行政院國家科學委員會補助專題研究計畫成果報告

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著床前遺傳診斷之研究

The research of preimplantation genetic diagnosis

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計畫類別：個別型計畫

計畫編號：NSC 89-2314-B-002-201

執行期間： 88 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：陳思原醫師

共同主持人：楊友仕教授

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
- □赴大陸地區出差或研習心得報告一份
- □出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

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一、中文摘要

著床前遺傳診斷可能讓我們在胚胎植入前，診斷一些遺傳疾病，而讓孕婦避免懷有不正常的胎兒。於6至12細胞期之胚胎做胚葉細胞切片是目前最常用的方法，然其傷害性及操作困難之問題仍然存在，因此如何提高其成功率，值得進一步的研究。最近非接觸半導體雷射被用來協助透明層的切割，可能提高取得細胞的效率。本研究為針對胚葉細胞切片的方法，比較半導體雷射和傳統用酸透明層鑽洞於胚葉細胞切片的效率及效果，並以無鈣鎂離子溶液來減少細胞之緊密結合。結果發現前者所需要的時間明顯的減短，取得完整細胞比率可達百分之百，切片後胚胎回到培養液可正常發育，且其發生完全孵化的比率比未做切片的對照組之胚胎明顯的高，可能是因為透明層打洞有協助孵化的作用，取得之細胞適合做分子生物學之診斷。因此雷射透明層切割有助於胚葉細胞切片的操作，其臨床價值值得做進一步的研究。

關鍵詞：著床前遺傳診斷，胚葉細胞，半導體雷射。

Abstract

The preimplantation genetic diagnosis (PGD) may detect a genetic disease prior to conception and allow a selective transfer of normal embryos to prevent carrying an abnormal fetus. Blastomere biopsy at the 6- to 12-cell stage is the most commonly used. However, the technique difficulty and the cell injury are still the problems. Recently, a non-contact diode laser system has been developed to cut the zona. This work is attempted to improve the efficiency and efficacy of the biopsy method. The embryos were pretreated with calcium-magnesium free medium. The blastomere was biopsied with the aid of laser for cutting of zona, in comparison with the acid-drilling method. The time for each biopsy was significantly shorter for the laser method than for the acid method. The success rate for obtaining an intact blastomere was 100% for the two methods. The growth of embryos was not affected after returning to the culture medium, compared to embryos without biopsy. The opening in the zona helped the blastocyst to escape, representing an

effect of assisted hatching. The intact blastomere retrieved is appropriate for use in molecular diagnosis. Thus, the laser-assisted technique is a convenient alternative for blastomere biopsy that may merit use in clinical practice and research.

Keywords : preimplantation genetic diagnosis, blastomere, diode laser.

Introduction and purpose

Preimplantation genetic diagnosis (PGD) has been introduced to the human application for prevention of hereditary diseases in the patients receiving IVF treatments since 1990 (1). Compared with conventional methods of prenatal diagnosis (amniocentesis or chorionic villi sampling), PGD has the advantages of detection of a genetic disease before conception and selective transfer of normal embryos to prevent the necessity and risk of artificial abortion when carrying an abnormal fetus. However, the complexity of procedures in PGD and the stringent requirement of molecular diagnosis for a single cell are its disadvantages (2).

The most common method used for PGD is blastomere biopsy. Regarding the methods for blastomere biopsy, the three-pipette technique, one pipette for holding the embryo, the other for drilling the zona using acidic Tyrode's solution, and another for aspirating the blastomere, is most commonly used in centers for PGD (1). However, that takes much time and labor to perform the procedures. Preserving the two-micromanipulator system, we simplified the conventional three-pipette technique to a two-pipette technique as an alternative method for blastomere biopsy in the mouse and human model (3,4). Recently, a non-contact laser system has been developed for zona cutting (5). The preliminary result from this report seems encouraging and merits further investigation for the clinical application.

The failure of biopsy may be due to a tight junction of blastomeres. The value of calcium-magnesium free medium to lessen a tight junction and avoid a tear of the cytoplasmic membrane deserves further research. Fluorescence in-situ hybridization (FISH) is mainly used in the diagnosis at the chromosomal level (6). It has been successfully used in the diagnosis of sex of embryos to prevent X-linked recessive disease by transferring female embryos. For the diagnosis of sex, FISH is better than PCR because FISH could also detect aneuploidy of sex chromosomes. PGD is still a great challenge in research and clinical use. In this project, we plan to evaluate the methods using laser cutting or acidic drilling for blastomere biopsy and the calcium-magnesium free medium for facilitating the procedure.

Results

In total, 61 extra embryos at the 2- to 4-cell stage were donated by 26 patients; of these embryos, 54 (90%) developed to 6-12 cells. Eighteen were biopsied using the laser-cutting method, 18 using the acid-drilling technique, and 18 for controls. The average time for each biopsy was significantly shorter for the laser method (80± 21 sec) than for the acid method (422± 120 sec). The success rate for obtaining an intact blastomere was same for the two methods (100% vs. 100%). Loss of blastomeres during fixation, frequency of incidence to observe nuclear material after fixation, and positive X/Y signals after FISH were not different between the two groups (90%, 90%). In those blastomeres (32) with positive FISH signals, 16 (50%) were identified to be XY male and 15 (47%) were XX female. 1 (3%) presented an abnormal number of X and Y signals (XYY).

The growing capacity to blastocyst was not different among the three groups (39%, 33%, 33%). Moreover, zona-drilled embryos in the two biopsied (22%, 17%) groups had a higher incidence of complete hatching than that of control embryos (0%) without micromanipulation. The hatching processes in the control embryos displayed expansion of the blastocyst, thinning of the zona pellucida, and extrusion of a cellular projection that penetrated the zona. In the zona-drilled embryo, the expanding blastocyst escaped from the drilled hole, and there was no thinning of the zona. There were no differences between the laser method and the acid method for ability and mode of hatching in embryos biopsied. These embryos did not lose a blastomere when cultured in vitro.

DISCUSSION

This study demonstrates that the laser method is more efficient than the conventional acid method for blastomere biopsy in human embryos. The shortened operating time might reduce exposure of suboptimal conditions outside the incubator with potential disadvantages for growth. Only two pipettes are needed for the laser method. An intact blastomere was successfully retrieved in 100% of embryos. No failure of biopsy may be due to the use of calcium-magnesium free medium to diminish a tight junction of blastomeres. The growth of embryos was not affected after returning to the culture medium.

The biopsied embryos unaffectedly achieved blastocyst formation, and apparently commenced the hatching process. These observations were similar to previous reports wherein zona drilling or partial zona dissection facilitated hatching of embryos (3,4,7). The blastomeres from either the laser-cutting method or the acid-drilling technique could be equally fixed and shown in FISH. The intact blastomere retrieved is

also thought to be suitable for polymerase chain reactions (PCR) in the detection of single-gene diseases. Thus, this laser-assisted technique is a convenient alternative for blastomere biopsy that may merit use in clinical practice and research.

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