HISTOLOGICAL EVALUATION AND IN SITU LOCALIZATION OF APOPTOSIS IN FRESH AND CRYOPRESERVED OVARIAN TISSUE
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Objective: Although apoptosis has significant impact on ovarian homeostasis and reproductive function, little is known about 1) the dynamics of this process; 2) the feasibility of using combined morphology and terminal deoxynucleotidyl transfrase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) for apoptosis detection and 3) the impact of cryopreservation on this process. We conducted this investigation to study these issues, using a porcine animal model.

Design: Eight non-pregnant, adult, female farm pigs were utilized for this study. The bilateral oophorectomy was performed at the Cleveland Clinic Foundation Biological Resources Unit in accordance with their standard operating procedures.

Materials/Methods: The ovarian tissues were divided into two parts; one part was immediately fixed while the other was cryopreserved using standard protocol for ovarian cortical strips. The cryopreserved specimens were subsequently thawed and then fixed in Bouin solution. All the specimens were sectioned, fixed and stained with H&E. Ovarian follicles were histologically categorized as healthy and atretic and evaluated for the presence of apoptosis. In situ examination of apoptosis was performed using TUNEL assay.

Results: 1) Apoptosis was found in the atretic, but not in the healthy follicles; 2) the nuclei of the granulosa cells, but not those of theca or stromal cells, were TUNEL positive; 3) some cells with histological features of necrosis and apoptosis were TUNEL negative, and 4) the distribution of apoptosis was not different between cryopreserved tissue and freshly fixed tissue.

Conclusions: 1) The presence of apoptosis in the atretic follicles suggests its involvement in follicular atresia; 2) necrosis may represent alternative pathway for granulosa cell death, and 3) combined histology and TUNEL assay is useful method in detecting apoptosis, and finally 4) cryopreservation does not affect the pattern of apoptosis in ovarian tissue.

Supported by: None