Improving Pregnancy Rates After Intracytoplasmic Sperm Injection by Using MIFT Technique : A New Option for Embryo Transfer

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Abstract : **To evaluate the efficacy of a new technique, micro–injected oocytes intrafallopian transfer (MIFT), involving a same day procedure. 9 patients with at least one patent fallopian tube were offered this procedure. Ovarian induction consisted of down regulation with GnRHa followed by ovarian stimulation with hMG except for one patient who received only hMG due to a poor response. HCG 10,000 IU was administered when at least 2 leading follicles exceeded 18 mm. in diameter and transvaginal oocyte retrieval was performed 34 – 36 hours thereafter. Intracytoplasmic sperm injection (ICSI) was performed on the oocytes of all patients with male factor infertility. MIFT was performed on the same day after ICSI by transfering 2 – 5 micro–injected oocytes into the fallopian tube by laparoscopy under general anesthesia. The implantation rate and pregnancy rate were 16.1% (5/31) and 55.6% (5/9) respectively. This study suggests that MIFT is effective, easy and should be considered as a new option which yields a high pregnancy rate.**

เรื่องย่อ : การเพิ่มอัตราการตั้งครรภ์ภายหลังการฉีดอสุจิเข้าภายในไข่โดยมิ๊ฟเทคนิค : ทางเลือกใหม่ของวิธีการย้ายตัวอ่อน

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การรักษาภาวะมีบุตรยากโดยการฉีดอสุจิเข้าภายในไข่ (ICSI) แล้วใส่กลับเข้าร่างกายทางท่อนำไข่ภายในวันเดียวกัน (MIFT) ทำการศึกษาเพื่อประเมินประสิทธิภาพของมิ๊ฟเทคนิคซึ่งเป็นวิธีใหม่ในการย้ายตัวอ่อนภายในวันเดียวกันในผู้ป่วยจำนวน 9 รายซึ่งมีท่อนำไข่ปกติอย่างน้อย 1 ข้าง ทำการฉีดเชื้ออสุจิเข้าภายในไข่ของผู้ป่วยทุกรายที่มีปัญหามีบุตรยากเนื่องจากความผิดปกติของฝ่ายชายตามด้วยการทำมิ๊ฟภายในวันเดียวกันโดยทำการย้ายตัวอ่อน 2-5 ตัวเข้าภายในท่อนำไข่ผ่านทางกล้องส่องตรวจอุ้งเชิงกราน พบอัตราการฝังตัวของตัวอ่อนและอัตราการตั้งครรภ์เท่ากับ 16.1% (5/31) และ 55.6% (5/9) ตามลำดับ การศึกษานี้บ่งชี้ว่ามิ๊ฟเป็นวิธีที่มีประสิทธิภาพทำได้ง่าย และน่าจะพิจารณาเป็นทางเลือกใหม่เนื่องจากประสบผลอัตราการตั้งครรภ์สูง

Since the first human birth following in vitro fertilization (IVF) in 1978, the technologies for assisting infertility patients have expanded greatly. A new approach for assisting reproduction, intracytoplasmic sperm injection (ICSI), was introduced in 1991. The limiting step in the success of IVF-ET programs is embryo implantation which remains between 4.4 - 9.7%[1,2] with a clinical pregnancy rate of 16.8 – 17.5%.[1,2,3] These relatively low rates of implantation mean that the majority of embryos fail to implant. Different methods have been found for increasing the implantation rate and pregnancy rate such as the transfer of more embryos, the use of a co-culture system, immunoglobulin therapy, fibrin sealant and various methods of embryo transfer.[4] Tubal embryo transfer may provide a greater chance of pregnancies than IVF – ET.[1,5,6] It is generally assumed that both an optimal intra-fallopian milieu and better synchronization between embryonal and endometrial development may enhance the development of embryos because it occurs within the natural environment. However, some investigators have reported that there was no statistically significant difference in the pregnancy rates acheived between tubal embryo transfer and IVF.[3,7,8] At Siriraj Hospital, pregnancy rates from PROST (pronuclear stage transfer) and conventional IVF were 25.5% and 16.8% respectively.[3] The aim of this study was to investigate the efficacy of a micro-injected oocytes intrafallopian transfer (MIFT) by a new "same day" technique for the infertile couples.

MATERIALS AND METHODS

ICSI procedures was performed on 9 patients who had indications for ICSI between January 1999 and February 2000. The indications for the procedure were a total motile sperm count of <500,000, abnormal forms > 85%, total motility < 20% or sperm that was obtained from testicular tissue in the azoospermic patient. Patients with at least one patent fallopian tube were offered MIFT for embryo replacement. For ovarian stimulation, all patients received a long protocol of gonadotropin–releasing hormone agonist (GnRHa) and human menopausal gonadotropin (hMG) except for one patient who received only hMG due to a poor response. Follicular development was monitored by transvaginal ultrasound. HCG 10,000 IU was administered when at least two leading follicles exceeded 18 mm. in diameter. Transvaginal oocyte retrieval was performed 34 – 36 h. post-hCG injection. Oocytes were treated with 80 IU/ml of hyaluronidase to complete removal of cumulus cells. A semen sample was collected immediately after oocyte retrieval and was classified using World Health Organization criteria (WHO).[9] Active motile spermatozoa were separated by the swim up method. Sperm from an azoospermic patient was obtained by biopsy of testicular tissue. ICSI was performed on oocytes that had extruded their first polar body (metaphase II oocytes). The micro-injected oocytes were incubated in culture media at 37^{0}C with 5% CO_2 before transfer.

Micro–injected oocytes intrafallopian tube transfer (MIFT) was done by transfering 2–5 micro-injected oocytes within 1 hr. post ICSI into the fallopian tube by laparoscopy under general anesthesia. Using a GIFT catheter placed approximately 4 cm into the fallopian tube via the fimbrial end. Supernumerary micro–injected oocytes were left in culture and checked 16 – 18 hours later for fertilization and the resulting zygotes were cryopreserved. The luteal phase was supported with an intravaginal progesterone suppository starting on the day of MIFT. A chemical pregnancy was confirmed 14 – 16 days after transfer by a rising serum β-hCG and transvaginal ultrasound was performed 2 weeks later to determine a clinical pregnancy by indicating a fetal heart beat and the number of gestational sacs present.

RESULTS

Table 1. Background details

	Mean ± S.D. (Range)
Number of patients	9
Patient age (years)	37.1 ± 1.5 (30 – 43)
Husband age (years)	39.9 ± 1.5 (33 – 46)
Infertility duration	6.1 ± 1.3 (2 – 13)
Dose of hMG ampules	41.4 ± 3.7 (22 – 60)
Day of hMG administration	11.9 ± 0.5 (10 – 15)

Table 2. Clinical outcome

Number of patient	Number of oocytes retrieved	Number of metaphase II oocytes	Number of micro–injected oocytes transferred	Result
1	30	25	3	Pregnancy
2	5	5	5	Pregnancy
3	6	5	5	Menstruation
4	20	18	3	Pregnancy
5	5	5	5	Pregnancy
6	4	4	3	Menstruation
7	3	3	3	Menstruation
8	2	2	2	Menstruation
9	2	2	2	Pregnancy
Mean ± S.D.	8.6 ± 3.3	7.7 ± 2.7	3.4 ± 0.4	

Table 3. Implantation rate Clinical pregnancy rate

Implantation rate	5/31 (16.1 %)
Clinical pregnancy rate	5/9 (55.6 %)

A total of 9 cycles were initiated in 9 patients. All patients had an indication for ICSI treatment. Various clinical parameters are shown in Table 1. 3 patients had subserous myomas. 1 Patient had both a subserous myoma and a submucus myoma 5 cm. in diameter. This patient underwent a hysteroscopic myomectomy 6 months before starting infertility treatment and she became pregnant thereafter.

Table 2 and Table 3 show the clinical outcome of treatment. ICSI was performed on a total of 69 oocytes. Following injection, 31 micro–injected oocytes were transferred. The mean number transferred was 3.4 ± 0.4 and 5 pregnancies (55.6%) were obtained. The implantation rate per transfer was 16.1%.

DISCUSSION

After Palermo performed the first intracytoplasmic sperm injection (ICSI) in 1991[10], the ICSI procedure has been applied routinely all over the world for male factor infertility with high fertilization rates. (about 80%) Although it produces higher fertilization rates, the limiting step in the success rate of clinical pregnancy is the step of embryo transfer due to low rates of embryo implantation. Even though most patients can produce embryos for transfer, the embryo implantation rate remains 4.4 – 9.4%[1,2], with a clinical pregnancy rate of about 16.8 – 17.1%.[1,2,3]

For embryo transfer, the use of tubal transfer and uterine transfer have yielded conflicting data. Many assisted reproductive technique (ART) programs report higher pregnancy rates after tubal transfer than after intrauterine transfer.[11] It is generally assumed that both an optimal intrafallopian milieu and a better synchronization between embryonal and endometrial development may enhance the development of oocytes or embryos. Moreover, embryos transferred into the fallopian tube would enter the uterine cavity with more appropriate timing. Also it has been postulated that implantation rates might be improved by the avoidance of uterine trauma which might occur during an intrauterine transfer procedure.

Levran et al. performed zygote intrafallopian transfer (ZIFT) and found that ZIFT achieved significantly higher pregnancy rates than intrauterine transfer (34.2% vs. 17.1% respectively).[1] Scott et al. used pronuclear stage transfer (PROST) for intrafallopian transfer and acheived 39% pregnancy rates.[5]

In 1998, we compared PROST and uterine transfer at Siriraj Hospital and concluded that the pregnancy rate using PROST was higher than that achieved by uterine transfer (25.5 and 16.8% respectively; $P = 0.139$) but there was no statistically significant difference between the two methods.[3]

Intrafallopian transfer can be provided by different modes according to the stage of transfer; PROST, TET and MIFT. PROST and TET are performed on day 1 and day 2 after ICSI. Both of them have many advantages : proof of normal fertilization, exclusion of polypoid embryos and the possibility of incubating immature oocytes. However, the disadvantages are that they need to be cultured for 1 and 2 days respectively. A suboptimal culture and adverse environmental factors present in laboratory air and released by common products used by laboratories have a detrimental effect on embryonic development and may reduce the implantation rate and pregnancy rate.[4,12] MIFT, a new procedure, has many advantages. It can be done on the same day as ICSI, it is more convenient, cost – effective and a low stress approach. Another advantage is that all oocytes are cultured for only 4 – 5 hr. after retrieval which avoids the negative effects of in – vitro culture especially in older patients (> 40 years old). These negative effects have reported as the hardening of the zona pellucida.[13,14] The main disadvantage of MIFT is the lack of control of fertilization. Therefore, the micro-injected oocytes which are chosen for transfer are there which show good oocyte morphology which correlates with good embryo quality.[15] In this study, we found that MIFT achieved a higher implantation rate of 16.1% compared with 8.7% per PROST and 4.4% per uterine transfer.[1] At Siriraj Hospital, MIFT also yielded a higher pregnancy rate of 55.6% than 25.5% per PROST and 16.8% per uterine transfer.[3] The mean number of micro–injected oocyte transfered was 3.4 but no multiple pregnancy occurred.

In conclusion, micro–injected oocyte transfer on the same day of oocyte retrieval is an effective and easy option as an infertility procedure. This new technique yielded a higher pregnancy rate than PROST and uterine transfer. It is undertaken as a single day–case episode and requires less embryological expertise and should be considered as a beneficial method of treatment for ICSI patients.

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