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In vitro maturation of guinea pig oocytes supplemented with Epidermal Growth Factor and Insulin-Like Growth Factor I

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Insights in oocyte maturation process in guinea pigs are essential for the development of *in vitro* culture systems in this species, since it represents an interesting animal model in reproduction field (Suzuki et al. Mol Reprod Dev 2003; 64, 219–25). The goal of this study was to elucidate the influence of both Epidermal Growth Factor (EGF) and Insulin-like Growth Factor I (IGF-I) on *in vitro* oocyte maturation (IVM) medium of guinea pig. We assessed meiotic and cytoplasmic oocyte maturation, in terms of cortical granules (CG) and mitochondrial distribution, apoptotic rate and steroidogenic response of cumulus-oocyte-complexes (COCs) after IVM. A pool of 500 COCs from adult guinea pigs were cultured in groups of 40 COCs in four replicates in TCM-199 with 2 mM/mL glutamine, 0.1 mg/mL sodium pyruvate and 0.003% BSA for 17h (38ºC, 5%CO_2) (Sigma Chemical Company). Oocytes were distributed in different combination doses of growth factors as follows: group 0 (without growth factors); group EI (50 ng/mL EGF + 100 ng/mL IGF-I); group EI-FCS [50 ng/mL EGF + 100 ng/mL IGF-I + 10% Fetal Calf Serum (FCS)] and group FCS (10% FCS). After IVM, 456 oocytes were randomly selected, fixed and stained with 10 µg/mL Hoechst 33342 to assess nuclear configuration [Metaphase II (MII)]. Among them, a total of 152 oocytes were denuded and stained with 100 µg/mL FITC-LCA for CG visualization (n = 93) or with 180 nm MitoTrackerRedCMXRos (Molecular Probes Inc) (n = 59) for mitochondria assessment. CG and mitochondria patterns were analyzed with laser- scanning confocal microscopy (Leica). Estradiol (E_2) and Progesterone (P_4) production by COCs was measured by ELISA assay (DEMEDITEC Diagnostics GmbH) in the maturation medium. In the rest of COCs (n = 44) apoptosis rate was visualized with TUNEL technique (Roche Diagnostics, SL) and analyzed with Image J software. Chi-square test and one-way ANOVA with Duncan *post-hoc* test were used. MII rate significantly increased in oocytes from EI and EI-FCS groups compared to 0 and FCS groups (78.3 and 83.7% *vs* 38.4 and 55.8%, respectively; $P < 0.05$). EI-FCS group showed higher rate of oocytes with peripheral migration of CG (76.9%) (compatible with cytoplasmic maturation) compared with 0 group (23.8%) ($P < 0.05$) whereas EI and FCS groups showed intermediate results (59.1 and 50.0%, respectively). There were no significant differences between groups in the mitochondrial distributions EI-FCS COCs´ showed the lowest apoptosis rate (6.6 ± 0.7%) and the highest E_2 (0.3 ± 0.01ng/mL) and P_4 (1.9 ± 0.05 pg/mL) production compared to the remaining experimental groups ($P < 0.05$). In our conditions, combination of 50 ng/mL EGF, 100 ng/mL IGF-I and 10% FCS seems to be a suitable medium for IVM system in guinea pig oocytes since it offers superior results of oocyte maturation and quality of CCs compared to the other groups studied included when FCS was added alone. Future studies using these oocytes for IVF and IVC are needed to assess the potential of such COCs. Funded by UTPL and UCM.

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