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• 英文關鍵字	Embryo；Polyamine；Implantation；Progesterone；Estrogen；Ornithine decarboxylase		
• 中文摘要	在哺乳動物細胞中含有三種多氨素:Putrescine、Spermidine 及 Spermine。在合成多氨素的過程中,ODC(Ornithine decarboxylase)爲起始及決定反應速度的酵素。DFMO(.alpha.-difluoro-methylornithine)爲 ODC 之不可逆性專一抑制劑。許多文獻已證實多氨素對細胞生長及分化扮演一重要角色,而早期未著床胚胎具快速分裂細胞群,因而多氨素也許在早期胚胎發育中也是一要角。目前多氨素對哺乳動物胚胎中生長之影響及其 ODC 基因表現仍未被清楚地探討,所以我們觀研在不同階段胚胎 ODC-mRNA 及 ODC 蛋白質含量的變化,還有多氨素對胚胎生長的可能影響。將 2-cell stage 的胚胎分別培養在 HTF 培養液或含 Putrescine、Spermidine、Spermine、MGBG、DFMO 或 DFMO 加 Putrescine 的培養液,結果抑制劑均無抑制的現象。ODC 蛋白質在 2-cell stage 就可以免疫染色法測得,顯示雖然 ODC 在胚胎早期就已表現了,但是 DFMO 卻不能抑制胚胎生長,可能因爲胚胎內已含有大量的 Putrescine 和其他多氨素,足夠以維持到 Blastocyst 的發育而不至於受抑制劑的影響。當以 RT-PCR 的方法來探測著床前胚胎內 ODC mRNA 的量,以目前的 One step PCR 方法,只能明顯偵測到 Blastocyst 期胚胎內 ODC-mRNA 的存在。 另外 Progestertone 與 Estrogen 對胚胎之著床有重要的影響。其主要作用點在子宮內膜細胞的增生肥厚。至於其對未著床的早期胚胎的影響則鮮少爲人所知。因此除了 ODC 之外,Progesterone 及 Estradiol 之受體是否存在未著床的胚胎內(作爲其是否會受此兩荷爾蒙調控的指標)也涵蓋在本研究之範圍內。經過多種不同方法的測試解決了高背景染色的困擾,終於建立了一個有效的免疫染色方法,來測定胚胎在不同發育時期其 ODC、Estradiol 和 Progesterone 的受體,以及 PCAN (分裂細胞特有胞核抗體)的存在。研究結果發現在培養皿內的不同發育期的胚胎,由 2-cell...到 Blastrula stage 皆有 ODC 與 PCNA 的存在而且似乎愈長愈強。相反的,Estradiol 及 Progestereone 的受體卻偵測不到。原本要以 Estradiol 及 Progesterone 來調節胚胎內 ODC,PCAN 等分子表現的實驗因無受體之存在而取消。		

- 英文摘要

In mammalian cells, there are three kinds of polyamine: Putrescine, spermidine and spermine. Ornithine decarboxylase (ODC) is the first and the rate limiting enzyme for polyamine synthesis. .alpha.-Difluoromethyl ornithine (DFMO) is a site specific and irreversible inhibitor of ODC. Data from numerous references show that polyamines are essential for cell growth, proliferation and differentiation. Early preimplanted embryos containing fast dividing cells. Polyamines may also play a role in the development of early embryos. The functional role(s) of polyamines and the expression of ODC gene during early development of the mammalian embryos are not well understood, efforts were made to study the expression of ODC-mRNA and ODC protein as well as to study the effect of polyamines on the embryo at different stages of development. On the other hand, progesterone and estradiol are important in preparing endometrium for embryo implantation. The question that if both hormones also play roles in the development of preimplanting embryos was asked. The initial effort was to examine whether progesterone- and estradiol-receptors were existed in the preimplanting embryo. Furthermore, efforts were also made to probe the possibility whether PCNA (proliferating cell nuclear antigen) might be used as marker for identifying healthy blastomeres. The data showed that DFMO did not appear to affect the growth of embryos. On the other hand, ODC protein molecules were detected in the 2-cell stage embryo as revealed by immunocytochemical study. This suggests that ODC was expressed during early embryo development prior to implantation although DFMO failed to suppress embryo development. It is thus speculated that there is abundant putrescine to support early embryo development without de novo synthesis. After elaborate efforts to overcome various difficulties, an immunocytochemical staining method was established for the detection of ODC, progesterone and estradiol receptors as well as PCNA molecules in the 2-cell, 4-cell, 8-cell, morula and blastrula stages of the preimplanting embryos. The data showed that both ODC and PCNA molecules did exist in the early preimplanting embryos. It appears that the intensity of immuno-staining increased as normal embryos developed. On the other hand, both progesterone and estradiol-receptors were not detected during all stages of preimplanting embryo development. Therefore, the study on the effects of these two hormones on ODC expression during the early embryo development was aborted. Nevertheless, the presence of ODC and PCNA molecules may remain to be useful markers for the vitality of a given embryos.