FOLLICLE STIMULATING-HORMONE (FSH) WITHDRAWAL INDUCES GERM CELL APOPTOSIS IN THE IMMATURE RAT TESTIS *IN VITRO*

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FSH is a key determinant of adult sperm output influencing both Sertoli and germ cell development. The aim of this study was to assess the impact of FSH action on Sertoli and germ cell proliferation and survival *in vitro*, and to identify FSH-regulated genes that may underpin these responses. Testis fragments from 17-day-old rats were cultured with recombinant human FSH for 2 or 24 h and then labelled with bromodeoxyuridine (BrdU) to identify proliferating cells. The testis fragments were then processed for analysis of cell numbers by stereology, BrdU incorporation by immunohistochemistry, and apoptosis by TUNEL. The TUNEL assay revealed that without FSH, spermatogonial apoptosis was induced to 195% and 179% ($P < 0.05$) compared to fragments with FSH after 2 and 24 h, respectively. No difference in apoptosis was observed in spermatocyte or Sertoli cell populations at these time points. No differences in Sertoli or germ cell proliferation were observed with or without FSH. To understand how FSH mediates spermatogonial apoptosis the response of 5 testicular genes of interest was examined. Expression of cyclin D2 (cell cycle, G_1-S), N-cadherin (N-Cad; adhesion molecule), Bax (pro-apoptotic), Bcl-w (anti-apoptotic), and stem cell factor (SCF; pro-apoptotic and other functions) was elevated to 151%, 348%, 209%, 258%, and 198%, respectively (all $P < 0.001$), in fragments cultured without FSH for 24 h, compared to fragments with FSH. No gene expression differences were observed at 2 h, except for SCF, which was elevated to 135% ($P < 0.01$). In conclusion, these studies have examined apoptosis and proliferation activities simultaneously in testis fragments *in vitro*, and demonstrated that FSH withdrawal induces both spermatogonial apoptosis and expression of testicular genes known to be involved in cell survival. This model will now be used to further investigate FSH-mediation of Sertoli and germ cell development.

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