Effect of Mitochondrial Function of Ovarian Granulosa Cells on *In Vitro* Fertilization and Embryo Transfer Outcomes in Obese Polycystic Ovary Syndrome Patients

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Abstract: *Objective:* To investigate the effect of abnormal ovarian granulosa cell metabolism on *in vitro* fertilization and embryo transfer (IVF-ET) outcomes in obese polycystic ovary syndrome (PCOS) patients. *Methods:* Patients with PCOS who met the study criteria were screened according to the inclusion criteria. A total of 32 patients with obese PCOS were recruited into the study group, and 39 patients with non-obese PCOS were recruited into the control group. The general data (age, body mass index, and years of infertility), insulin resistance index (HOMA-IR), follicle-stimulating hormone (FSH), luteinizing hormone (LH), granulosa cell mitochondrial function, and IVF-ET outcome of patients in the study group and control group were retrospectively analyzed. *Results:* The differences in age and years of infertility between the study group and the control group were insignificant ($P > 0.05$), and the body mass index (BMI) of the study group and control group was 30.5 ± 1.24 kg/m^2 and 22.3 ± 1.12 kg/m^2, respectively, in which the difference was statistically significant ($P < 0.05$); the HOMA-IR of the study group was significantly higher than that of the control group ($P < 0.05$); the reactive oxygen species (ROS) in the study group was significantly higher than that in the control group ($P < 0.05$), and the ATP content in the study group was significantly lower than that in the control group ($P < 0.05$); comparing the FSH and LH levels between the two groups, the difference was not statistically significant ($P > 0.05$); the rate of IVF-ET failure was significantly higher in the study group than in the control group. *Conclusion:* PCOS is a complex endocrine disorder, and obesity is one of the independent risk factors for the development of PCOS.

Keywords: Polycystic ovary syndrome; Mitochondrial function; Ovarian granulosa cell; *In vitro* fertilization and embryo transfer

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by oligo-ovulation or anovulation, polycystic ovarian changes, hyperandrogenemia, and/or hyperinsulinemia, and it is a common cause of infertility. Obesity (body mass index ≥ 30 kg/m^2) is an independent risk factor for PCOS and is strongly associated with the clinical outcomes in patients with PCOS; weight gain and increased risk of obesity-related diseases have been reported in patients with PCOS. Due to abnormal ovarian function in patients with PCOS, patients with fertility requirements are usually treated clinically by *in vitro* fertilization and embryo transfer (IVF-ET); however, the incidence of IVF-ET failure in patients with PCOS is as high

as 30%–50%. It is now believed that endocrine disruption in PCOS patients in IVF-ET can affect IVF-ET outcomes by affecting granulosa cell mitochondrial function [1-6]. It has been shown that abnormal mitochondrial function is associated with decreased ovarian reserve and granulosa cell apoptosis due to abnormal mitochondrial function plays an important role in the development of PCOS [7]. However, no studies related to obesity and abnormal mitochondrial function in ovarian granulosa cells of PCOS patients have been reported in literature. Therefore, we intend to analyze the relationship between obesity and abnormal ovarian granulosa cell mitochondrial function in patients with PCOS and provide new ideas to improve IVF-ET outcomes in PCOS patients.

2. Data and method
2.1. General data
Patients with PCOS who met the study criteria were screened according to the inclusion criteria. The exclusion criteria included (1) presence of endometriosis, endometrial lesions, or ovarian tumors; (2) presence of insulin resistance or hyperinsulinemia; (3) family history of tumors; and (4) a history of PCOS-related diseases. A total of 32 patients with obese PCOS who met the criteria were recruited into the study group, and 39 women with non-obese PCOS, who were outpatients and hospitalized during the same period, were recruited into the control group.

2.2. Methods
(1) All study subjects were tested by drawing venous blood on the 5th day of menstruation, and urine specimens were collected for biochemical analysis. General clinical data and laboratory indices were recorded, and BMI was calculated using the formula.

(2) Controlled ovarian hyperstimulation (COH) was performed using the standard long protocol in all participants. Pituitary descending regulation was routinely started at mid-luteal phase (18–21 d of menstruation) with the administration of gonadotropin-releasing hormone agonist (GnRH-a) Diphereline (IpsenPharma Biotech, France) 0.05 mg/d or 0.1 mg/d subcutaneously. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) levels were measured after 10–14 days of dosing, and follicle development was monitored by vaginal ultrasound. When FSH < 5 mIU/mL, LH < 5 mIU/mL, E2 < 50 pg/mL, and sinus follicle diameter < 5 mm, meeting the criteria for downregulation, ovulation treatment was started with recombinant human gonadotropin (Gonal-f, Merck Serono, Switzerland) 150-300 IU/d subcutaneously. Based on the patient's age, BMI, antral follicle count (AFC) of both ovaries, and anti-Müllerian hormone (AMH), basal FSH and LH were used to determine the starting dose and medication time of gonadotropin (Gn). Serum LH, E2, and progesterone (P) levels, as well as follicle and endometrial development were monitored regularly during the dosing period, and the dose was adjusted according to the hormone levels and follicle development. Gn was discontinued when 60%–70% of follicles ≥ 18 mm or ≥ 14 mm in diameter or 40%–50% of follicles ≥ 20 mm or ≥ 14 mm in diameter and 200–300 pg/mL of serum E2/14 mm follicles were measured in both ovaries, and 250 μg of recombinant human chorionic gonadotropin (rhCG, Ovidre, Merck Serono Switzerland) was injected subcutaneously at around 2100 hour on the same night. Vaginal ultrasound-guided ovulation was performed 36 hours later. Patients' blood and follicular fluid were collected, fasting plasma insulin concentration was measured, luteinized granulosa cells were extracted, and the corresponding kits were used to detect and assess granulosa cell mitochondrial function (e.g., adenosine triphosphate [ATP] content and reactive oxygen species [ROS] levels).

2.3. Observational index
The general clinical data, including age, BMI, and years of infertility, were compared and analyzed between

the two groups; FSH, LH, insulin resistance index (HOMA-IR), granulosa cells mitochondrial function, and IVF-ET outcomes were compared and analyzed between patients in the study group and the control group.

2.4. Statistical analysis

All data in this study were processed using SPSS 22.0 statistical software. Measurement data were expressed by mean ± standard deviation (s) and tested by t test; counting data adopted rate (%) to express and were tested by chi-squared test (χ^2).

3. Results

3.1. Comparison of clinical data between the two groups

The differences in age and years of infertility between the study group and the control group were insignificant ($P > 0.05$). The BMI of the control group (22.3 ± 1.12 kg/m^2) was lower than that of the study group (30.5 ± 1.24 kg/m^2), and the difference was statistically significant ($P < 0.05$). **Table 1** shows the comparison of clinical data between the two groups.

Table 1. Comparison of clinical data between the two groups

Group	Age (years)	Years of infertility (years)	Body mass index (kg/m^2)
Control group (n = 39)	24.3 ± 2.12	3.3 ± 1.13	22.3 ± 1.12
Study group (n = 32)	26.3 ± 2.12	3.4 ± 1.22	30.5 ± 1.24
t	1.232	1.469	8.234
P	> 0.05	> 0.05	< 0.05

3.2. Comparison of mitochondrial function in granulosa cells between the two groups

ROS in the study group was significantly higher than that in the control group ($P < 0.05$). The ATP content in the study group, compared with the control group, was significantly lower than that in the control group ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of mitochondrial function in granulosa cells between the two groups

Group	ATP (pmol/mg)	ROS (DCF fluorescence intensity)
Control group (n = 39)	1.64 ± 0.34	27,339 ± 4,684
Study group (n = 32)	0.35 ± 0.04	46,825 ± 5,352
t	11.232	11.369
P	< 0.05	< 0.05

Abbreviations: ATP, adenosine triphosphate; DCF, dichlorofluorescein; ROS, reactive oxygen species.

3.3. Comparison of *in vitro* fertilization and embryo transfer outcomes between the two groups

The FSH and LH levels in IVF-ET cycles between obese and non-obese individuals were not statistically significant ($P > 0.05$); HOMA-IR was significantly higher in the study group than in the control group ($P < 0.05$); and the rate of IVF-ET failure was significantly higher in the study group than in the control group ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of IVF-ET outcomes between the two groups of patients

Group	FSH (u/L)	LH (mIU/ml)	Failure rate of IVF-ET (n, %)	HOMA-IR (mU/L)
Control group (n = 39)	8.3 ± 2.12	15.3 ± 1.12	2 (5.13)	0.36 ± 0.06
Study group (n = 32)	6.3 ± 2.12	14.3 ± 1.22	9 (24.13)	0.89 ± 0.12
t	1.232	1.369	8.234	6.987
P	> 0.05	> 0.05	< 0.05	< 0.05

Abbreviations: FSH, follicle-stimulating hormone; HOMA-IR, insulin resistance index; IVF-ET, *in vitro* fertilization and embryo transfer; LH, luteinizing hormone.

4. Discussion

Infertility is defined as the absence of pregnancy after more than 12 months of regular unprotected intercourse. Infertility is a major global reproductive health problem with an incidence of 10%–15%. Anovulation is an important cause of infertility in women, in which PCOS is the second leading cause after tubal infertility. PCOS is the most important disease related to reproductive endocrine and metabolic disorders in Chinese women of reproductive age, and its prevalence rate is 5%–10%, with a gradually increasing trend. In PCOS, the follicles are in a state of abnormal development, mainly due to follicle over-recruitment, dominant follicle selection disorder, and pre-ovulation follicle failure. These problems can cause ovarian dysfunction, which in turn can affect pregnancy outcomes. However, we have found that the quality of eggs obtained and embryos formed in infertile PCOS patients is reduced during IVF, resulting in low fertilization rates, low pregnancy rates, and high miscarriage rates. Therefore, there is an urgent scientific need to investigate how to improve the quality of eggs and embryos by improving the clinical symptoms of PCOS, thus increasing the success rate of IVF-ET.

Granulosa cells play an important role in the development, maturation, fertilization, and embryonic development of oocytes, which require large amounts of ATP to provide energy. When granulosa cells surround oocytes, they not only affect the cytoplasmic maturation of oocytes during follicular development, but also complete the response to gonadotropin through their own unique FSH and LH receptors, as well as participate in the maturation promoting effect of gonadotropin on oocyte nucleus. Data from several studies have suggested that abnormal ovarian granulosa cell function is closely associated with abnormal folliculogenesis in PCOS. Obesity increases the degree of sex hormone disorders and metabolic dysfunction, which may have an impact on reproductive performance in obese women. Fat cells secrete cytokines that contribute to a pro-inflammatory state and the initiation of oxidative damage. In this study, the mitochondrial function of ovarian granulosa cells in obese PCOS patients and non-obese PCOS patients was analyzed, and it was found that the mitochondrial function of ovarian granulosa cells in obese PCOS patients was significantly lower than that in non-obese PCOS patients. Abnormal mitochondrial function is a common mechanism in the occurrence and development of various metabolic diseases. Obesity may lead to the disturbance of cell energy metabolism by causing insulin resistance and hyperinsulinemia, thus resulting in abnormal mitochondrial function and affecting the metabolism and development of granulosa cells [8-11]. In addition, obesity may also lead to impaired function of post-insulin receptor signaling pathway, thus inducing hyperinsulinemia and insulin resistance. This may be related to the pathogenesis of PCOS and follicle maturation disorder. Some studies have shown that in obese PCOS patients, mitochondrial function abnormalities can be found in some of these patients by observing the morphology of granulosa cells and the insulin level in culture medium. Mitochondria are important sites for energy metabolism and substance metabolism in cells. Cell metabolism disorders caused by obesity and hyperinsulinemia may lead to mitochondrial dysfunction and affect the development process of granulosa cells. Animal model studies have demonstrated that high-fat diet can induce the overgrowth and abnormal expression of mitochondrial

proteins in mouse ovarian granulosa cells as well as granulosa cell apoptosis. This study found that the mitochondrial function of ovarian granulosa cells in obese PCOS patients decreased, which may interfere with oocyte development and ultimately affect the outcome of IVF-ET [12-15]. However, due to the small sample size of this study, there may be statistical errors.

In the present study, human luteinized ovarian granulosa cells were extracted, and the corresponding kits were used to detect and assess granulosa cell mitochondrial function (*e.g*., ATP content and ROS levels) as well as to analyze the correlation with IVF-ET outcomes. We found that obesity affects ovarian granulosa cell mitochondrial function in PCOS patients and may be associated with abnormal ovarian function and reproductive endocrine disorders in PCOS patients. We also analyzed the possible factors involved in the high follicular rate and low embryo quality in infertile PCOS patients. There was a limitation due to the small sample size; thus, further expansion of the sample size is needed. Meanwhile, relevant animal experiments can be conducted to further investigate the mechanisms related to follicular development and maturation.

In conclusion, PCOS is a complex endocrine disorder, and obesity is one of the independent risk factors in its development.

Disclosure statement

The authors declare no conflict of interest.

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