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GnRH Expressions during Fetal Ovarian Development in Mouse

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GnRH has been known to be produced in ovary as well as hypothalamus and could exert autocrine and/or paracrine regulations on ovarian function. However, its local expressions and exact roles on fetal ovary are still unknown. To identify its expressions in ovarian development of fetus, we carried out RT-PCR with the RNA samples from the fetal ovaries dissected out microsurgically. Noon of the day on which the copulatory plug was detected was designated day 0.5 of gestation. And to exactly determine the sex of the fetus harvested during the different gonadal stages, genomic DNA PCR was also performed with specific primers for sexing. The expression of GnRH in 12, 15, 18, 20 g.d. are investigated. The RT-PCR product (375bp) from the tissues of fetal ovaries was the same with that of hypothalamus. The expressions of GnRH in fetal ovary were gradually increased from 12 to 20 g.d. Interestingly, however, the GnRH expression in day 1-neonatal ovary was not detected. Therefore, it is suggested that the endogeneous GnRH synthesized in fetal ovary could play a role in gonadal development. (BSRI-4437)

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Evaluation of Apoptosis on Granulosa Cells in Porcine Atretic Follicles

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Ovarian follicular atresia in mammals is a widespread degenerative process by which the follicles lose their integrity and the major portion of oocytes is lost. Recently, this enigmatic process of cell death has been regarded as apoptosis in several mammalian species. However, it was not evaluated yet obviously whether granulosa cell death in porcine atretic follicles is apoptosis. To identify the apoptosis in porcine granulosa cells, we carried out DNA fragmentation assay, in situ localization of apoptotic cells and endonuclease assays. Cellular DNA samples prepared from granulosa cells of follicles were labeled on the 3'-ends and resolved by gel electrophoresis. Internucleosomal cleavage of DNA was readily apparent in granulosa cells collected from atretic follicles, whereas only intact high mw DNA was present in healthy follicles. Quantitative analysis of the amount of radioactivity incorporated into low mw DNA fragments indicated a 5- to 6-fold increases in samples prepared from granulosa cells of atretic compared to healthy follicles. Apoptotic cells were widely localized specifically on the granulosa cell layers in atretic follicles, whereas there were scattered weak signals detected on the cells in healthy follicles. Endonuclease activities in granulosa cells were mainly Ca^{2+} -Mg^{2+}dependent rather than Ca^{2+} or Mg^{2+} dependent. The Ca^{2+}-Mg^{2+}dependent endonuclease activity was also significantly higher in the cells of atretic follicles than that of healthy follicles. We concluded that the granulosa cell death in porcine atretic follicles could be evidently defined as apoptosis. (KOSEF-HRC-41)