國科會專題計劃成果報告

計劃名稱

中文：早期人類胚胎於體外培養系統中染色體的變化

英文：The Fate of Chromosomal Mosacism in Early Haman Embryos Grown in in Vitro Culture System.

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中文摘要

許多受精卵的染色體是不正常的，而這些懷孕的結果常導致流產，臨床上治療不孕症病患，常發現高齡婦女做體外授精，胚胎植入時，其成功率比年輕之女性低，是否其胚胎中的染色體組成有較高的異常率，造成著床失敗。本研究運用螢光原位雜交方法，對體外授精並培養三天的胚胎，取出其分裂球，做染色體 13,18,21,X,Y **的分析，並將病人的年齡層分為二組，年輕之一組為** 25 **至** 34 **歲病人的胚胎，高齡之一組為** 35 **至** 44 **歲病人的胚胎，結果發現後者(年齡層較高病人的胚胎，結果發現後者(年齡層較高者)的染色體異常，包括單套染色體，多套染色體及鑲嵌型的比例，均明顯高於前者(年齡層較輕者)，由此顯示，高齡婦女(≧35 歲)婦女於體外授精，胚胎植入術中，其著床率及懷孕成功率低於年齡層較輕者(25 至 34 歲)，可能和高齡者的胚胎染色體異常率較高有關。**

關鍵詞：人類早期胚胎，體外培養系統，螢光原位雜交，染色體異常

Abstract

Many conceptions are thought to be chromosomally abnormal and often these pregnancies end in miscarriage. Current advancement in assisted reproductive technologies has made possible culture of human embryos in vitro. Therefore, it is now possible to examine the chromosomes of early human preimplantation embryos. However, it is difficult to obtain metaphase chromosomes for such embryos, so conventional karyotyping does not yield very useful information. Fluorescence in situ hybridization (FISH) has enabled us to examine the chromosomes of tissues where metaphase spreads cannot be obtained, such as embryos or cancer tissue (Harper et al. 1995).

Haper have used this technique to examine early human embryos and found that four groups of chromosome patterns are present: normal embryos (uniformly diploid), abnormal embryos (uniformly abnormal, uch as Downs, Turners, etc), mosaic embryos (two cell lines present, such as XX and XO) and chaotic embryos (all nuclei show different chromosome complements).

Recently, the technique of embryo biopsy has become mature with some application of pre-implantation diagnosis to human reproduction (Handyside and Delhanty, 1977). According to Haper and Delhanty (1977), examination of at least two blastomeres for early human embryos can minimize the chance of misdiagnosis due to chromosomal mosacism.

In this study, fluorescence in situ hybridization analysis of early human embryos using simutaneously probe for chromosome X, Y, 13,18 and 21 was done.

Forty-seven cleavage-stage human embryos obtained by IVF were analyzed by fluorescence in situ hybridization. The embryos also were analyzed by two different age group (25 to 34 Y/O and 35 to 44 Y/O).

The results showed that in the 35 to 44 Y/O group, aneuploidy was significantly increased when compared with the young age group. It is suggested that implantation failure in older women could be due to aneuploidy.

Keywords: Fluorescence in situ hybridization; Human embryos biopsy, chromosome anomalies.

MATERIALS AND METHODS

24 embryos from 25 to 34 Y/O group and 23 embryos from 35 to 44 Y/O group were studied by fluorescence in situ hybridization. All the embryos were donated by the patient and consent form was written. The embryos were produced by in vitro fertilization and all theembryos were the excess embryos that were not used after embryo transfer. Embryo biopsy was performed at the eight cell stage and two to three blastomeres were aspirated gently using micro- manipulation instrument. Blastomeres were fixed on glass slide, dehydrated and stored at -20℃ until analysis. All fixed embryos were analyzed by FISH using simultaneously X, Y, 18, 13 and 21 chromosome-specific probes following Munne et al. The incidence of trisomy and monosomy 13 and 21 were calculated as the number of affected embryos divided by

the number of embryos analyzed with the 13/21 probe.

RESULTS

Aneuploidy was found to increase with maternal age from 6.7% in embryos of women 25 to 34 years old to 18.2% in women 35 to 44 years old ($P<0.01$). The increase in neuploldy mostly was due to aneuploidy of chromosome 21. This study revealed that aneuploidy is the chief abnormality in IVF patient when maternal age is over 35 years old. This is because the oocytes of old women are more prone to nondisjunction caused by meiotic errors. A rate of 18.2% aneuploidy for five chromosomes may seem to be high, but many aneuploid embryos must be eliminated before a clinical pregnancy is recognized.

DICUSSION and SELF-EVALUATION

In this study, we get the experience of in vitro culture of human embryos from fertilized egg to 4 cells stage in human tubular fluid medium and culture of 4-8 cells stage to morula-blastocyte stage in blastocyst medium. The techniques of embryo biopsy at eight cells stage were also practiced, firstly using mice embryo, then human embryos. Fluorescence in situ hybridization technique for blastomeres to detect aneuploidy of chromosome 13, 18, 21, X, Y were performed to get the preliminary result that older women have more high aneuploidy rate than younger women. Whole morula and blastocyte for fluorescence in situ hybridization were practiced and the preliminary result revaled nearly 25% mosacism rate. This phenomenon need fur ther studied to see the mosacism is chiefly located in the traphectoderm layer. And inner cells for growing to fetus are less suffered from mosacism.

REFERENCE

1.Haper, J.C., Coonen, E. (1994). Identifica tion of the sex of human preimplantat ion embryos using fluorescence in situ h ybridization. Hum Reprod 9, 721-4.

2.Hopman, A.H.N., Ramaekers. (1988). In situ hybridization as a tool to study num erical chromosome aberrations. Histochem istry, 89:307-16.

3.Munne, S., Alikan; M. (1995) Embryo morphology, developmental rate and mate -rnal age are corrected with chromosomal abnormalities. Fertil Steril, 64:382-91.