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• 中文摘要

卵細胞的功能缺陷，常成爲受孕力降低及女性不孕症的重要影響因素。因此，對於造成卵細胞老化或缺陷的形成因素的探討更顯爲急迫。在卵子生成(Oogenesis)及成熟過程(Maturation)，以及胚胎發育及分化過程，都須藉由粒線體所提供的能量(ATP)，才能夠順利完成各個成長過程。粒線體的功能會受基因異常、缺氧及氧化性壓迫而造成呼吸功能缺陷，並且直接減少 ATP 的合成。高反應性氧分子及自由基這會經由缺陷性的呼吸作用而產生，並且對細胞內的 DNA，RNA，蛋白質等產生廣泛性的氧化性破壞。目前，我們已經初步由榮民總醫院及國泰醫院收集了 24 例由接受試管嬰兒治療而未授精之卵細胞(Unfertilized oocytes 或 Degenerative oocytes)、各階段分裂停止之授精卵(如 1 cell, 2-cell 或 4-cell)，以及 12 例顆粒細胞的標本。並針對這些卵細胞的粒線體 DNA 的斷損突變及解毒酵素的基因突變(包括 Glutathione-S- transferase GSTM1, GSTT1, GSTP1)基因型的鑑定及表現量加以定量。實驗結果於無法授精的卵細胞及其顆粒細胞中出現 mtDNA 斷損片段約 3400bp，3940bp，4590bp，且同病患的 GSTM1 gene 爲 Null mutation。由結果顯示病患帶有 Null type mutation 的 GSTM1 gene，常伴隨有粒線體 DNA 的斷損突變。以 Primer-shift PCR 的方法分析斷損突變，其片段長度隨引子的設計作平移，目前我們正進行 DNA 定序分析。此外，本研究更進一步針對不孕症治療中進行超排卵步驟對卵巢及卵細胞及受精卵所產生的影響進行研究。以 C57BL/6J mice 注射 5 IU/mice 的 PMSG 及 HCG 分別注射同劑量，不同的注射週次(從 1 週次至 6 週次)，將取出之卵巢、排出之受精卵、輸卵管及子宮，分析因超排卵所引發之傷害，分析其粒線體 DNA 的斷損突變包括點突變及片段突變，並且以 RT-PCR 定量熱休克蛋白(Heat sock protein HSP70 及 HSP90) mRNA 的表現量。由結果顯示 C57BL/6J mice 隨著刺激週次的增加，其排卵率、受精率、胚胎分裂率皆呈現降低，胚胎死亡率增加。六週次的刺激超排卵遠較於一週次的刺激超排卵產生大量的 HSP70 mRNA 大量表現及 Mice mtDNA 片段突變(Length mutation)的增加大量表現。我們期望本計畫之研究成果能使我們對於粒線體基因突

變在卵細胞及卵丘細胞的老化及受孕力降低上所扮演的角色有進一步的瞭解。此外，藉此超排卵的研究能更進一步了解以賀爾蒙治療所引發的傷害，並且能尋找出更適當的治療方式，使不孕症的療程能有更進一步的療效。

- 英文摘要

Females of most mammalian species, including humans, experience reproductive declined with age. The fertilization ability and developmental competence of human embryos appear to be directly related to the metabolic capacity of mitochondria. Mitochondrial dysfunctions resulting from a variety of intrinsic and extrinsic influences, including genetic abnormalities, hypoxia and oxidative stress, can profoundly deplete the level of ATP generation in oocytes, which in turn may result in aberrant chromosome segregation or development arrest. Deletions and point mutations in oocyte mitochondrial DNA may subtend metabolic deficiencies or replication disorders in some infertile women and in women with advanced age. We hypothesized that some unfertilized oocytes and cleavage-arrest embryos from women undergoing IVF would contain the mtDNA mutations (deletions, point mutation, and depletion) and that oocytes and ovaries from older women, whose oocytes tend to have low development potential, would be more likely contain mtDNA mutations than oocytes from younger women. In this study, we collected 24 oocytes and embryos from women undergoing IVF program in the department of OB/GYN in Taipei-Veterans General Hospital and Cathy Hospital. The samples were collected due to unfertilization or cleavage-arrest in 1 cell, 2-cell, and 4-cell stage. By using the PCR and RT-PCR methods, we examined the genetic integrity of human degenerative oocytes and embryos. Large-scale deletions and point mutation of mtDNA and the mutations of detoxification enzymes including glutathione-S-transferase (GST M1, GSTP1). There are several types of deleted mtDNA were generated from the aged oocyes and cumulus cells. Three types of length mutation were generated from 3400bp, 3940bp, and 4590bp. The primer shift PCR was also proceeded on for excluding miss-annealing amplification. The one who carried the deleted mtDNA was examined with a null type of GSTM1. The present study aims to shed light of abnormal oocytes ovulated by aged females. In order to reach this goal, cellular and biochemical traits of ovulated from C57B/6J female mice retrieved after exogenous ovarian hyperstimulation for different cycles from one to six cycles. Decreased numbers of ovulated oocytes were collected from the treated mice with repeated ovarian stimulation for six cycles. Furthermore, the fertilization rate, cleavage rate, and development capacity were also decreased. The expression of heat shock protein HSP70 was dramatically increased in the overstimulated ovaries. Large scale deletions of mice mtDNA were also examined in the overstimulated ovaries. Taken together, the results will underlie the mutation of mtDNA and gene expression in mitochondria may play some roles in pathophysiology of oocyte senescence.