探討人類卵巢濾泡液對鼠胚胎發育及著床能力之影響

Study on the Promoting Factors for Mouse Embryo Development and Implantation in Human Follicular Fluid

中文摘要

以試管嬰兒(in vitro fertilization; IVF)的技術來幫助不孕症夫婦孕育下一代是目前很重要的治療方式。但目前胚胎植入後之著床率只有 15%~30%左右，還是低於一般正常夫婦，其主因被認爲與胚胎的發育及著床有關，也使得對胚胎發育及著床調控的了解成爲刻不容緩之課題。當以試管嬰兒技術來培養胚胎時，是否由於缺少濾泡液之參與，才造成囊胚之發育率及著床懷孕率一直無法提高，此乃是目前亟待釐清的問題。各種研究顯示濾泡液內之各種成分可促使卵成熟、促使濾泡細胞之增殖（proliferation）與分化（differentiation）、吸引精子，促進卵與精子的受精能力，甚或提高懷孕率。因此本論文的主要主題是探討並尋找人類濾泡液中是否有可以促進胚胎發育及著床因子之存在，並對於其作用機轉加以研究。本研究將濾泡液作不同方式加熱處理後，分別與 2 細胞(two cells)期鼠胚(ICR mouse embryos)進行培養，發現皆有促進胚胎發育之潛能。其中以 100℃、30 分鐘處理之濾泡液組(heated human follicular fluid, hFF100)，其增加胚胎發育至囊胚(blastocyst)的比例爲對照組的 1.18 倍，具有顯著性差異($p<0.05$) 。而囊胚孵化(hatching)則爲對照組的 1.38 倍，具有顯著性差異($p<0.05$) 。進一步以 Matrigel 進行著床(implantation)評估，亦有 增加著床之能力，爲對照組之 1.03 倍，但經統計運算後則無明顯差異($p>0.05$)。將 hFF100 以單向電泳 SDS-PAGE 分析則發現有 3 個主要蛋白質，其分子量約爲 53kDa、40kDa 及 21kDa。當以 JC-1 螢光染色探討各胚胎期之粒線體功能時,發現經 10%hFF100 培養鼠胚後,比對照組有表現較高的粒線體膜電位(mitochondrial membrane potential, $\triangle\Psi$)，顯現出其粒線體功能較活躍，而且對外加 100 M 之 H_2O_2 處理也較有保護效果。我們在脂質過氧化物(lipid peroxide)螢光染色的實驗中也發現，在 10%hFF100 培養下之鼠胚比對照組產生的量少，因此證實 hFF100 濾泡液具有保護胚胎降低 ROS 傷害的機制。此外，我們更進一步利用人類胎盤滋養層細胞(trophoblast cells)的培養來確認 hFF100 對胎盤細胞的影響。當胎盤滋養層細胞與 10% hFF100 培養 48 小時後，其 ATP 產量較對照組增加 0.13 倍，而且於著床時入侵(invasion)所需因子 matrix metalloproteinase-2 (MMP-2)、matrix metalloproteinase-9 (MMP-9)及 tissue-inhibitors metalloproteinase-1 (TIMP-1)之 mRNA 表現亦呈增加之現象，在著床黏附因子 integrin -5 及血管新生因子 vascular endothelial growth factor (VEGF)之 mRNA 也有明顯增加，因此可進一步證實 hFF100 應可促進胚胎表現與著床相關之因子進而促進其著床。綜合以上實驗結果，我們認爲經過 100℃、30 分鐘處理的人類濾泡液，確實可增加著床前胚胎之發育，並可保護胚胎減少受 ROS 之傷害，以及加強粒線體功能，使得胚胎能順利發育到囊胚，進而孵化

以及著床，因此應可進一步利用在人類胚胎之培養上,增加胚胎發育至囊胚之數目,進而提高著床率及懷孕率，造福罹患不孕症之夫婦。

英文摘要

In vitro fertilization (IVF) techniques is the very important treatment that help infertile couples breeding next generation. The implantation rate of embryo in human IVF and embryo transfer treatment has been reported to approximately around 15 to 30%, which is still lower than in normal fertile couples. The main reason considered about the low pregnancy rate and blastocyst development rate in IVF treatment course could possibly be due to pre-implantational embryo culture without follicular fluid in vitro. Follicular fluid is known to promote oocyte maturation, proliferation and differentiation of follicular cells and affect sperm capacitation, acrosome reaction, sperm quality, sperm-oocyte fusion, and can even increase the pregnancy rate. In this study, we searched for the factors that could promote embryo development and implantation in human follicular fluid and elucidate the regulation mechansim. After processing the human follicular fluid by heating at different temperature at 56℃ (hFF56) and 100℃ (hFF100) for 30 min, we examined the blastocyst development rate, hatching rate, and implantation rate by 10% (v/v) of both types of follicular fluids supplementation with two-cell embryo of ICR mouse. 1.18 fold increased of blastocyst development rate ($p<0.05$) and 1.38 fold increased of hatching rate ($p<0.05$) were found in the hFF100 treated embryos. The implantation rate was found to be 1.03 fold increased compared with control group by using Matrigel invasion assay, but no significantly difference between two groups ($p>0.05$). There are three different bands in the hFF100 fraction after SDS-PAGE electrophoresis, approximate molecular weights of 53kDa, 40kDa and 21kDa, respectively. Higher mitochondrial membrane potential (△Ψ) was detected in the hFF100-treated embryos by JC-1 staining. We also found the lower lipid peroxides contents of hFF100 pre-treated embryos followed 100 M hydrogen peroxide treatment. The more active mitochondria and protection from ROS damages were addressed in the hFF100 treated embryos. Furthermore, we also used the trophoblast cell as the cell culture model to identify the effects of the hFF100 on embryo implantation. After 48 hours cultured with the 10 %(v/v) hFF100, the ATP production increased 0.13 fold compared with control. Increased mRNA expressions of the factors involved in embryo invasion and implantation were detected including MMP-2, MMP-9, TIMP-1, integrin -5, and VEGF. According to our findings, we suggested that the hFF100 treatment could improve embryo development potential and implantation capability.In conclusion, heated human follicular fluid to 100℃ for 30 min could definitely increase the developmental potential of mouse pre-implatational embryos and

increase the protective effect against ROS damage as well as enhance mitochondria function. These effects of hFF100 could aid the pre-implantation embryo more efficiency to develop to blastocyst, hatching and even implantation. These finding could support that follicular fluid supplementation in the human IVF culture to improve the blastocyst formation and finally enhance the implantation and pregnancy rate of infertile couples to bear their own baby.