Methods: The Mor2 double-stranded RNA (dsRNA) was prepared species-specifically and microinjected into the cytoplasm of denuded germinal vesicle (GV) oocytes. Oocytes were cultured for 48 h (porcine) and 16 h (mouse) for in vitro maturation in appropriate culture media. We measured relative changes in morphology, maturation rates and changes in mRNA levels after microinjection of Mor2 dsRNA.

Results: We confirmed gene-specific knock down of Mor2 mRNA in both species after Mor2 RNAi. In contrast to our previous finding that mMor2 RNAi resulted in GV arrest, we found that pMor2 RNAi in porcine oocytes resulted in metaphase I (MI) arrest (58%). To determine whether this difference between mouse and porcine RNAi is due to differences in culture media or in culture time, we firstly cultured mouse oocytes in porcine media after RNAi. After mMor2 RNAi and in vitro culture for 16 hours in the porcine media, mouse oocytes were developed to MII stage (62%) and there was no statistical difference compared to that of non-injected (76.8%) and buffer-injected (73.3%) control groups. This disparity appears to be come largely from component of medium. We tried to culture in M199 media after mouse mor2 dsRNA microinjection. To evaluate role of cumulus cells in oocyte maturation, we did pMor2 RNAi in porcine oocytes in the presence of cumulus cell rather than in denuded oocytes. We found that the pMor2 RNAi in porcine cumulus oocyte complex resulted in development of oocytes to the MII stage despite of Mor2 insufficiency introduced by RNAi.

Conclusion: We found that GV arrest after mMor2 RNAi in the mouse oocyte is overcome by medium components and MI arrest after pMor2 RNAi in the porcine oocyte is overcome by the presence of cumulus cells during in vitro maturation. Therefore, we concluded that the mouse and porcine oocytes are having different metabolic systems in relation to malate dehydrogenase for oocyte maturation. This fact could be a basis for differences in maturation rates in vitro in two species. Further scrutinized studies on metabolic pathways would led us in finding better culture system to improve oocyte maturation rates in vitro, especially in more challenging species like the porcine.

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