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Effect of epidermal growth factor on nuclear and cytoplasmic *in vitro* maturation of guinea pig oocytes

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The guinea pig may represent an animal model for research on ovarian infertility and improvement of the in vitro maturation (IVM) conditions is needed in this species. The aim of the present work was to immunolocalize the Epidermal Growth Factor (EGF)-Receptor in the guinea pig ovaries and to study the effect of EGF on meiotic and cytoplasmic maturation, and apoptotic rate in cumulus-oocyte-complexes (COCs). Immunohistochemistry was performed in paraffined ovaries using a rabbit polyclonal antibody EGF-R (1:100; Santa Cruz Biotechnology) and the ABC Vector Elite kit (Vector Laboratories). For the IVM, COCs were collected by aspiration of follicles >700µm under a stereoscopic microscope. They were cultured at 37°C in 5% CO2 during 17 h with TCM-199 supplemented with glutamine, pyruvate, BSA, and different concentrations of EGF (Sigma) [0 (control), 10, 50 or 100 ng/mL] or 10% Fetal Calf Serum (FCS). After IVM, 564 oocytes were fixed and stained with 10 µg/mL Hoechst to assess nuclear configuration in terms of Metaphase II (MII) rate. A total of 143 oocytes were treated progressively with 0.5% pronase, 4% paraformaldehyde, 0.02% Triton X-100, 7.5% BSA and 100 µg/mL FITC-LCA for cortical granule (CG) staining. Also, 78 oocytes were stained with 180 nm MitoTracker RedCMXRos (Molecular Probes Inc) for active mitochondria visualization. CG and mitochondria patterns were analyzed with laser scanning confocal microscopy (Leica TCS SP2). Apoptosis rate in cumulus cells (n=58 COCs) were visualized with TUNEL (In Situ Cell Death Detection Kit, Roche) and analyzed with Image J software. Chi-square test was used to compare nuclear maturation, CG and mitochondria migration rates. The apoptotic index was analyzed by a one-way ANOVA using Duncan post-hoc test. Positive immunostaining for EGF-R was found in granulosa and theca cells and oocytes in all follicular stages. MII were significantly higher in oocytes supplemented with 50 ng/mL EGF group (75.9%) compared to other experimental groups (43.5, 51.8, 53.7 and 59.5% for 0, 10, 100 ng/mL EGF and 10% FCS, respectively, $P<0.05$). Group matured with 50 ng/mL EGF showed higher rate of oocytes with peripheral migration pattern of CG (compatible with cytoplasmic maturation) compared to control group (71.9 vs. 32.4%; $P<0.05$) and migrated mitochondrial pattern compared to the control group and the group supplemented with 100 ng/mL EGF (80.0% vs. 27.8% and 31.3%, respectively; $P<0.05$). Apoptotic rate was lower in 50 ng/mL EGF (17.2±0.9%) and 10% FCS (16.0±1.2%) groups related to the control one (28.7±1.4%) ($P<0.05$). In conclusion, the presence of EGF-R in guinea pig ovaries, suggests that EGF may exert a direct effect on ovarian function. A dose of 50 ng/mL EGF seems to be the most appropriate concentration for IVM of guinea pig oocytes, since it improves nuclear and cytoplasmic oocyte maturation and reduces apoptosis in the cumulus cells.

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