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IVF versus ICSI in sibling oocytes from patients with polycystic ovarian syndrome: a randomized controlled trial

Jiann-Loung Hwang[1,2,5], Kok-Min Seow[1,4], Yu-Hung Lin[1,3], Bih-Chwen Hsieh[1,3], Lee-Wen Huang[1], Heng-Ju Chen[1], Shih-Chia Huang[1], Chin-Yu Chen[1], Pei-Hsin Chen[1] and Chii-Ruey Tzeng[2]

[1]Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, [2]Department of Obstetrics and Gynecology, Taipei Medical University, Taipei, [3]College of Medicine, Fu Jen Catholic University, Sinchuang City, Taipei County and [4]Department of Obstetrics and Gynecology, E-Da Hospital/I-Shou University, Kaohsiung County, Taiwan

[5]To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital, No. 95, Wen Chang Road, Shih Lin District, Taipei 111, Taiwan. E-mail: m001015@ms.skh.org.tw

BACKGROUND: This study compares the fertilization rate and embryonic development of oocytes randomly inseminated by conventional IVF or ICSI in patients with polycystic ovarian syndrome (PCOS) and normozoospermic semen during IVF cycles. METHODS: Sibling oocytes were randomized to be inseminated either by ICSI or IVF. Fertilization rate (two pronuclei/COC), day 2 embryonic morphology and rate of development were assessed. RESULTS: A total of 1089 cumulus–oocyte complexes (COC) were collected in 60 cycles (mean ± SD, 18.2 ± 7.2). Totals of 541 and 548 COC were inseminated by IVF and ICSI respectively, with a significantly higher fertilization rate in the ICSI group (ICSI versus IVF, 72.3 ± 15.5 versus 44.8 ± 25.1%). No fertilization failure occurred in the group of oocytes inseminated by ICSI, whereas the COC in nine patients (15%) inseminated by IVF had complete fertilization failure. The day 2 embryonic morphology and rate of development were not different regardless of the insemination method. CONCLUSIONS: Our results suggested that another randomized controlled study, randomizing patients instead of sibling oocytes, should be undertaken to compare the pregnancy rate per started cycle and to see whether ICSI should be performed on all, or at least on a portion of, oocytes for patients with PCOS undergoing IVF cycles.

Key words: ICSI/IVF/PCOS/sibling oocytes

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogeneous syndrome characterized by chronic anovulation and hyperandrogenism (Franks, 1995). PCOS is the most prevalent cause of anovulatory infertility in reproductive age women (Franks, 1995). IVF is an effective treatment after repeated failure of ovulation drugs administration (Dor *et al.*, 1990; Homburg *et al.*, 1993). During IVF cycles, the fertilization rate had been reported to be significantly lower in patients with PCOS than in patients with other causes of infertility (Dor *et al.*, 1990; Urman *et al.*, 1992; Homburg *et al.*, 1993; MacDougall *et al.*, 1993; Kodama *et al.*, 1995; Sengoku *et al.*, 1997; Doldi *et al.*, 1999). High incidence of unpredictable total failure of fertilization was reported in patients with PCOS (Kodama *et al.*, 1995; Marci *et al.*, 2001).

It has been suggested that ICSI, a technique that initially developed to overcome severe male factor infertility (Palermo *et al.*, 1992; Devroey and Van Steirteghem, 2004), can be applied in other non-male factor infertile patients such as previous complete fertilization failure or a low fertilization rate in conventional IVF cycles (Fishel *et al.*, 2000; Oehninger and Gosden, 2002; Mahutte and Arici, 2003). Split insemination of oocytes by IVF and ICSI was suggested in patients with unexplained infertility undergoing IVF treatment to eliminate fertilization failure (Hershlag *et al.*, 2002). Will ICSI be beneficial for patients with PCOS during their IVF cycles? The hypothesis is that, by using ICSI, the fertilization rate will increase. The present study was conducted in patients with PCOS and normozoospermic semen undergoing IVF treatment by insemination of sibling oocytes randomly using conventional IVF or ICSI from the same semen sample. The primary outcome measure was fertilization rate. The secondary outcome measures were complete fertilization failure rate, embryonic morphology and rate of development.

Materials and methods

Patients

There were 66 patients with PCOS who entered the IVF programme for the first time after failure of conception with four to six previous attempts of ovulation induction, between January 1998 to January 2000. The diagnosis of PCOS was made with the following criteria:

(i) chronic anovulation; (ii) characteristic ultrasonographic appearance—enlarged ovary with >10 peripherally located follicles of 3–8 mm in diameter around a dense central stroma (Adams *et al.*, 1985); (iii) at least one of the following abnormal hormonal profiles: elevated serum LH (>10 mIU/ml), elevated LH:FSH ratio (>2), elevated serum testosterone (>0.8 ng/ml). The mean age and duration of infertility was 32.5 ± 2.3 years and 4.5 ± 1.3 years respectively. All of the male partners had normal semen quality with a sperm count of $\geq 20 \times 10^6$ sperm cells/ml, ≥4% morphologically normal sperm cells (Kruger's strict criteria) and ≥50% progressively motile sperm cells. The study was approved by the Ethics Committee of Shin Kong Wu Ho-Su Memorial Hospital. All couples were required to give informed consent in writing. The risks of ICSI were fully explained to the couples. The exclusion criteria were: (i) age >38 years; (ii) day 3 FSH levels >12 mIU/ml; (iii) fewer than six cumulus–oocyte complexes (COC) retrieved.

Ovarian stimulation

All patients received ovarian stimulation with a GnRH agonist desensitization protocol. A GnRH agonist, buserelin acetate (Supremon; Hoechst, Germany), 500 μg/day was administered ≥2 weeks after a progestin-induced withdrawal bleeding or spontaneous menstruation and decreased to 250 μg/day upon gonadotrophin administration until the day of hCG (Pregnyl; NY Organon, The Netherlands) injection. HMG (Pergonal; Serono, Switzerland), 150 IU/day, was administered for 6 days after pituitary desensitization (serum E_2 <50 pg/ml and no ovarian cyst >10 mm) and the dose was adjusted thereafter according to the clinical response. HCG was given when there were at least three leading follicles >18 mm with adequate serum estradiol (E_2) levels. The serum E_2 levels had to be <3600 pg/ml to lessen the chance of severe ovarian hyperstimulation syndrome (Chen *et al.*, 1997). Transvaginal oocyte retrieval was performed 36 h later.

Sperm preparation, oocyte retrieval, insemination or injection, embryo evaluations

The culture medium used was P-1 medium (Irvine Scientific, USA) supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific). The cultures were done in a humidified atmosphere of 5% CO_2 in air at 37°C. After completion of oocyte retrieval, the first COC retrieved was allocated either to ICSI or to IVF according to blocked randomization, and the rest of the COC were allocated alternately to ICSI or IVF i.e. one to ICSI, then one to IVF, and so on (Tournaye *et al.*, 2002). The oocyte–cumulus complexes (COC) were preincubated for 3–6 h before insemination or injection. Both procedures were done simultaneously. The semen sample on the day of oocyte retrieval was verified to be normal. Sperm were prepared using swim-up technique, and were inseminated at a concentration of 50 000 motile sperm/ml. The cumulus cells were removed in the group of COC treated by ICSI, and only the metaphase II (MII) oocytes were injected as described by Van Steirteghem *et al.* (1993). Embryos were checked for pronuclei 16–18 h later. The fertilization rate was expressed as numbers of two pronuclei (2PN) per numbers of COC. The embryos were evaluated 24 h later (42 h after insemination or injection) by another laboratory staff member who did not know the insemination method of oocyte. The embryo classification was modified from the system described by Cummins *et al.* (1986). Grade 1 embryos consisted of symmetrical blastomeres of approximately equal size and without anucleate fragments. Grade 2 embryos had blastomeres of even or uneven size and had <10% of the volume of embryos filled with anucleate fragments. Grade 3 embryos had anucleate fragments occupying between 10 and 50% of the volume of the embryos. Grade 4 embryos had anucleate fragments >50% of the volume of the embryos.

Embryo transfer and pregnancy

Embryo transfer was performed on day 2 after insemination or injection. The best embryos (grade 1 or 2 with 4-cell stage) were selected for transfer. Supernumerary embryos were frozen. Luteal phase was supported by 600 mg of vaginally administered micronized progesterone (Utrogestan; Laboratories Piette International S.A., Belgium). Clinical pregnancy was defined as a visible fetal heart beat on ultrasonography at 7 weeks gestation.

Statistical analysis

A power calculation with an α error of 5% and a β error of 20% was performed by PASS statistical package 6.0 (NCSS, USA). On the basis of our previous experience and the background literature, it was assumed that given a fertilization rate of 50% in the IVF group and 65% in the ICSI group, then ≥170 oocytes would need to be inseminated in each study arm.

Paired and unpaired *t*-tests and χ^2-test were used for the statistical analysis where appropriate. $P < 0.05$ was considered significant. Analysis was performed using the SPSS statistical package for Windows 10.0 (SPSS Inc., USA).

Results

Figure 1 shows the stages of the randomized controlled trial. A total of 66 patients was assessed for eligibility. Among them, one refused to participate and one did not meet the criteria. Of the remaining 64 cases (64/66), four dropped out from the study. Two were due to fewer than six COC collected and two were oocyte retrieval cancellation for fear of severe ovarian hyperstimulation syndrome. There were 1089 COC collected in the 60 completed cycles (60/66), with a mean (SD) of 18.2 ± 7.2 COC per cycle. The number of COC inseminated by IVF was 541 (a mean of 9.0 ± 3.6 COC per cycle) and 548 COC were treated by ICSI (a mean of 9.1 ± 3.6 COC per cycle) (Table I).

Of the COC treated by ICSI, 456 COC (83.3 ± 12.6%) were found to be metaphase II oocytes. The fertilization rate (2PN/COC) was significantly higher in the group of COC treated by ICSI, compared to the group inseminated by IVF (Table I). No fertilization failure occurred in the group of oocytes treated by ICSI, while fertilization failure occurred in nine patients (15%) treated by conventional IVF (79 COC). The rate of complete fertilization failure was significantly higher in the COC ($n = 541$) inseminated by IVF, compared to the MII oocytes ($n = 456$ from 548 COC) inseminated by ICSI. The odds ratio of the patients undergoing IVF insemination and having fertilization failure was 1.176, with a 95% confidence interval of 1.058–1.308. The number needed to treat (NNT) was 5.68. There was no significant difference in the numbers of oocytes per patient inseminated by IVF between the patients with or without complete fertilization failure (8.8 ± 2.4 versus 9.1 ± 3.8, 79 COC in nine patients versus 462 COC in 51 patients). As shown in Table II, no significant difference existed in the rates of MII oocytes and fertilization rate in oocytes treated by ICSI, between patients with no fertilization failure ($n = 51$, 469

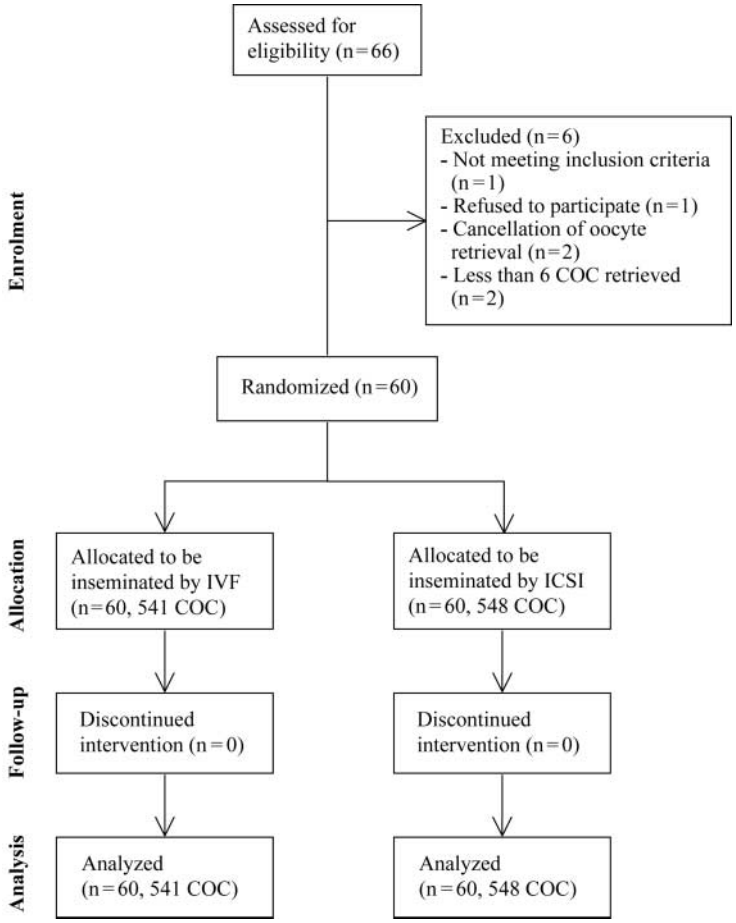

Figure 1. Flow diagram of the stages through the randomized controlled trial.

COC, 390 MII oocytes, 336 2PN) and those patients with fertilization failure ($n = 9$, 79 COC, 66 MII oocytes, 56 2PN) by IVF.

Table III showed the comparison of embryonic morphology and rate of development 42 h after insemination in 51 couples without fertilization failure after either conventional IVF or ICSI. There was no significant difference between the two groups.

Discussion

In the present study we have randomly inseminated the sibling oocytes in patients with PCOS, whose husband had normal semen quality by World Health Organization (1992) and Kruger's strict criteria, either by conventional IVF or ICSI. We found that: (i) the percentage of matured oocytes were not different between patients who had complete fertilization failure or no fertilization failure by IVF; (ii) the oocytes inseminated by ICSI had a significantly higher fertilization rate than those inseminated by conventional IVF; (iii) the complete fertilization failure by conventional IVF was as high as 15%, whereas there was no fertilization failure in oocytes inseminated by ICSI; (iv) despite the insemination method, the developmental potential in terms of day 2 embryonic morphology and rate of cellular cleavage were similar.

Although more oocytes are usually retrieved, reduced fertilization rate has been described for patients with PCOS undergoing IVF, compared to patients with tubal infertility or normal ovaries (Dor *et al.*, 1990; Urman *et al.*, 1992; Homburg *et al.*, 1993; MacDougall *et al.*, 1993; Kodama *et al.*, 1995; Sengoku *et al.*, 1997; Doldi *et al.*, 1999). The lower fertilization rate might be due to poor oocyte quality resulting from long-term abnormal hormonal milieu in PCOS patients such as raised tonic levels of LH, insulin resistance, abnormal insulin-like growth factor system and overproduction of androgens (Kodama *et al.*, 1995; Sengoku *et al.*, 1997; Tarlatzis and Grimbizis, 1997; Ludwig *et al.*, 1999; Stadtmauer *et al.*, 2001). The mechanism by which endocrine imbalance causes impairment of oocyte quality is not well understood. Even in non-PCOS patients, elevated LH levels have been reported to significantly reduce the fertilization and cleavage rates (Stanger and Yovich, 1985). The percentage of MII oocytes in our study was comparable to the couples with male infertility and normal ovarian function treated by ICSI in our programme (data not shown) and others (Devroey and Van Steirteghem, 2004). Our study showed that the percentage of MII oocytes (inseminated by ICSI) from patients who had fertilization failure by IVF was similar to that of patients who had no fertilization failure by IVF. It suggested that nuclear maturity was not the main

Table I. Fertilization in sibling oocytes inseminated by conventional IVF or by ICSI

	IVF	ICSI	*P*
All patients ($n = 60$)			
Total no. of COC	541	548	
No. of COC per patient	9.0 ± 3.6	9.1 ± 3.6	
Total no. of MII oocytes	–	456	
No. of MII oocytes/no. of COC (%)	–	83.3 ± 12.6	
Total no. of 2PN oocytes	246	392	
No. of 2PN oocytes/no. of COC (%)	44.8 ± 25.1	72.0 ± 15.0	<0.001[a]
Without fertilization failure ($n = 51$)			
Total no. of COC	462	469	
No. of COC per patient	9.1 ± 3.8	9.2 ± 3.8	
Total no. of MII oocytes	–	390	
No. of MII oocytes/no. of COC (%)	–	83.2 ± 12.9	
Total no. of 2PN oocytes	246	336	
No. of 2PN/no. of COC (%)	52.8 ± 17.8	72.3 ± 15.5	<0.001[a]
With fertilization failure in IVF ($n = 9$)			
Total no. of COC	79		
No. COC per patient	8.8 ± 2.4		
Total no. of MII oocytes	–		
No. of MII oocytes/no. of COC (%)	–		
Total no. of 2PN oocytes	0		
No. of 2PN oocytes/no. of COC (%)	0		

COC = cumulus–oocyte complexes; MII = metaphase II; 2PN = two-pronuclear oocytes.
Expressed as mean ± SD per patient where appropriate.
[a]Paired *t*-test.

Table II. Fertilization in oocytes treated by ICSI according to patients whose sibling oocytes having or not having fertilization failure by IVF

	No fertilization failure ($n = 51$)	Fertilization failure ($n = 9$)	P^a
Total no. of COC	469	79	
No. of COC per patient	9.2 ± 3.8	8.8 ± 2.2	
Total no. of MII oocytes	390	66	
No. of MII oocytes/no. of COC (%)	83.2 ± 12.9	84.0 ± 11.9	NS
Total no. of 2PN oocytes	336	56	
No. of 2PN/no. of COC (%)	72.3 ± 15.5	70.5 ± 12.4	NS

2PN = two-pronuclear oocytes; COC = cumulus–oocyte complexes; NS = not significant.
Expressed as mean ± SD per patient where appropriate.
[a]Unpaired *t*-test.

cause of failed fertilization or low fertilization rate. Similar implications have been reported (Sengoku *et al.*, 1997; Ludwig *et al.*, 1999). Ludwig *et al.* (1999) retrospectively compared 51 ICSI cycles of 31 PCOS patients having concomitant male factor infertility with 105 ICSI cycles of age-matched controls using the same ovarian stimulation protocol. The percentages of MII oocytes were not different. The clinical pregnancy rate was similar whereas the abortion rate was significantly higher in the PCOS patients. They suggested that some cytoplasmic factors, not nuclear maturity, might negatively influence the developmental potential of embryos and the outcome of pregnancy in PCOS patients. Sengoku *et al.* (1997) compared the chromosome normality of unfertilized oocytes in patients with PCOS with those of

Table III. Comparison of embryonic morphology and rate of development (day 2) in 51 couples with fertilization after conventional IVF and ICSI

	IVF	ICSI	P^a
Total no. of zygotes	246	336	
Total no. of embryo cleavage	229	325	
Embryo quality (mean ± SD, %)			
Grade 1 embryo	31.6 ± 24.0 (74)	28.9 ± 20.9 (94)	NS
Grade 2 embryo	32.9 ± 28.4 (69)	36.9 ± 20.0 (112)	NS
Grade 3 embryo	27.0 ± 21.3 (61)	26.4 ± 14.2 (89)	NS
Grade 4 embryo	8.4 ± 12.1 (25)	7.9 ± 8.7 (30)	NS
Developmental stage (42 h after insemination) (mean ± SD, %)			
2-cell	34.3 ± 31.1 (68)	29.4 ± 14.7 (92)	NS
3-cell	13.9 ± 14.9 (32)	10.5 ± 11.5 (35)	NS
4-cell	49.3 ± 22.4 (113)	52.0 ± 13.6 (170)	NS
≥ 5-cell	4.8 ± 8.5 (16)	8.3 ± 10.1 (28)	NS

Values in parentheses are raw numbers.
NS = not significant.
[a]Paired *t*-test.

patients with tubal infertility. The age, duration of infertility and ovarian stimulation protocol were similar. The unfertilized oocytes showed no significant difference in chromosome number between the two groups. The fertilization rate was significantly lower in the patients with PCOS that was not attributable to chromosome aberrations or oocyte immaturity (Sengoku *et al.*, 1997). Oocyte intrinsic abnormalities, such as abnormal expression of growth differentiation factor-9, may contribute to aberrant folliculogenesis in PCOS women (Teixeira Filho *et al.*, 2002).

For most IVF programmes, the incidence of fertilization failure occurred in 5–10% of IVF cycles and 2–3% in ICSI cycles (Mahutte and Arici, 2003). Because the couples enrolled in our study had normal semen quality by World Health Organization (1992) and Kruger's strict criteria, a good fertilization rate and low incidence of fertilization failure should be expected (Mahutte and Arici, 2003). However, the complete fertilization failure rate by IVF was as high as 15% in our study, in contrast to no fertilization failure occurring in the oocytes inseminated by ICSI. The sibling oocytes inseminated by IVF had a significantly lower fertilization rate, compared to those inseminated by ICSI. It is suggested that ICSI could overcome the low fertilization rate and high complete fertilization failure rate in PCOS patients during their IVF cycles. Because ICSI bypasses the sperm–zona pellucida interaction, our results imply that some abnormality in the zona pellucida might be present in PCOS patients. Kodama *et al.* (1995) reported a complete fertilization failure rate of 18% in PCOS patients (76 cycles) inseminated by conventional IVF, as compared to 5% in the control group (408 cycles). A failed fertilization rate of 12.2 and 13.7% was reported by other two studies (MacDougall *et al.*, 1993; Marci *et al.*, 2001).

The quality of embryos obtained in terms of day 2 embryonic morphology and rate of cellular development were comparable, irrespective the method of insemination. Staessen *et al.* (1999) conducted a comparative study in sibling oocytes inseminated by IVF or ICSI from couples with tubal infertility and normozoospermic semen. Ménézo B2 medium (bioMérieux, France) was used. They found that

the embryonic morphology was similar, whereas the proportion of 4-cell stage embryos was higher in the sibling oocytes inseminated by ICSI than those inseminated by IVF. The different sources of oocytes might explain the different results of the two studies. Differences in cleavage characteristics could also be dependent on type of culture media used. Micromanipulation technique appears to have no negative effect on the embryonic development. However, it should be noted that a slightly increased incidence of chromosomal abnormalities have been found in 1082 karyotypes of ICSI children (Aytoz *et al.*, 1998; Bonduelle *et al.*, 1998, 2002a,b, 2003).

Stadtmauer *et al.* (2001) performed ICSI in PCOS patients undergoing IVF because they experienced poor fertilization rate by conventional IVF in a number of patients unless ICSI was used. Our study shows that another randomized controlled study, randomizing patients instead of sibling oocytes, should be undertaken to compare the pregnancy rate per started cycle and to see whether ICSI should be performed on all, or at least on a portion of, oocytes for patients with PCOS undergoing IVF cycles.

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