

OOCYTE-SECRETED FACTOR(S) REGULATE APOPTOSIS OF BOVINE CUMULUS CELLS

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Paracrine factors secreted by the oocyte affect cumulus cell proliferation and differentiation. These factors may also act in an anti-apoptotic manner, maintaining the low incidence of cellular apoptosis within cumulus cells. The purpose of this study was to determine whether the incidence of apoptosis within cumulus cells is regulated by oocyte-secreted factors (OSF). Bovine cumulus-oocyte complexes (COC) aspirated from abattoir-derived ovaries were randomly allocated to 3 treatments: (1) groups of 5 intact COC; (2) groups of 5 oocyctomised complexes (OOX), where oocytes were removed microsurgically; and (3) groups of 5 OOX co-cultured with 25 denuded oocytes (DO). To examine a dose effect of OSF, OOX were also cultured with increasing numbers of DO (OOX+5DO, OOX+25DO, OOX+50DO). In both experiments, complexes were cultured in their respective treatments for 24 h in 50 µl of oocyte maturation medium (+/- rFSH; 0.1 IU/mL). Apoptosis was assessed using TUNEL, with all nuclei counterstained with propidium iodide (PI). Nikon TE2000 laser confocal scanning microscopy was used to visualise and quantify the incidence of apoptosis (TUNEL/PI). The proportion of apoptotic cells was determined by image analysis. Oocyte removal lead to a significant increase in cumulus cell apoptosis (OOX, 67% apoptotic; COC, 15%; $P < 0.001$). However, the incidence of apoptosis in OOX was restored to COC levels when co-cultured with DO (18%; $P > 0.05$, COC v. OOX+DO). FSH prevented apoptosis in all treatment groups ($P < 0.05$) decreasing the incidence by 27% in OOX and by 12% in COC. Cumulus cell apoptosis within OOX (+/- FSH) was reduced in a dose dependent manner by treating with increasing numbers of DO. These results indicate for the first time that oocyte-secreted factor(s) regulate the incidence of apoptosis within cumulus cells.