行政院國家科學委員會專題研究計畫成果報告

男性不孕症的基因診斷
Genetic Diagnosis in Male Infertility

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一、中文摘要

自從單一精子注射應用在人類的生殖科技，男性不孕症的遺傳診斷相形重要，如何能解決不孕，而且能避免孕育出不健康的下一代，除了發展生殖科技能得到更好的結果，如利用體外培養 spermatocyte 以完了成數分裂 診斷出遺傳問題避孕遺傳到下一代更是重要。

目前因染色體不正常而造成的男性不孕症，已可藉胚胎著床前的診斷來挑選正常的胚胎來殖入，然而對於精子生成中的遺傳因子之缺損卻無法診斷與避免，本研究即著重在染色體 Yq11 的 micro-deletion 的診斷與臨症狀的表現與基因表現來探討。

關鍵詞：男性不孕症、Yq11 區域

Abstract

Intracytoplasmic sperm injection procedure is a mile stone for treatment of severe male infertility patients. This procedure was first applied in severe oligoospermic patients. However, now spermatid injection was also applied successfully in the animal model. Recently applying spermatocyte with in vitro maturation, fertilization, blastocyst formation and also pregnancy was achieved in mice. Setting a new treatment regimen in male infertility in human is on the way. However, arrest of spermatogenesis and spermiogenesis in some patients is related to some gene derangement. It is very important to detect, differentiate and correlate the clinical phenotype with the genes that were involved in the process of the spermatogenesis. In this project we designed 12 sets of PCR primers in 2 reaction. We try to detect the Yq11 microdeletion. RT-PCR and m-RNA in situ also was developed to detect at which stage the arrest of spermatogenesis happened. We try to correlate the clinical phenotype, gene expression and gene derangement.

Keywords: Male Infertility, Basic Protein Y11 gene

二、緣由與目的

由於單一精子注射在 1994 年正式被廣於的使用在男性不孕症的病人，為治療男性不孕症開創了一個新紀元，近來利用 spermatid 行單一精子注射在動物實驗上已相當成功，而利用 spermatocyte 精母細胞以 GV 或 MI 的卵子行單一精子注射，而利用卵子的成熟過程來催化精母細胞的減數分裂已成功的應用在老鼠的身上，所以在治療男性不孕症的方法更為多樣且有效。

然而在診斷上也由於治療的方法更為積極後，也相形重要，要知道若有重大遺傳問題，而只有藉助治療，只是將更多的遺傳疾病，傳遞到下一代，所以本篇研究，其目的在於診斷，藉而找出找出更多的男性不孕症的遺傳因子，可供臨床上辨別是否要進入生殖科技的治療程序。

三、結果與討論

We have included 113 oligozospermia patients in this project. All patients had blood

chromosome karyotyping and Yq11 microdeletion detection. There are 17 in 74 azoospermic patients with chromosome anomaly. In these 17 patients, 10 patients are Klinefelter's syndrome. The other 7 patients are sex chromosome anomaly. In the 39 severe oligoospermic patients. Only 2 patients had chromosome anormaly. One had marker chromosome which turned out to be chromosome 15 inversion duplication from FISH study. The other one is mosaic trisomy chromsome 18. These patient was scheduled and went through IVF + ICSI + PGD. Pregnancy was achieved and amniocenetesis was done with a normal female baby. About the Yq11 microdeletion, there are 7 patients with big deletion in the 74 azoospermic patients. However, there are 6 patients who had microdeletion detected. There is no difference in the deletion size between the azo and oligoospermic patients.

About the study of gene expression, we had 2 testis specific expression genes from other research group. Tissue was fixed in DEPC treated paraformaldhyde. This part is still on going. From the study of spermatid and sperm detection in the testis section. We have done for 37 non-obstructive azoospermia patients. There are 5 patients who have sperm detected. As the spermatid detection, there are 13 patients who had spermatid detected from the FISH study. If correlated to the pathology report, these 5 patients who had sperm detected in the FISH section had the spermatid report only. From this part we had poster published in the ASRM meeting in Oct. '99 and was selected as prize poster.

四、計劃成果自評

Since powerful ICSI was applied in assisted reproduction technology. Genetic study for the male infertility patients attracts more and more attention from the geneticist. Chromosome anomaly is one of the genetic derangement for these patients. However, chromosome Yq11 microdeletion is also another focus for this disease. In this project we tried to correlate the clinical phenotype and gene derangement. We have collected 127 and 37 oligozospermic patients' DNA and testis biopsy sample. We have done the first part study for Yq11 microdeletion and haploid cell detection in the testis biopsy by applying FISH.

We had poster presentation in ASRM this year in USA to present the data of our part one of this study. We were selected as prize poster.