重複性卵巢超排卵刺激引發老鼠粒線體 DNA 突變及氧化性傷害

Repeated Ovarian Superovulations Induce Mouse Mitochondrial DNA Mutations and Oxidative Damages

中文摘要

利用促性腺刺激素(gonadotropin)誘導超排卵(superovulation)，是目前人工生殖技術中很重要的治療方式。超排卵是以荷爾蒙刺激卵巢，使大量的濾泡細胞生長並發育到 metaphase Ⅱ。臨床上即是利用這樣超排卵刺激下所取得的成熟卵進行人工受精(IVF)。於哺乳類中，發現以標準劑量單次刺激超排卵降低受孕力及胚胎植入前和植入後存活率。若以高劑量刺激，則非整倍數卵(oocyte anueploidy)、胚胎死亡率(embryo mortality)、胎兒發育遲緩障礙(fetal growth retardation)和先天性異常(congenital abnormalities)的機率升高。本論文將針對重複性刺激卵巢超排卵造成的卵細胞缺陷或對卵巢傷害性的成因及機轉加以探討。因此我們利用 gonadotropin 給予老鼠一到六週次重複性的刺激，針對卵細胞的成熟及品質及卵巢或其他器官(主要是肝臟組織)的細胞及組織中是否有氧化性傷害(oxidative damages)的堆積。結果發現於多週次刺激組的老鼠卵巢產生嚴重充血並水腫，且排卵數量及卵細胞品質降低。而多週次刺激組的胚胎中 apoptosis 的數目增加，且胚胎的發育能力(即胚胎於體外發育至囊胚期)降低，細胞質內粒線體凝集現象，此爲老化的卵細胞的特性。此外，我們檢測卵巢和肝臟組織中 lipoperoxides，8-OH-dG 與 carbonyl proteins 的含量。實驗發現三者含量隨刺激週次的增加而顯著性增加。此外，我們更進一步分析粒線體 DNA 突變的產生，發現有 675bp 粒線體 DNA 突變片段。並且，隨刺激週次的增加其含量有增加的趨勢。這顯示了多週次刺激對老鼠產生組織的破壞，並且影響胚胎與卵細胞的品質。因此，對於多週次的超排卵治療造成卵細胞老化或缺陷的形成因素及引發傷害的機轉的研究更顯爲急迫。藉此尋找更適當的治療方式，使不孕症的療程能有更佳的療效。

英文摘要

Superovulation by injection of exogenous gonadotropin is still the fundamental method to produce in vivo derived mature oocyte for in vitro fertilization therapy. In previous reports, declined fertility and pre-and post-implantation mortality have been shown after one ovarian stimulation cycle by using standard doses of gonadotropin. Furthermore, increased frequencies of anomalies such as oocyte aneuploidy, embryo mortality, congenital abnormalities and fetal growth retardation have been reported. In this study, we want to address which factors contributing to adverse oocyte competence or ovarian function and capacity. We also examined oxidative damages that repeated ovarian superovulation induces in mouse ovary and proposed the molecular mechanisms. We stimulate the mouse with one to six cycles by

gonadotropin. After stimulation, sever edema and hyperemia was observed in the mouse ovaries with increased cycle numbers of stimulations. Upsurge embryo degeneration and reduced developmental competence of the stimulated mouse embryo had found. The collected embryos failed to growth into blastocyst stage by three to six cycle stimulations. On the other hand, the numbers of ovulated oocytes were decreased in the groups with ovarian multiple stimulation. More aggregated mitochondria were found in the cytoplasm of the repetitively stimulated oocytes. Furthermore, higher amount of oxidative damages including 8-OH-dG, lipoperoxide contents (e.g. malondialdehyde), and carbonyl proteins were also revealed in the livers with more cycle numbers of stimulation. The higher proportions of mtDNA mutations were also found. The detected molecular size of mutated band was approximately about 675 bp. However, the classification of the relationship between oocyte competence and ovarian responses to stimulation in the mouse may provide insights into the origin of oocyte defects and the biology of ooplasmic ageing that could be of clinical relevance in the diagnosis and treatment of human infertility.