

Encircling Granulosa Cells Protect Against Hydrogen Peroxide-induced Apoptosis in Rat Eggs Cultured in Vitro

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Abstract—Increased oxidative stress due to in vitro culture conditions affect quality of denuded eggs during various assisted reproductive technologies (ARTs) programs. Presence of intact granulosa cells could protect eggs from oxidative stress damage under in vitro culture conditions. Therefore, present study was aimed to investigate whether encircling granulosa cells could protect against hydrogen peroxide (H₂O₂)-induced egg apoptosis in ovulated cumulus oocyte complexes (COCs) cultured in vitro. For this purpose, ovulated COCs were collected from oviduct after superovulation induction and some of them were denuded. COCs and denuded eggs were cultured with or without various concentrations of H₂O₂ for 3 h in vitro. The morphological changes, Bax/Bcl2, cytochrome c levels and DNA fragmentation were analyzed both in COCs and denuded eggs. Our result suggests that H₂O₂-induced morphological apoptotic features in a concentration-dependent manner in denuded eggs cultured in vitro. The H₂O₂ (20 μM) induced overexpression of Bax as well as cytochrome c, suppressed Bcl2 expression and induced DNA fragmentation in treated eggs cultured in vitro. Presence of encircling granulosa cells protected against H₂O₂-induced morphological apoptotic features, reduced Bax expression, cytochrome c level and DNA fragmentation in eggs. These data suggests that encircling granulosa cells protect ovulated eggs from oxidative stress damage caused due to H₂O₂ under in vitro culture conditions.

Keywords: Granulosa cells, Hydrogen peroxide. Bax/Bcl2, DNA fragmentation, Egg apoptosis.