Comparison of Pronuclear Stage Embryo Transfer and *In Vitro* Fertilization-Embryo Transfer Following Intracytoplasmic Sperm Injection

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Abstract

To determine whether the mode of embryo transfer (PROST *vs* IVF) affected the outcome in intracytoplasmic sperm injection (ICSI) cycles, 237 ICSI cycles (106 PROST and 131 IVF) were analyzed. Several parameters, including patient age, duration of infertility, amounts of hMG used, number of mature eggs retrieved and injected, fertilization rate, number of embryos transferred, and clinical pregnancy rate, were compared. Most of the variable factors were not significantly different, except the mean numbers of transferred embryos which were significantly higher in the PROST group. The clinical pregnancy rate showed no statistical difference between PROST and IVF cycles (25.5 and 16.8% ; p = 0.139). This study suggests that even the pregnancy rate in PROST cycles was slightly higher than IVF cycles, but there was no statistically significant difference between the two groups.

Key word : Pronuclear Stage Embryo Transfer - *In Vitro* Fertilization-Embryo Transfer - Intracytoplasmic Sperm Injection - Comparison

After Palermo performed the first intracytoplasmic sperm injection (ICSI) in 1991[1], the ICSI procedure has been applied routinely all over the world. ICSI indications have also evolved with time. Initial indications were low sperm count, poor morphology and low motility. Other indications were failure of fertilization with conventional *in-vitro* fertilization (IVF) or subzonal insemination (SUZI)[2,3], globozoospermia[4] and azoospermia with spermatozoa extracted from epididymis or testis[5,6]. Published reports on success rates with ICSI indicated clinical pregnancy rates of approximately 20-40 per cent when ICSI was used in IVF cycles[7,8].

In the ampullary part of the fallopian tube; ciliary movements of the endothelial cells, contractions of the fallopian tube and secretions of the tube then assist in the transport of the human embryo to the uterine cavity. Yovich et al[9] replaced embryos (pronuclear stage transfer, PROST) directly

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into the fallopian tubes which represented physiological environment that enhanced its survival. Previous studies suggested a higher pregnancy rate following PROST than IVF, as well as reports of higher pregnancy rates in both fresh and frozen embryo transfer cycles using PROST *vs* IVF[10-13]. In contrast, some reports suggested no benefit from the use of tubal as opposed to uterine transfer[14-16].

The purpose of this study was to compare the results of ICSI cycles in which tubal transfer (PROST) was used, to IVF with uterine transfer.

MATERIAL AND METHOD

A total of 237 ICSI procedures were carried out from January to December 1997 at Siriraj Hospital. The patients were treated with ICSI when the total motile sperm count was < 500000, the number of abnormal forms was > 85 per cent, total motility was < 20 per cent, or when patients had a history of fertilization failure with conventional IVF. PROST was used for embryo replacement in 106 cycles, and IVF used in 131 cycles. Patients with patent fallopian tubes were offered either PROST or IVF for embryo replacement. Patients with blocked tubes or other pelvic pathology limiting tubal access or tubal mobility were offered IVF only.

1. Ovarian stimulation

The combination of gonadotropin releasing hormone agonist (GnRHa) and human menopausal gonadotropin (hMG), short protocol was used. Buserelin (Suprefact®) was administered intranasally from day 1 of the cycle until the day of hCG injection. The daily dose was 600 microgram. Human menopausal gonadotropin (Humegon®, or Pergonal®) 150-300 IU per day was given intramuscularly from day 3. Follicular development was monitored by transvaginal ultrasound. When at least two follicles reached 18 mm in diameter, hMG was stopped, and 10000 IU of hCG (Pregnyl, or Profasi) was given intramuscularly and oocyte retrieval scheduled for 34-36 hours after hCG injection.

2. Oocyte retrieval

Using intravenous pethidine and diazepam, oocyte retrieval was performed 34-36 hours after hCG injection under transvaginal ultrasound guidance by 18 G, single lumen ovum pick up needle with suctional pressure 100-120 mmHg. Oocytes were collected and trimmed of excess cumulus cells using 27G needles, then treated with 80 IU/ml hyaluronidase for 30-60 seconds to complete removal of cumulus cell. The adhering corona radiata was removed by aspirating the oocytes in and out of a glass pipette with a diameter ranging from 180 to 220 micron. The oocytes were washed 4 times and incubated in culture medium under oil until further processing. ICSI was performed on oocytes that had extruded their first polar body (metaphase II oocytes), 3-4 hours after their retrieval.

3. Sperm preparation

Sperm collection was performed by masturbation just before oocyte retrieval. Active motile spermatozoa were separated by the mini-Percoll gradient centrifugation method[17].

4. Intracytoplasmic sperm injection[1]

ICSI was carried out using commercial pipettes. Injection pipettes with an inner diameter of approximately 5-6 micron were used to aspirate spermatozoa from the sperm collection droplet and to place them into the 10 per cent polyvinylpyrrolidone. Sperm with flagellar motion were immobilized by touching the midpiece with the injection pipette, then loaded into the injection pipette and injected into the center of the oocyte after aspirating some ooplasm. The injected oocytes were washed four times and incubated in culture medium at 37°C in an atmosphere of 5 per cent CO_2.

5. Assessment of fertilization

At 18-20 hours after the ICSI procedure, the oocytes were checked for the presence of one, two or more pronuclei. Normal fertilization was defined by the presence of two pronuclei. PROST transfers were carried out by laparoscopy under general anesthesia. Using a GIFT catheter placed approximately 4 cm into the fallopian tube *via* fimbrial end. The patients were admitted for at least 1 day. For IVF cycles, embryos were cultured for a further 24 hours and they were scored for their number of cells and overall quality aspect. The transfers were performed using Jansen Anderson bulb tip embryo transfer catheter without anesthesia. The patients went home after 1 hour rest.

6. Follow-up

Luteal phase support consisted of hCG 1500 IU given intramuscularly every 3 days for 3-4 doses or micronized progesterone 200 mg given twice a day. Serum beta hCG was determined 12 and 14 days after transfer. Patients with positive beta hCG were followed with transvaginal ultrasound to confirm the presence of a clinical pregnancy and to determine the number of gestational sacs 4 weeks after transfer.

7. Statistical analysis

The data were analysed using Student's *t*-test and Fisher's exact test, with $p < 0.05$ was required for statistical significance.

RESULTS

Various parameters of the PROST and IVF groups are shown in Table 1. Mean age of the couples, duration of infertility, number of days of hMG administration, dose of hMG used, and number of follicles ≥ 14 mm diameter, were not significantly different.

Comparison of treatment outcome between PROST and IVF cycles for embryo transfer are shown in Table 2. The number of retrieved oocytes per cycle and fertilization rates were not significantly different between the two groups. However, the number of embryos transferred were higher in the PROST group (4.2 ± 1.2 *vs* 3.4 ± 1.1, $p < 0.01$). The clinical pregnancy rate was somewhat higher in PROST cycles, compared to IVF cycles, but there was no statistically significant difference (25.5% *vs* 16.8%, $p > 0.05$). Outcome of pregnancy is shown in Table 3, without significant difference between PROST and IVF groups.

Table 1. Background details*.

	PROST	IVF
Number of cycles	106	131
Patient age (y)	36.1 (23-43)	35.1 (24-42)
Husband age (y)	39.1 (25-60)	38.4 (27-59)
Infertility duration (y)	6.9 ± 3.3	7.9 ± 3.5
Days of hMG administration	8.5 ± 1.4	8.2 ± 1.6
Dose of hMG (ampoules)	34.9 ± 10.3	32.4 ± 8.3
Number of follicles ≥ 14 mm	8.6 ± 7.0	9.4 ± 5.6

* Data on age is mean (range), and other values are presented as means ± SD.

Table 3. Outcome of pregnancy.

	PROST	IVF
Number of pregnancies	27	22
Abortions (%)	6 (22.2)	6 (27.3)
Ectopic pregnancies (%)	0	1 (4.5)
Term deliveries (%)	17 (63.0)	13 (59.1)
Ongoing pregnancies (%)	4 (14.8)	2 (9.1)

DISCUSSION

Until the advent of ICSI, there was no generally effective treatment for infertile couples when there was a truly severe andrological component. In most such couples, the fertilization rate using standard IVF was generally zero or at best < 20 per cent, and fertilization improved only minimally after insemination of eggs at higher sperm concentration in a microdroplet or by micromanipulation of eggs using partial zona dissection or subzonal sperm insemination. ICSI has been introduced successfully for some patients suffering from severe oligoasthenoteratozoospermia, previous fertilization failure and azoospermia[18,19].

For embryo transfer, the use of tubal transfer (PROST) and uterine transfer has yielded conflicting data. Some studies demonstrated a higher pregnancy rate with tubal transfer because it resulted in a more synchronized entry of embryos into the

Table 2. Outcome of treatment.

	PROST(106)	IVF(131)	P value
Number of oocytes retrieved	10.5 ± 7.2	11.0 ± 9.3	0.65
Fertilization rate (%)	55.6	58.1	0.81
Number of embryos transferred	4.2 ± 1.2	3.4 ± 1.1	< 0.01
Clinical pregnancies (%)	27 (25.5)	22 (16.8)	0.139

uterine cavity[10-13]. In contrast, none of the prospectively controlled studies showed any advantage to intratubal transfers over intrauterine transfers[15, 16]. The present study demonstrated that even the intratubal transfer of ICSI - derived embryos seemed to obtain higher clinical pregnancy rate than with intrauterine transfer, but no significant statistical difference was found.

There are many factors associated with the success rate of ICSI, for example, patient age, ovarian stimulation protocol, total embryos transfer and embryo transfer technique. Advanced patient age can decrease ovarian response and pregnancy rate[20] but this is not due to diminished capacity of the uterus to sustain embryo implantation. The higher rate of aneuploidy in oocytes and diminished number of eggs after ovarian stimulation in older women contribute significantly to the reduced ongoing pregnancy rate in older women. In this study, we compared patient age, male partner's age, period of infertility, ovarian stimulation protocol, mean hMG used and mean oocytes retrieved in both groups (PROST and IVF), we found that they were identical between both groups, and the fertilization rate was not different either. The number of embryos transferred was significantly higher in PROST than in IVF cycles. We have usually transferred 1-2 embryos more in PROST cycle for its invasive procedure. There may be some other factors that enhance pregnancy rates such as quality of embryos available for transfer but this could not be assessed in the PROST cycle. However Sutter et al[21] suggested that oocyte morphology did not correlate to embryo quality after ICSI. Intratubal transfer of ICSI - derived embryos is a heavy procedure, it requires more medical staff and equipment, and some complications may occur with the procedure[22]. However, in an institution where embryo culture system is suboptimal, or in patients with cervical stenosis, the result of transcervical transfer will be poor and intratubal transfer could be a more useful procedure.

In conclusion, PROST procedure after ICSI seems to obtain a clinical pregnancy rate higher than IVF cycle, but the difference is not statistically significant.

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เปรียบเทียบการใส่ตัวอ่อนที่เกิดจากการฉีดอสุจิเข้าไปในไข่ ระหว่างการใส่เข้าไปในท่อนำไข่และใส่เข้าโพรงมดลูก

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การรักษาภาวะมีบุตรยากโดยการฉีดตัวอสุจิเข้าไปในไข่โดยตรง (ICSI) เมื่อเป็นตัวอ่อนแล้ว ใส่กลับโดยการใส่เข้าไปในท่อนำไข่ (PROST) หรือใส่กลับเข้าไปในโพรงมดลูก (IVF) เพื่อศึกษาดูว่า การใส่ตัวอ่อนทั้ง 2 วิธีนี้ มีอัตราการตั้งครรภ์แตกต่างกันหรือไม่ จึงได้ศึกษาในคู่สมรสที่รักษาด้วยวิธีฉีดอสุจิเข้าไปในไข่จำนวน 237 ราย แบ่งเป็นการใส่กลับตัวอ่อนเข้าไปในท่อนำไข่ 106 ราย และใส่กลับเข้าโพรงมดลูก 131 ราย ได้เปรียบเทียบอายุของผู้ป่วย ระยะเวลาของการมีบุตรยาก จำนวนยาฮอร์โมน hMG ที่ใช้ จำนวนไข่ที่สุกและฉีดอสุจิ อัตราการปฏิสนธิ จำนวนตัวอ่อนที่ใส่กลับ และอัตราการตั้งครรภ์ พบว่าทั้ง 2 กลุ่ม มีลักษณะต่างๆ ใกล้เคียงกัน ยกเว้นจำนวนตัวอ่อนที่ใส่กลับ ซึ่งในกลุ่มที่ใส่ตัวอ่อนกลับเข้าไปในท่อนำไข่ มีจำนวนสูงกว่าอย่างมีนัยสำคัญทางสถิติ ส่วนอัตราการตั้งครรภ์ พบว่าทั้ง 2 กลุ่ม ไม่มีความแตกต่างอย่างมีนัยสำคัญ (25.5 และ 16.8%, p = 0.139) จากการศึกษานี้สรุปได้ว่าการใส่ตัวอ่อนกลับเข้าทางท่อนำไข่ แม้จะมีอัตราการตั้งครรภ์สูงกว่าการใส่ตัวอ่อนกลับเข้าโพรงมดลูก แต่ก็ไม่มีนัยสำคัญทางสถิติ

คำสำคัญ : การใส่ตัวอ่อนที่เกิดจากการฉีดอสุจิเข้าในไข่ – การใส่เข้าไปในท่อนำไข่และใส่เข้าโพรงมดลูก – การเปรียบเทียบ

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