Original Article
Patients with polycystic ovary syndrome have successful embryo arrest

Baoli Yin, Haoying Hao, Duo Wei, Xiaobing Song, Juanke Xie, Cuilian Zhang

Reproductive Medicine Center, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, P. R. China

Abstract: In this retrospective study, we investigate the relationship between embryo arrest and polycystic ovary syndrome (PCOS) during in vitro fertilization-embryo transfer (IVF-ET). In this study, 667 subjects were enrolled, including 330 patients with PCOS and 337 subjects without PCOS. The subjects underwent in vitro fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET) cycles at the Reproductive Medical Centre of Henan Provincial Hospital from January 2009 to December 2012. Four protocols were used to stimulate the ovaries, including long protocol, super-long down-regulation protocol, short protocol and antagonist protocol. Oocytes were retrieved using transvaginal ultrasound guidance. Pronuclei were checked on the next morning after IVF/ICSI. Cleavage stage embryo was assessed after 62-66 hours. Women with PCOS had significantly elevated body mass index, basal luteinizing hormone, estradiol and testosterone compared with normal women. Basal Follicle stimulating hormone level in PCOS patients was lower compared with that in control group. After IVF-ET, PCOS patients had more available oocytes than subjects in control group. PCOS patients had slightly lower fertilization rate than the controls in IVF cycles, but in ICSI cycles, fertilization rate in PCOS patients was significantly higher than that in controls. For either IVF or ICSI, the embryo arrest rate was not changed by PCOS. Moreover, there was no significant difference in embryo arrest rate between both groups adopting different stimulation protocols. Interestingly, embryo arrest rate was not correlated with testosterone for patients in PCOS group. The data indicated that patients with PCOS had successful early embryo arrest during IVF-ET.

Keywords: Polycystic ovary syndrome, embryo arrest, testosterone, in vitro fertilization, intracytoplasmic sperm injection, embryo transfer

Introduction

During in vitro fertilization-embryo transfer (IVF-ET), the pronuclei can be observed 18-20 hours after insemination or intracytoplasmic sperm injection (ICSI) in humans [1]. After 38 and 62 hours, the embryo is at 4-cell and 8-cell stages, respectively. Normal velocity of embryo cleavage is crucial for embryo selection during IVF-ET. During IVF, no more than 50% embryos can develop into blastocyst stage [2]. About 10-15% IVF embryos are arrested at 2-4-cell cleavage stage without any morphological or biochemical signs of apoptosis [3-8]. If these embryos are transferred, most of them are unable to further develop [9].

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathy for women at reproductive ages [10]. Many studies have demonstrated that oocyte quality and embryo development may be affected by PCOS [11]. However, it is still obscure whether these deleterious effects can induce developmental arrest of early embryos during IVF. In this retrospective study, we analyze the relationship between PCOS and embryo arrest during IVF-ET.

Material and methods

Patients

In the present study, we reviewed 667 patients (330 patients with PCOS and 337 patients without PCOS) who underwent IVF/ICSI-ET at the Reproductive Medical Centre of Henan Provincial People's Hospital from January 2009 to December 2012. The diagnosis of PCOS was based on Rotterdam criteria [12]. All participants had not received any medication for three months before IVF. Their ages ranged from 20 to 40 years and their body mass indexes were

Table 1. Clinical characteristics of normal subjects and PCOS patients (means ± SD)

	Normal (n = 330)	PCOS (n = 337)	P vaule
Age (years)	33.3 ± 0.26	32.8 ± 0.22	0.672
BMI (kg/m^2)	22.4 ± 0.16	24.4 ± 0.21*	0.000
Basal FSH (IU/L)	7.54 ± 0.15	6.03 ± 0.10*	0.000
Basal LH (IU/L)	4.24 ± 0.16	7.42 ± 0.29*	0.000
Basal estradiol (pg/ml)	162 ± 4.83	190 ± 7.23*	0.001
Testosterone (ng/ml)	0.83 ± 0.020	1.30 ± 0.046*	0.000

Note: PCOS, polycystic ovary syndrome; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone. *, $P < 0.05$.

between 20 and 30. There were no other etiologies for the PCOS cases, such as congenital adrenal hyperplasia, 21-hydroxylase deficiency, androgen-secreting tumors, Cushing's syndrome, thyroid disease, and hyperprolactinemia. Women who underwent IVF/ICSI-ET because of tubal or male factors were enrolled as controls without other diseases such as ovary tumor, uterus tumor, endometriosis, hyperthyroidism, hyperprolactinemia, and diabetes. This work was approved by the Ethics Committee of Henan Provincial People's Hospital of Zhengzhou University. Written informed consents were obtained from all patients or their families.

Ovarian stimulation and embryo transfer

Group 1 (72 subjects) underwent long protocol: Gonadotropin-releasing hormone-agonist Decapeptyl) (Ipsen, UK) was administered approximately 1 week before the expected period day (day 21 of a 28-day cycle) for pituitary down-regulation. Then, ovary was stimulated by recombinant follicle stimulating hormone (FSH) (Gonal-F; Serono, Switzerland). Group 2 (23 subjects) underwent super-long down-regulation protocol: 3.75-1.875 mg long-acting triptorelin was injected on day 2-4 of the cycle. After 28-30 days, 3.75-1.875 mg long-acting triptorelin was given again. Ovarian stimulation was initiated with recombinant FSH after 28-30 days. Group 3 (32 subjects) underwent short protocol: From day 3 of menses, 0.1 mg Decapeptyl and recombinant FSH were both administered daily until the day of human chorionic gonadotropin injection. Group 4 (33 subjects) underwent antagonist protocol: On day 2 of menses, 150-300 IU recombinant FSH or clomiphene were given, if endometrium thickness was < 5 mm, the diameter of the largest follicle was < 10 mm and estradiol level was < 50 pg/ml. Gonadotropin dosage should be adjusted or supplemented after 5 days. Once

the diameter of the dominant follicle reached 14 mm, 0.25 mg gonadotropin-releasing hormone-agonist Cetrotide (Serono, Switzerland) was administered. When the diameter of 3 or more follicles reached 17 mm, 6,000-10,000 IU human chorionic gonadotropin (Livzon Pharmaceutical Group Co., Ltd., Guangdong, China) was administered. After 36 hours, oocytes were retrieved using transvaginal ultrasound guidance.

Embryo assessment

Pronuclei were checked on the next morning after IVF/ICSI (Day 1, 16-20 hours after insemination). Cleavage stage embryo was assessed on Day 3 (62-66 hours after insemination). Good quality embryos should satisfy the following criteria: normal development velocity; even blastomere size; and less than 5% fragment. If only 2 to 4 blastomere were presented for Day 3 embryos, the embryos would be classified as development arrest [13].

Seventy-two hours after oocyte retrieval, one to two good quality embryos were transferred to the uterus. If serum level of human chorionic gonadotropin was > 10 IU/L on day 14 after embryo transfer, the subject was regarded biochemically pregnant. If gestational sac was visualized 4 weeks later, the subject was considered clinically pregnant.

Statistical analysis

All statistical analysis was performed by SPSS 13.0 (Chicago, USA). Quantitative variables were described as means ± SD. Comparison between groups was performed by One-way ANOVA. Nominal and frequency data were analyzed using Chi-square test or Fisher's exact test as appropriate. *P* values less than 0.05 were considered statistically significant for all statistical tests.

Results

The pregnant results are similar between two groups and embryo arrest is not affected by PCOS

To investigate the effect of IVF-ET, the clinical parameters and IVF-ET outcomes of normal subjects and PCOS patients were analyzed.

Table 2. IVF-ET outcome in normal subjects and PCOS patients (means ± SD)

	Normal (n = 330)	PCOS (n = 337)	P vaule
Available oocytes	11.47 ± 0.34	14.03 ± 0.36*	0.000
Fertilization rate (%)	0.75 ± 0.01	0.76 ± 0.01	0.408
2PN rate (%)	0.63 ± 0.01	0.65 ± 0.01	0.271
Oocyte cleavage rate (%)	0.73 ± 0.01	0.74 ± 0.01	0.332
Arrest rate (%)	0.08 ± 0.01	0.06 ± 0.01	0.178
Transferred embryos	2.2 ± 0.03	2.2 ± 0.02	0.405
Implantation rate (%)	0.22 ± 0.02	0.21 ± 0.02	0.785
Clinical pregnancy rate (%)	0.39 ± 0.0	0.35 ± 0.03	0.338

Note: IVF-ET, in vitro fertilization-embryo transfer; PCOS, polycystic ovary syndrome; 2PN, 2 pronucleus. *$P < 0.05$.

Table 3. Fertilization rate and arrest rate after IVF or ICSI in normal subjects and PCOS patients (means ± SD)

	Normal (n)	PCOS (n)	P vaule
IVF Fertilization rate (%)	0.77 ± 0.01 (160)	0.75 ± 0.02 (182)	0.366
ICSI Fertilization rate (%)	0.73 ± 0.02 (170)	0.77 ± 0.01 (155)*	0.044
Arrest rate in IVF	0.07 ± 0.01 (160)	0.07 ± 0.01 (182)	0.578
Arrest rate in ICSI	0.08 ± 0.01 (170)	0.06 ± 0.01 (155)	0.211

Note: IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; PCOS, polycystic ovary syndrome. *$P < 0.05$.

Women with PCOS had significantly elevated body mass index, basal luteinizing hormone, estradiol and testosterone compared with normal women. Basal FSH level in PCOS patients was lower compared with that in control group (**Table 1**). After IVF-ET, PCOS patients had more available oocytes than subjects in control group (P < 0.05). However, no significant differences were found for fertilization rate, 2 pronucleus rate, cleavage rate, embryo quantity, implantation rate, clinical pregnancy rate or embryo arrest rate between PCOS patients and subjects in control group (**Table 2**). These data suggested that the pregnant results were similar between two groups and embryo arrest was not affected by PCOS.

Embryo arrest rate is not associated with testosterone level, age and insemination manners

To evaluate the relationship between embryo arrest and PCOS, different insemination manners were used, including IVF and ICSI. The results showed that PCOS patients had slightly lower fertilization rate than the controls in IVF cycles, although the difference was not significant (P = 0.366). However, in ICSI cycles, fertilization rate in PCOS patients was significantly

higher than that in controls (P = 0.044). In addition, embryo arrest rate was not affected by PCOS for either IVF or ICSI (**Table 3**). In addition, the embryo arrest rate of patients ≤ 35 years old was similar to that of patients ≥ 35 years old in both groups (**Table 4**). Interestingly, different stimulation protocols did not affect the embryo arrest rate for both groups (**Table 5**). Correlation analysis showed a positive correlation between embryo arrest and testosterone level for PCOS patients > 35 years old ($r^2 = 0.404$, $P < 0.05$). However, the embryo arrest rate was similar between PCOS patients and control subjects (**Table 6**). These data indicated that the embryo arrest rate was not associated with testosterone level, age and insemination manners.

Discussion

Ludwig and colleagues retrospectively reviewed 31 PCOS patients (51 cycles) and age-matched control subjects (105 cycles), and discovered that there was no difference in pregnancy rate but the clinical abortion rate for PCOS patients was higher than normal subjects [14]. The authors suggested that only cytoplasmic but not nuclear maturity was influenced in PCOS patients [14]. Consistent with these results, the present study showed no difference in 2 pronucleus rate between the two groups. During IVF treatment, PCOS was prone to have decreased fertilization rate and even fertilization failure [15-21]. It is proposed that long standing hyperandrogenemia, high luteinizing hormone level and insulin resistance could result in compromised oocyte quality [14, 15, 20, 22, 23]. Our study showed that, compared with controls, PCOS patients had slightly lower fertilization rate in IVF cycles, but significantly higher fertilization rate in ICSI cycles. Hwang and colleagues indicated that there were certain abnormalities in zona pellucida of PCOS patients that resulted in decreased fertilization rate [24]. ICSI could avert these abnormalities and thus increase fertilization rate. However, bad oocyte quality and subsequently compromised embryo quality could not be remedied by ICSI.

Table 4. The effect of age on the arrest rate in normal subjects and PCOS patients(means ± SD)

Age (year)	Normal		PCOS	
	IVF	ICSI	IVF	ICSI
≤ 35	0.079 ± 0.013 (n = 106)	0.096 ± 0.014 (n = 126)	0.064 ± 0.010 (n = 152)	0.065 ± 0.012 (n = 135)
> 35	0.061 ± 0.015 (n = 54)	0.051 ± 0.027 (n = 44)	0.077 ± 0.023 (n = 30)	0.048 ± 0.027 (n = 20)

Note: PCOS, polycystic ovary syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

Table 5. The effect of different stimulation protocols on the arrest rate in normalsubjects and PCOS patients (means ± SD)

Protocols	Normal		PCOS	
	IVF	ICSI	IVF	ICSI
Long protocol	0.071 ± 0.015 (n = 72)	0.091 ± 0.022 (n = 73)	0.071 ± 0.104 (n = 74)	0.068 ± 0.021 (n = 58)
Ultra-long protocol	0.077 ± 0.026 (n = 23)	0.057 ± 0.040 (n = 13)	0.103 ± 0.033 (n = 18)	0.043 ± 0.029 (n = 15)
Short protocol	0.089 ± 0.022 (n = 32)	0.079 ± 0.021 (n = 44)	0.036 ± 0.021 (n = 21)	0.072 ± 0.041 (n = 14)
Antagonist protocol	0.062 ± 0.020 (n = 33)	0.088 ± 0.025 (n = 40)	0.061 ± 0.014 (n = 69)	0.062 ± 0.017 (n = 68)

Note: PCOS, polycystic ovary syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

Table 6. Correlation between arrest rate and testosterone levels

Age (year)	Normal		PCOS	
	IVF	ICSI	IVF	ICSI
≤ 35	0.070	-0.037	0.095	0.061
> 35	-0.030	-0.016	0.404*	-0.121

Note: PCOS, polycystic ovary syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection. *$P < 0.05$.

On the other hand, only metaphase II oocytes were inseminated in ICSI cycles, which could eliminate the immature oocytes.

Zhong and colleagues showed that abnormalities of endocrine profile could decrease fertilization rate and cleavage rate [25]. Hyperandrogenemia is a key feature of PCOS. High androgen level could stimulate the proliferation of granulosa cells and suppress their apoptosis, independent of the role of gonadotropin [26]. Self-sterilization of the ovary depends on the apoptosis of granulosa cells. Apoptosis of granulosa cells is closely related to follicular atresia. As a result, apoptosis of granulosa cells is a prerequisite for the selection of dominant follicles. Because of the higher testosterone level, the ovaries of PCOS patients could recruit a large number of small antral follicles that are destined to undergo atresia. In addition, the oocytes of these small follicles could be congenitally flawed.

No difference was found for embryo arrest rate between PCOS patients and controls. However, women with PCOS who were > 35 years old showed positively correlated testosterone level and embryo arrest rate. On one hand, high testosterone level is hazardous to embryo quality in PCOS patients. On the other hand, embryo quality gradually deteriorates as the age of patients increases. Follicles that are destined to undergo atresia are recruited because of high testosterone level. In addition, ovarian stimulation protocols were not associated with embryo arrest. It is possible that short term medication cannot alter developmental potential of oocytes. In summary, embryo arrest rate is not affected by PCOS in IVF-ET.

Disclosure of conflict of interest

None.

Address correspondence to: Cuilian Zhang, Reproductive Medicine Center, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, No. 7 Weiwu Road, Zhengzhou 450003, P. R. China. Tel: +86-371-65897665; Fax: +86-371-65897665; E-mail: gezhaojia6677@163.com

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