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## Apoptosis of Rat Ovarian Granulosa Cells during Follicular Development

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**Objective:** To evaluate the apoptosis pattern and apoptosis associated genes at different follicular development stages.

**Materials & Methods:** After down-regulation of ovarian function with GnRHa, granulosa cells were collected from rats at different times of following treatment paradigms: specify each stage [stage E (estrogen), EF (E + FSH), and EF hCG (EF + hCG)]. The collected cells were divided into three groups: freshly isolated cells and cells cultured in serum-free medium with or without E2 for 24 hrs. Regulation of apoptosis of granulosa cells was evaluated by FITC-dUTP stain, QC-PCR, and western blot analysis.

**Results:** Apoptosis rate of freshly isolated granulosa cells increased according to follicular development. After *in vitro* culture of the cells, the apoptosis rate increased at all development stages, especially stage EF hCG ( $p < 0.05$ ). The expression of active caspase-3 showed good correlation with apoptosis rate ( $r^2 = 0.93$ ,  $p < 0.01$ ). While Bax mRNA did not change, Bcl-2 mRNA significantly decreased according to follicular development ( $p < 0.05$ ). p53 mRNA significantly increased during follicular development ( $p < 0.05$ ) and closely matched with apoptosis rate ( $r^2 = 0.7$ ,  $p < 0.05$ ). After *in vitro* culture, Bax and p53 mRNA tended to increase and showed a good correlation with apoptosis rate ( $r^2 = 0.64$ ,  $p < 0.05$  and  $0.86$ ,  $p < 0.01$ ), but Bcl-2 mRNA decreased at all stages and had no correlation. Fas and TNF- $\alpha$  mRNA levels increased significantly at stage EF ( $p < 0.05$ ) and then decreased. The expression of Fas and caspase-8 mRNA showed good correlation with apoptosis rate ( $r^2 = 0.78$ ,  $p < 0.01$ ;  $r^2 = 0.59$ ,  $p < 0.05$ ). After *in vitro* culture of granulosa cells for 24 hrs at each stage, however, the mRNA levels of these genes decreased compared to control at all stages and had no correlations with apoptosis rate.

**Conclusions:** Apoptosis of rat ovarian granulosa cells increases according to follicular development stages and may be mediated by various apoptosis associated genes. After *in vitro* culture, however, apoptosis of the cells may be induced mainly by pro-apoptotic genes.