นิพนธ์ต้นฉบับ

The comparison of Subzonal insemination in IVF program.

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Objective : *To evaluate and compare the fertilization ability of oocytes undergoing Subzonal Insemination (SUZI) or Partial Zona Dissection (PZD) after failed fertilization in conventional IVF.*

Design : *72 oocytes that failed to fertilize 18 hours after insemination in conventional in vitro fertilization were randomly allocated into three groups: Group 1 undergoing SUZI, Group 2 PZD, Group 3 controlled reinsemination.*

Setting : *Chula IVF center Dept. of OB & GYN, Chulalongkorn University Hospital.*

Patients : *Infertile women who attended the IVF Clinic. Sixteen patients who had unfertilized oocytes were counselled about the availability of the assisted fertilization procedure.*

Intervention : *SUZI, PZD, or reinsemination was done by using microtools attached to a hydraulic micromanipulatior system.*

Mean outcome measures : *Pronuclei and cleavage rates were recorded and compared between groups.*

Results : *The fertilization rates for PZD, SUZI, and controlled reinsemination, were 9/26 (35 %), 4/21 (19 %) and 4/25 (16 %), respectively.*

Conclusion : *The oocytes undergoing PZD have a higher fertilization rate compared to Subzonal Insemination for assisted fertilization after failure to fertilize in an IVF program.*

Key words : *Subzonal insemination, SUZI, Partial zona dissection, PZD, reinsemination, Unfertilized oocyte.*

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กำธร พฤกษานานนท์, วิชชุดา อานนท์กิจพานิช, วิสุทธิ์ บุญเกษมสันติ, ประมวล วีรุตมเสน. การศึกษาเปรียบเทียบการช่วยการปฏิสนธิ โดยวิธีการฉีดอสุจิเข้าในชั้นใต้เปลือกหุ้มไข่ ในไข่ที่ไม่ปฏิสนธิจากกระบวนการปฏิสนธินอกร่างกาย. จุฬาลงกรณ์เวชสาร 2539 มกราคม; 40(1): 15-21

วัตถุประสงค์ : *เพื่อศึกษาเปรียบเทียบวิธีการช่วยการปฏิสนธิ โดยวิธีการฉีดอสุจิเข้าในชั้นใต้เปลือกหุ้มไข่ (Subzonal insemination : SUZI) และวิธีตัดช่องที่เปลือกหุ้มไข่ (Partial Zona dissection : PZD) ในไข่ที่ไม่ปฏิสนธิจากกระบวนการปฏิสนธินอกร่างกาย*

รูปแบบการวิจัย : *ไข่ที่ล้มเหลวจากการปฏิสนธิ ภายหลังจาก 18 ชั่วโมง ในการปฏิสนธินอกร่างกายจำนวน 72 ใบได้ถูกแบ่งออกเป็น 3 กลุ่ม โดยวิธีการสุ่ม กลุ่มที่ 1 ใช้วิธีการฉีดอสุจิ (SUZI) กลุ่มที่ 2 ใช้วิธีการตัดช่อง (PZD) และกลุ่มที่ 3 เป็นกลุ่มควบคุมใช้วิธีใส่อสุจิเข้าไปใหม่*

สถานที่ : *หน่วยรักษาผู้มีบุตรยาก ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

วัสดุและวิธีการ : *ผู้ป่วยซึ่งเข้ามารับการรักษา โดยวิธีปฏิสนธินอกร่างกาย จำนวน 16 คน ซึ่งมีไข่ที่ไม่ปฏิสนธินอกร่างกายโดยใช้เครื่องมือจุลหัตถกรรม ซึ่งติดกับกล้องจุลทรรศน์ ตามวิธี SUZI, PZD หรือเป็นกลุ่มควบคุม*

วิธีการวัดผล : *อัตราการปฏิสนธิเป็นตัวอ่อนระยะโปรนิวคลิอาย (Pronuclei) และระยะการแบ่งเซล เปรียบเทียบระหว่างกลุ่ม*

ผลการศึกษา : *อัตราการปฏิสนธิโดยวิธีการ SUZI, PZD และกลุ่มควบคุม เท่ากับ 4/21 (19%), 9/26(35 %) และ 4/25(16%) ตามลำดับ*

สรุป : *ไข่ซึ่งได้รับการช่วยการปฏิสนธิ โดยวิธีการตัดช่องที่เปลือกหุ้มไข่ (PZD) มีอัตราการปฏิสนธิสูงกว่าวิธีการฉีดเชื้ออสุจิเข้าในชั้นเปลือกหุ้มไข่ และวิธีการเติมเชื้อเข้าไปใหม่ ในไข่ที่ไม่ปฏิสนธิจากกระบวนการปฏิสนธินอกร่างกาย*

Successful in vitro fertilization and embryo transfer (IVF-ET) depends on many factors. After successful oocyte retrieval, failure to achieve fertilization of any of the retrieved oocytes can occur in approximately 10% to 20% of all IVF cycles.[1] The majority of these cases are unanticipated and are without clear etiology, as only a small percentage are due to known male factors, with the remainder probably divided between undetected poor sperm quality, poor oocyte quality, or unidentified factors that may impair sperm-oocyte interaction. Several inves-tigators also stressed the importance of hormonal regulation during the fertilization and implantation period.[2] Recent developments in gamete micromanipulation techniques have improved the successful treatment of difficult infertile problems where previously this was little hope. Techniques such as subzonal microinsemination (SUZI),[3,4] partial zona dissection (PZD),[5] and more recently intra-cytoplasmic injection of a single spermatozoon (ICSI),[6,7] have been used with varying success. However, reinsemination of the unfertilized oocyte is widely practiced in many centers, in-cluding our own. In general, results from such reinseminations have either been encouraging or controversial.

The purpose of this study was to evaluate the use of SUZI and PZD to fertilize oocytes that had failed to fertilize previously during routine IVF insemination and to compare the outcome with conventional repeat in vitro reinsemination of sibling oocytes.

Materials and methods

Patient selection

Unfertilized oocytes from sixteen patients which underwent treatment for infertility by in vitro fertilization at the Department of Obstetrics and Gynecology, Chulalongkorn University Hospital were used for the study.

Sperm assessment was performed ac-cording to the recommendation of the World Health Organization (WHO),[8] and prepared by discontinuous percoll gradient.[9]

IVF laboratory methods

After standard controlled ovarian hyper-stimulation and transvaginal oocyte retrieval, insemination was performed with 50,000 to 100,000 spermatozoa/oocyte in culture tubes. All gamete incubation was carried out in a modular plastic chamber within a CO_2 incubator in a humidified environment of 5% O_2, 5% CO_2 and a temperature of 37°C

Examination of the oocytes for evidence of spontaneous fertilization was carried out 16-18 hr. after insemination. Fertilization was con-firmed by the presence of two pronculei and/or two polar bodies followed by subsequent cleavage.

Oocytes that failed to fertilize were allo-cated to undergo either reinsemination or SUZI or PZD, respectively. The oocytes were examined to ensure that they satisfied the criteria for one of the procedures. The criteria included the presence of one polar body,and a normal cyto-plasmic appearance with the absence of demon-strable pronuclei. Newly acquired spermatozoa

were used for either reinsemination or SUZI or PZD. For reinsemination, each oocyte was reinseminated with 50,000 to 100,000 spermatozoa in a fresh 1 ml sample of HTF medium and incubated in the same controlled environment prior to examination approximately 24 hr. later for evidence of fertilization.

Micromanipulation methods

Micromanipulation for both SUZI and PZD was carried out on a Nikon Diaphot inverted microscope (Nikon, Tokyo, Japan) equipped with a hydraulic manipulator (Narishegi, Tokyo, Japan) and a warm plate, thus allowing for the procedure to be carried out at a controlled temperature of 37° C. (Figure 1)

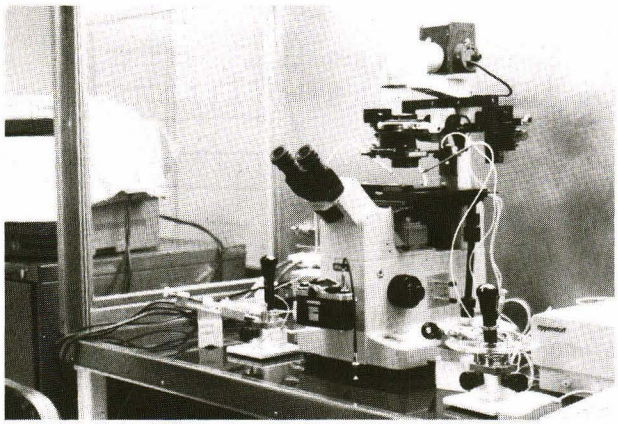

Figure 1. Hydraulic micromanipulator attached to an inverted microscope used for assisted fertilization at Chula IVF center, Department of OB & GYN.

The oocyte SUZI technique was carried out as previously discribed by Fishel[4] with four to six spermatozoa injected into the perivitelline space. For PZD, a small opening at the zona pellucida was done as described by Cohen.[5] All oocytes were cultured after the procedure and incubated in a similar condition as those used for IVF. Examination for fertilization and cleavage was carried out approximately 24 and 48 hr. later. (Figure 2.)

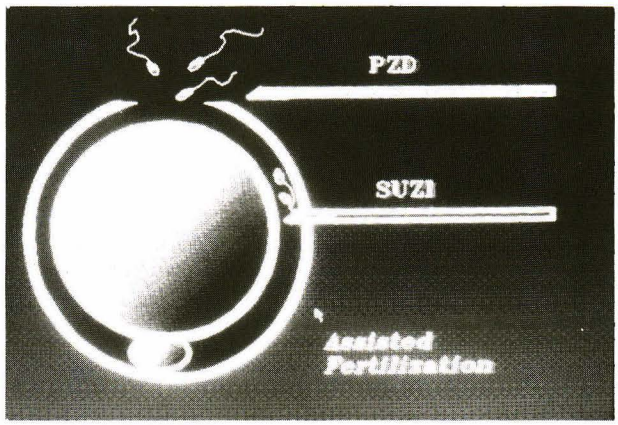

Figure 2. The methods for assisted fertilization by Subzonal insemination (SUZI) and Partial zona dissection (PZD).

Statistical analysis

Statistical analysis was carried out by use of the Student's t-test for comparison of means and by chi-square test as applicable. A p value < 0.05 was regarded as significant.

Results

Seventy two oocytes from 16 patients that failed to fertilize 16-18 hours after insemination in conventional in vitro fertilization were randomly allocated into three groups : Group 1 underweent SUZI: Group 2 underwent PZD, and Group 3 used controlled reinsemination.

A summary of the analysis of semen samples utilized in these cycles is shown in Table 1. The mean of semen parameters did not show a definite male factor infertility.

Table 1. Summary of semen parameters.

	Mean ± SD	Range
Sperm count (million/ml)	52.5 ± 30.2	12 - 115
Motility (%)	41.7 ± 18.3	19 - 64
Progressive motility (%)	57.1 ± 22.4	28 - 29
Abnormal forms (%)	49.2 ± 28.1	15 - 88

The fertilization rates following SUZI (19%) and controlled reinsemination (16%) were similar. In contrast, the rate following PZD (35%) was significantly better (Table 2). Polyspermy were found both in SUZI and PZD but not in the reinsemination group. Damaged oocytes were also found in both groups, but were more numerous in SUZI.

Table 2. Results of subzonal insemination (SUZI), partial zona dissection (PZD), and control reinsemination (RI) in unfertilized oocytes.

	SUZI	PZD	RI
No. of oocytes	21	26	25
Fertilized	4(19%)	9(35%)*	4(16%)
Polyspermy	1	1	0
Damaged	2	1	0
Embryo cleavage	3(75%)	8(88.9%)	3(75%)

*Statistical significance, $p < 0.05$

Discussion

Assisted fertilization by the micromanipulation of gametes has been a revolutionary advance in the management of those couples who cannot achieve satisfactory fertilization with conventional IVF.

Although oocyte reinsemination following fertilization failure after an initial insemination attempt appears to be widely practiced in most IVF-ET programs, very little success is published about it in the literature. The few available reports in the literature are generally contradictory. While Trounson[10] reported a low fertilization rate, others reported variable outcome between 21.9% and 70.8% following reinsemination. However, pregnancies following embryo replacement derived from reinsemination have been reported only by Ben Rafael[11] and Boldt.[12]

Plachot[13] reported that 30% of unfertilized oocytes carried some chromosomal abnormalities, while Oehninger[14] reported oocyte morphological defects in 37% of cycles with delayed fertilization. Also, "occult" male factor was reported by Oehninger[14] to be a factor contributing to some failure of oocyte fertilization in vitro. A combination of both factors, oocyte and sperm defects, was also reported in about 15% of delayed fertilization cycles by Oehninger.[15]

While the cause of fertilization failure may be multifactorial, our observation indicated that PZD was more successful than SUZI and reinsemination. We speculate that these successes were cases in which "zona hardening" or "occult" male factor may not have existed. Our result show that reinsemination did not result in a significantly improved fertilization rate was in agreement with the report of Trounson.[10]

In conclusion, the result of this study suggests that couples with a poor rate or complete failure of fertilization in one treatment cycle and where the semen parameters appears to be suboptimal should be referred to a center with facilities for oocyte micromanipulation. This allows the application of a second line of action which from this study, should be PZD instead of reinsemination, if fertilization fails to occur in such subsequent cycles.

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