Comparative Study of Intracytoplasmic Sperm Injection and In Vitro Fertilization with High Insemination Concentration in Sibling Oocytes in the Treatment of Unexplained Infertility

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Introduction: *Some unexplained infertile couples may have fertilization failure; the in vitro fertilization (IVF) with high insemination concentration (HIC) and intracytoplasmic sperm injection (ICSI) were reported to give better fertilization rate when compared to standard IVF procedure.*
Objective: *To compare the fertilization rate, abnormal fertilization rate, total fertilization failure rate, and pregnancy rate between the IVF with HIC and ICSI for the treatment of unexplained infertility.*
Material and Method: *The prospective study in 36 unexplained infertile couples in Thammasat University Hospital between 2005-2007 was performed by equally dividing sibling oocytes in assisted reproductive technology cycles with controlled ovarian hyperstimulation protocol in each patients into two groups; group I were inseminated with 200,000 sperms (IVF with HIC) and group II underwent ICSI procedure. The embryos from the better fertilization group were selected to transfer. The fertilization rate, the abnormal fertilization rate, the implantation rate, the pregnancy rate and total fertilization failure rate were compared by Chi-square test and Fisher's exact test with statistical significance if $p < 0.05$.*
Results: *The fertilization rate, the abnormal fertilization rate, the implantation rate, the pregnancy rate and total fertilization failure rate of group I and II were 56.97% vs. 70.29% ($p = 0.15$), 6.56% vs. 1.26% ($p = 0.003$), 17.50% vs. 16.67% ($p = 0.64$), 38.46% vs. 36.36% ($p = 0.67$) and 13.89% vs. 0.0% ($p = 0.04$) respectively.*
Conclusion: *ICSI exhibited least total fertilization failure rate (0.0% vs. 13.89%) and gave less abnormal fertilization rate (1.26% vs. 6.56%) with statistical significance in unexplained infertility when compared to the IVF with HIC procedure. Therefore, applying of ICSI procedure may increase the success chance of embryo transfer and pregnancy outcomes.*

Keywords: *In vitro fertilization (IVF) with high insemination concentration (HIC), Intracytoplasmic sperm injection (ICSI), Unexplained infertility, Total fertilization failure*

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The prevalence of infertility was generally 3.5-16.7%[1,2]. The causes of infertility were male factors, the female factors, both or unexplained. The prevalence of unexplained infertility was about 5-15%[3,4]. In this group, abnormality was not found in the infertility investigations such as semen analysis, assessment of ovulation, assessment of uterine factor, assessment of tubal patency and antisperm antibody test. The treatments of unexplained infertility include ovulation induction, intrauterine insemination (IUI) and assisted reproductive technology(ART). There were recommendations that the ART should be performed after three IUI cycles failure with the pregnancy rate of 12.2-32.4%[5,6]. However, 17-20% of total fertilization failure rate of in vitro fertilization (IVF) were reported[7,8],

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this may be due to the fact that sperm and oocyte did not fertilize naturally even in vitro, and this problem cannot be detected by conventional infertility investigations. The ICSI was used to reduce the total fertilization failure rate in this case[9-12] and some studies reported a better fertilization rate of ICSI compared with IVF[11,12]. IVF with high insemination concentration technique (HIC) was also reported to improve the fertilization rate[13] but it may have the polyspermy fertilization. One study reported that IVF with HIC and ICSI could rescue the unfertilized oocytes of conventional IVF in unexplained infertility[14]. There is no study to compare the IVF with HIC and ICSI in the treatment of unexplained infertility with no previous history of fertilization ability. Therefore, the present study was performed in the unexplained infertile couples with no previous history of fertilization ability. The present study had the objective to compare the IVF with HIC and ICSI procedure for the better treatment of unexplained infertility.

Material and Method

The objective of the present study was to compare the fertilization rate, abnormal fertilization rate, total fertilization failure rate, cleavage rate, implantation rate, pregnancy rate, and outcome of pregnancy obtained from the IVF with HIC and ICSI treatments in unexplained infertility.

This prospective study was performed in Thammasat University Hospital. Thirty-six unexplained infertile couples who attended the Thammasat fertility clinic between 2005 and 2007 were included. Twenty-two to 125 unexplained infertile couples were studied and reported previously[10-12].

The inclusion criteria were the volunteer unexplained infertile couples with normal semen analysis (WHO criteria plus Kruger's strict criteria in morphology assessment), and with no abnormalities found in the ovulatory, uterine, tubal, and cervical factors. There must be at least six oocytes in metaphase II after oocyte retrieval and the couples had given informed consent to join the present study.

After control ovarian hyperstimulation by either long or short protocols with GnRH agonist (Suprefact® nasal spray, Hoechst AG, Germany) and recombinant FSH (Gonal-F®, Serono, Italy) and ovulation induction with hCG (Pregnyl®, Organon, Netherland) 10,000 units when at least two leading follicles diameter reached 18 millimeters by transvaginal ultrasonography. The oocyte retrieval was performed transvaginally 36 hours after hCG injection with double lumen needle No. 16 (Cook®,Cook, Australia) under ultrasound guided (Aloka SSD-500, Aloka, Japan). The sperm was collected by ejaculation and then analysis was done and prepared by gradient centrifugation technique with sperm preparation medium (Sil-Select Plus™, Fertipro, Belgium). The oocytes in metaphase II were equally allocated into two groups, to IVF with HIC or to ICSI according to randomization and the rest were allocated alternately to ICSI or to IVF with HIC sequentially until the last one. Group I was inseminated by in vitro fertilization (IVF) with 200,000 sperms (high insemination concentration, HIC). Group II was inseminated by ICSI standard method with micromanipulator (Narishige®, Narishige, Japan).

The fertilized oocytes were checked 16-18 hours after insemination. The group which had higher fertilization rate was continuing cultured and prepared for transfer and another group with lower fertilization rate was cryopreserved by slow freezing protocol with cyopresevation operating machine (Planner®, Planner product, United Kingdom) for frozen-thaw cycle. If there were equal fertilization rates, the fertilized oocytes in IVF with HIC group were the first priority to be cultured for embryo transfer. The embryos transfer were performed at 8 cells stage or blastocyst stage into intrauterine cavity with embryo transfer catheter (K-JET®, Cook, Australia) under transabdominal ultrasound guide (Aloka SSD-500, Aloka, Japan). The luteal support was performed by progesterone vaginal suppository (Crinone®, Serono, Italy) once a day until the date of serum hCG was checked. After the embryos were transferred, the patients had bed rest for 1 hour and the serum hCG was checked at 14 days after transfer. In pregnancy cases, the luteal support were continued until 12 weeks gestational age, the clinical pregnancy was checked by transvaginal ultrasound (Aloka SSD-500, Aloka, Japan) at 6 weeks gestational age.

Data were expressed as mean $\pm$ standard deviation (SD) ($\bar{x} \pm$ SD) for continuous data and percentage (%) for the objective outcome rate. The comparative analysis of the fertilization rate, abnormal fertilization rate, total fertilization failure rate, cleavage rate, implantation rate, pregnancy rate and pregnancy outcome in both groups were performed by Chi-square test and Fisher's exact test with statistical significance if $p < 0.05$.

Results

Thirty-six unexplained infertile couples were studied, of which 77.78% was primary infertility and

22.22% was secondary infertility. The duration of infertility was 4.15 ± 2.09 years, the mean age of female partners was 33.25 ± 4.22 years, and the mean age of male partners was 35.92 ± 4.25 years. For the protocol, 72.22% of the control ovarian hyperstimulation were performed by long protocol and 27.78% by short protocol with 1,876.39 ± 600.77 units of Gonadotropin per cycle. The mean numbers of oocyte retrievals per cycle were 14.28 ± 7.30 with 13.31 ± 6.89 metaphase II and 0.97 ± 0.40 metaphase I. The semen analysis were the followings; 126.51 ± 88.02 millions per milliliter of sperm count, 76.56 ± 21.56% of motility and 25.90 ± 9.80% of normal morphology respectively (Table 1).

The comparative analysis revealed that the mean numbers of oocyte inseminated were 6.78 ± 4.59 per cycle by IVF with HIC and 6.64 ± 4.47 per cycle by ICSI (p = 0.85). The fertilization rate of IVF with HIC and ICSI were 56.97% and 70.29% (p = 0.15), the abnormal fertilization rate (3PN, 4PN) of IVF with HIC and ICSI were 6.56% and 1.26% with statistical significance (p = 0.003). The total fertilization failure rate of IVF with HIC and ICSI were 13.89% and 0% with statistical significance (p = 0.04), and the cleavage rate were 98.00% and 98.43% respectively (p = 0.51). The mean number of embryo transfers were 2.22 ± 0.94 per cycle in IVF with HIC and 2.50 ± 0.88 per cycle in ICSI respectively (p = 0.77), whereas the implantation rate per embryo transfer were 17.50% and 16.67%, (p = 0.64), the pregnancy rates were 38.46% and 36.36% (p = 0.67), and the outcomes of pregnancy were 40% and 50% singleton, 40% and 25% twins, 20% and 25% abortion, respectively (p = 0.62, 0.56, 0.70, Table 2).

Discussion

The fertilization rate of IVF with HIC was less than that of ICSI (56.97% and 70.29%) but with no statistical significant difference which was the same as those of a previous study[10] but different from those of other studies of which ICSI had the better fertilization rate when compared with conventional IVF[11,12]. This may be due to the IVF with HIC increased the fertilization rate when compared with conventional IVF[13] and may be due to too small a sample size in the present study to reach the statistical significance when comparing IVF with HIC and ICSI. But when compared with the other cause of infertility such as moderate male factor, some studies reported that the fertilization rate of conventional IVF was statistically less than those of ICSI (37.4% vs. 64.3%) whereas those of IVF with HIC was not statistically different from those of ICSI (59.6% vs. 67.6%)[15]. Therefore, the present study may also give the same result direction. The abnormal fertilization rate (3PN, 4PN) of IVF with HIC was higher than ICSI (6.15% vs. 1.26%) with statistical significance (p < 0.05). The reason for this difference may be due to polyspermy fertilization in IVF with HIC was greater than the abnormal fertilization caused by the injury of ICSI procedure to the oocyte. The total fertilization

Table 1. Demographic data of the 36 unexplained infertile couples

	n (%)	$\bar{x}$ ± SD
Age (years)		
Male partner	-	35.92 ± 4.25
Female partner	-	33.25 ± 4.22
Type of infertility		
Primary	28/36 (77.78)	-
Secondary	8/36 (22.22)	-
Duration of infertility (years)	-	4.15 ± 2.09
Control ovarian hyperstimulation		
Long protocol	26/36 (72.22)	-
Short protocol	10/36 (27.78)	-
Dosage of gonadotropin (units/cycle)	-	1,876.39 ± 600.77
Numbers of oocyte retrieval/cycle	514 (100.00)	14.28 ± 7.30
Metaphase II	483/514 (93.97)	13.31 ± 6.89
Metaphase I	31/514 (6.03)	0.97 ± 0.40
Semen analysis		
Concentration (millions/milliliter)	-	126.51 ± 88.02
Motility (%)	-	76.56 ± 21.56
Normal morphology (%)	-	25.90 ± 9.80

Table. 2 Results of the IVF with HIC and ICSI in the 36 unexplained infertility

	IVF with HIC		ICSI		p-value
	n (%)	$\bar{x} \pm$ SD	n (%)	$\bar{x} \pm$ SD	
Numbers of metaphase II oocyte to be inseminated[a]	244/483 (50.52)	6.78 ± 4.59	239/483 (49.48)	6.64 ± 4.47	0.85
Fertilization rate	139/244 (56.97)	3.86 ± 3.66	168/239 (70.29)	4.67 ± 3.21	0.15
Total fertilization failure rate	5/36 (13.89)	-	0/36 (0)	-	0.04*
Abnormal fertilization rate	16/244 (6.56)	-	3/239 (1.26)	-	0.003*
3PN	15/244 (6.15)	-	3/239 (1.26)	-	0.005*
4PN	1/244 (0.41)	-	0/239 (0)	-	0.99
Cleavage rate	49/50 (98)	95.61 ± 13.43	63/64 (98.43)	98.96 ± 5.10	0.51
Numbers of embryo transfer per case	40/13	2.22 ± 0.94	60/22	2.50 ± 0.88	0.77
Implantation rate	7/40 (17.50)	23.15 ± 38.41	10/60 (16.67)	18.40 ± 31.47	0.64
Pregnancy rate[b]	5/13 (38.46)	-	8/22 (36.36)	-	0.67
Outcome of pregnancy					
Singleton	2/5 (40)	-	4/8 (50)	-	0.62
Twins	2/5 (40)	-	2/8 (25)	-	0.56
Abortion	1/5 (20)	-	2/8 (25)	-	0.70

[a] Total numbers of metaphase II oocyte in some cases were odd number therefore resulted in unequally divided after randomization

[b] 35 cases had embryos transfer, 1 case did not have transfer due to abnormal cleavage embryos

* p-value < 0.05

failure rate of IVF with HIC was also higher than ICSI (13.89% vs. 0%) with statistical significance ($p < 0.05$). This was similar to those of previous studies and may be due to some of unexplained infertility usually caused by the problems of naturally unfertilization[10-12]. The cleavage rate, the implantation rate, the pregnancy rate of both IVF with HIC and ICSI, were 98.00% vs. 98.43%, 17.50% vs. 16.67%, 38.46% vs. 36.36% with pregnancy outcome of 40% vs. 50% singleton, 40% vs. 25% twins and 20% vs. 25% abortion respectively. None of these parameters were statistically significantly different, which were the same as those of other studies with other causes of infertility[16,17].

Conclusion

In unexplained infertility, there may have some subgroups with the problems of naturally unfertilization even in vitro. Even though the IVF with HIC gave no significant difference in fertilization rate, it still exhibited some percentage (13.89%) of higher total fertilization failure and higher abnormal fertilization rate with statistical difference from those of ICSI. Therefore, the ICSI procedure with none of total fertilization failure rate and less abnormal fertilization rate as found in the present study may be the method of choice in this ART group.

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การศึกษาเปรียบเทียบการรักษาด้วยวิธีการฉีดเชื้ออสุจิเข้าในเซลล์ไข่และการปฏิสนธินอกร่างกายด้วยจำนวนอสุจิเข้มข้นสูง ในคู่สมรสที่มีภาวะมีบุตรยากชนิดที่หาสาเหตุไม่ได้

เจริญไชย เจียมจรรยา, พฤหัส ต่ออุดม, นันทนา ก๋ำนารายณ์

บทนำ: *คู่สมรสที่มีภาวะมีบุตรยากชนิดที่หาสาเหตุไม่ได้บางรายมีปัญหาที่มีการปฏิสนธิล้มเหลวทั้งหมด การรักษาด้วยการปฏิสนธินอกร่างกายด้วยจำนวนอสุจิเข้มข้นสูง (In vitro fertilization with high insemination concentration, HIC) และการฉีดเชื้ออสุจิเข้าในเซลล์ไข่ (Intracytoplasmic sperm injection) มีรายงานว่า มีอัตราการปฏิสนธิดีกว่าการปฏิสนธินอกร่างกาย (In vitro fertilization) ตามวิธีการมาตรฐาน*

วัตถุประสงค์: *เพื่อศึกษาเปรียบเทียบอัตราการปฏิสนธิ อัตราการปฏิสนธิที่ผิดปกติ อัตราการปฏิสนธิล้มเหลวทั้งหมด และอัตราการตั้งครรภ์ ระหว่างการรักษาด้วยการปฏิสนธินอกร่างกายด้วยจำนวนอสุจิเข้มข้นสูง (In-vitro fertilization, IVF with HIC) และการฉีดเชื้ออสุจิเข้าในเซลล์ไข่ (Intracytoplasmic sperm injection, ICSI) ในคู่สมรสที่มีภาวะมีบุตรยากชนิดที่หาสาเหตุไม่ได้*

วัสดุและวิธีการ: *การศึกษาไปข้างหน้าในคู่สมรสที่มีบุตรยากชนิดที่หาสาเหตุไม่ได้จำนวน 36 คู่ ที่มารับการรักษาในโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ระหว่างปี พ.ศ. 2548-2550 โดยการกระตุ้นไข่ด้วยวิธีการ control ovarian hyperstimulation แล้วแบ่งไข่ที่เก็บได้ในแต่ละรายออกเป็น 2 ส่วนเท่า ๆ กันแบบสุ่ม ส่วนที่1 ทำการปฏิสนธิด้วยเชื้ออสุจิเข้มข้นสูงจำนวน 200,000 ตัว ส่วนที่ 2 ทำการฉีดเชื้ออสุจิเข้าในเซลล์ไข่ แล้วคัดเลือกตัวอ่อนจากกลุ่มที่มีอัตราการปฏิสนธิสูงกว่าใส่กลับคืนเข้าในโพรงมดลูก แล้วทำการศึกษาเปรียบเทียบอัตราการปฏิสนธิ (fertilization rate) อัตราการปฏิสนธิผิดปกติ (abnormal fertilization rate) อัตราการฝังตัวของตัวอ่อน (implantation rate) อัตราการตั้งครรภ์ (pregnancy rate) และอัตราการปฏิสนธิล้มเหลวทั้งหมด (total fertilization failure rate) โดยใช้สถิติ Chi-square test และ Fisher's exact test และตั้งระดับความสำคัญทางสถิติไว้ที่ $p < 0.05$*

ผลการศึกษา: *เมื่อเปรียบเทียบอัตราการปฏิสนธิ, อัตราการปฏิสนธิผิดปกติ, อัตราการฝังตัวของตัวอ่อน, อัตราการตั้งครรภ์ และอัตราการปฏิสนธิล้มเหลวทั้งหมด ระหว่างกลุ่มที่ 1 และ 2 ได้ผลดังนี้ 50.97% ต่อ 70.29% ($p = 0.15$), 6.56% ต่อ 1.26% ($p = 0.003$), 17.50% ต่อ 16.67% ($p = 0.64$), 38.40% ต่อ 36.36% ($p = 0.67$) และ 13.89% ต่อ 0% ($p = 0.04$) ตามลำดับ*

สรุป: *การฉีดเชื้ออสุจิเข้าในเซลล์ไข่ (ICSI) มีอัตราการปฏิสนธิล้มเหลวทั้งหมด และอัตราการปฏิสนธิที่ผิดปกติน้อยกว่าของวิธีการปฏิสนธินอกร่างกายด้วยจำนวนอสุจิเข้มข้นสูง (IVF with HIC) อย่างมีนัยสำคัญทางสถิติในคู่สมรสที่มีบุตรยากชนิดที่หาสาเหตุไม่ได้*