

(myoma, endometrial abnormalities) did not influence on the incidence of vaginal bleeding.

Conclusions: The incidence of vaginal bleeding was 27.1% and the ovarian involvement of endometriosis might influence on the incidence of vaginal bleeding during postoperative GnRH agonist and add-back therapy with tibolone.

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Cryoprotection of VEGF is Mediated by Antiapoptotic Effect in Rat Granulosa Cells

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Background & Objectives: Vascular endothelial growth factor (VEGF) has been known as a cytoprotective agent in hematopoietic cells, neurons, myocytes, which protects these cells from apoptosis. And recently, VEGF also has shown to have cytoprotective effect in granulosa cells, which are vulnerable to apoptosis. Although the mechanism of cell death during cryopreservation is incompletely understood, apoptosis through caspase activation was suggested as a possible mechanism of cell death in cryopreserved cord blood hematopoietic cells and hepatocytes. This study was conducted to evaluate the involvement of apoptosis in the freezing and thawing process and the possible protective effect of VEGF in rat ovarian granulosa cells.

Method: Granulosa cells were obtained from PMSG stimulated Sprague Dawley rats by needle puncture under microscope. Granulosa cells were cultured in DMEM/12 medium with or without VEGF 50 ng/ml for 24 hours. Then the granulosa cells were frozen and thawed, and finally cultured for an additional 24 hours. Cell viability was determined using Trypan blue exclusion test and Annexin-V/ propidium iodide (PI) staining was performed for each step to distinguish viable, early, and late apoptotic, and necrotic cells. DNA degradation was evaluated by PI/RNase staining. Apoptotic cell death was confirmed by caspase-3 colorimetric assay. For the evaluation of dose-dependency, different doses of VEGF treatment (25, 50, 100, 200 ng/ml) were also applied. Statistical analysis was done using two-way ANOVA and Bonferroni multiple comparison test.

Results: After freezing-thawing process with additional 24-hour culture, VEGF treated group showed significantly higher number of viable granulosa cells ($p=0.0036$) and a significant decrease of the percentage of cells with degraded DNA (subdiploid DNA content) ($p=0.001$) than untreated group. VEGF treatment reduced late apoptosis induced by freezing and thawing process ($p=0.008$) without any effect to early apoptosis rate, suggesting that VEGF might delay apoptosis progression. Confirming this effect of VEGF, the expression of caspase-3 was significantly decreased in VEGF treated group ($p=0.0078$). Furthermore, VEGF treatment reduced early apoptosis during subsequent 24-hour culture after thawing ($p=0.0001$) resulting in higher rate of viable granulosa cells after cryopreservation ($p=0.001$). Among the different doses of VEGF treatment, 50 ng/ml of VEGF showed the highest protective effect against the

freezing and thawing induced damage.

Conclusions: Granulosa cell damage induced by the freezing and thawing process is mediated, at least in part, by the apoptotic process. Our preliminary results suggest that VEGF treatment before cryopreservation decreases the granulosa cell damage in rats, which is mediated by the antiapoptotic effect of VEGF.

P-29 인간 배아줄기세포의 분화과정에서 미토콘드리아의 변화에 대한 연구

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Background & Objectives: 미토콘드리아는 ATP를 생성하는 세포내 소기관으로 생리학적 기능과 특성에 따라 다양한 형태학적인 변화를 나타낸다. 본 연구에서는 인간 배아줄기세포에서의 분화과정에서 관찰되는 미토콘드리아 미세구조의 변화를 투과전자현미경을 이용하여 관찰하였으며, 미토콘드리아의 증식 및 분화와 관련이 있는 mitochondrial transcription factor A (mTFA)의 발현양상을 real time RT-PCR 방법으로 분석하였다.

Method: 미토콘드리아의 미세구조관찰을 위해 미분화 및 분화과정의 배아줄기세포를 glutaraldehyde로 전고정한 후 osmium tetroxide로 후고정하여 Epon으로 포매하였다. 초박편절단기로 thin section한 후 uranyl acetate와 lead citrate로 이중 염색한 후 관찰하였다. mTFA의 발현양상을 살펴보기 위해 인간 배아줄기세포주를 MEF feeder layer와 공배양하여 미분화 colony, 8일간 부유배양한 배아체, DMEM/F12 + 10% FBS에서 분화시킨 세포로부터 RNA를 추출하여 RT-PCR으로 cDNA를 합성한 후 real-time PCR을 수행하였다.

Results: 미분화 상태의 배아줄기세포는 그 크기가 작고 구형이며 cristae와 matrix가 발달하지 않은 원시형태의 미토콘드리아를 가지고 있음이 관찰되었다. 그러나 분화과정에서 미토콘드리아는 그 길이가 길어지고 cristae가 발달되었으며 matrix에서도 전자밀도가 높아짐을 관찰할 수 있었다. 또한 분화가 진행됨에 따라 mTFA의 발현양이 미분화 줄기세포에 비해 통계적으로 유의하게 증가하는 것을 확인할 수 있었다 ($p < 0.05$).

Conclusions: 인간 배아줄기세포의 분화과정에서 미토콘드리아의 분화와 증식이 수반되며, 이들의 변화가 특정 세포로의 유도분화 과정에서 중요한 역할을 수행하는 것으로 생각된다.