

94. KINETICS OF MEIOTIC MATURATION DIFFER BETWEEN PRE-PUBERTAL AND ADULT PORCINE OOCYTES

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Oocytes from pre-pubertal pigs have lower developmental competence (DC) than those from adult pigs during in vitro embryo production [1]. Oocyte DC depends on success of nuclear (meiotic) and cytoplasmic maturation. Comparison of these oocytes groups provides an excellent model to better understand these developmental competence differences. The objective of this study was to determine whether meiotic maturation kinetics differ between pre-pubertal versus adult oocytes and their response to dibutyryl cAMP (dbcAMP) meiotic inhibitor. Oocytes were aspirated from 3-8 mm follicles of pre-pubertal and adult pig ovaries, washed three times in maturation media (MM) and transferred to 50 µL droplets of MM. Oocytes were matured for 44 h in medium 199 supplemented as previously described [2], either in presence or absence of 1 mM dbcAMP. After 24 h oocytes were washed in MM without dbcAMP and transferred to 50 µL MM droplets without dbcAMP. Groups of oocytes were fixed at 0, 16, 22, 40 and 44 h of IVM in a solution of ethanol and acetic acid (3:1, v/v) for 48 h then stained with 1% (w/v) orcein. Nuclear structures were then assessed by phase contrast microscopy. Eight separate replicates were examined with approximately 25 oocytes examined per time point per replicate. Pre-pubertal oocytes progress faster throughout meiotic stages germinal vesicle (GV) and metaphase 1 (M1), finishing meiosis by reaching metaphase 2 at the same rate as adult. dbcAMP maintains higher rates of pre-pubertal and adult oocytes in GV and M1 meiotic stages, however pre-pubertal oocytes still reach these stages earlier than adult oocytes. In conclusion, increased time in meiotic stages appears to be important for attainment of developmental competence during in vitro maturation. Likewise, increased response of adult oocytes to dbcAMP indicates they are more equipped to utilize or receive more dbcAMP to increase meiotic time, hence DC.

(1) Torres CRL, Rath D (1992) *Theriogenology* **37**: 283. (2) Grupen CG, *et al.* (2002) *Mol. Reprod. Dev.* **62**(3): 387–396.