Pre-Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(10) 5146–5154

DOI: 10.1177/0300060519860680
journals.sagepub.com/home/imr
SAGE

Follicular output rate tends to improve clinical pregnancy outcomes in patients with polycystic ovary syndrome undergoing *in vitro* fertilization-embryo transfer treatment

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Abstract

Objective: This study aimed to examine the relationship between the follicular output rate (FORT) and clinical outcomes in patients with polycystic ovarian syndrome (PCOS).

Methods: A total of 841 patients with PCOS undergoing *in vitro* fertilization-embryo transfer (IVF-ET) were divided into three groups according to their FORT (low, middle, and high). Controlled ovarian hyperstimulation and clinical outcomes were compared retrospectively.

Results: Serum estradiol levels on the day of human chorionic gonadotropin (3780.5, 3599.9, and 3375.7 pg/mL) and the number of retrieved oocytes (17.5, 16.1, and 14.8) decreased from the high to low FORT groups. Pre-ovulatory follicle counts were significantly higher in the high FORT group than in the middle and low FORT groups. The number of retrieved oocytes, high-quality embryo rate, and clinical pregnancy rate decreased from the high to low FORT groups. The incidence of moderate and severe ovarian hyperstimulation syndrome (OHSS) in the middle FORT group was significantly lower than that in the high and low FORT groups.

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Conclusions: FORT may be used to predict clinical outcomes of IVF/intracytoplasmic sperm injection-embryo transfer in patients with PCOS. Efforts should be made to prevent OHSS in patients with PCOS and a high or low FORT in controlled ovarian hyperstimulation cycles.

Keywords
Polycystic ovarian syndrome, follicular output rate, ovarian hyperstimulation syndrome, in vitro fertilization, embryo transfer, oocyte, pregnancy

Date received: 9 November 2018; accepted: 10 June 2019

Introduction

Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disease and its etiology is largely unknown.[1] Currently, PCOS is treated by three types of therapy and the third-line therapy is *in vitro* fertilization-embryo transfer (IVF-ET). The most challenging part of IVF is superovulation. For patients with PCOS, superovulation often results in more, but low quality, ova and there is a high incidence of ovarian hyperstimulation syndrome (OHSS). Low-quality ova may lead to low embryo quality and reduced clinical pregnancy. OHSS is an iatrogenic disease after ovulation, and the pathogenesis is not yet fully understood. OHSS often results in the development of multiple follicles, abnormally increased estrogen levels, and increased secretion of vascular endothelial growth factor (VEGF) by granulosa cells.[2] This increases intravascular permeability, leading to outflow of intravascular fluids from blood vessels and interstitial fluid accumulation. Clinically, manifestations of OHSS include abdominal distention, oliguria, hydrothorax, ascites, thrombosis, and liver and kidney injury. These adverse complications are hard to predict and often result in severe physical and psychological trauma to the patient.

Although a number of indicators have been proposed or used to predict ovarian reactivity, such as the antral follicle count (AFC), basal follicle-stimulating hormone (FSH) levels, and anti-Müllerian hormone (AMH) levels,[3–7] these predictive indicators have certain limitations. Currently, there is no indicator that can predict the ovarian response to ovulation and oocyte developmental potential simultaneously.[3–7]

Genro et al.[8,9] proposed the concept of the follicular output rate (FORT). In 2012, Gallot et al.[8] studied patients with regular menstrual cycles and found that the FORT was a quantitative indicator reflecting ovarian follicular developmental potential, and that a higher FORT was associated with a better pregnancy outcome. Additionally, Hassan et al.[10] investigated patients with unexplained infertility and showed that the number of high-quality embryos and the clinical pregnancy rate increase with FORT. Therefore, the FORT is an independent variable affecting the outcome of pregnancy. In patients with PCOS, the number of follicles for the IVF-ET ovulation process is prone to ovarian hyperactivity. Although many oocytes can be retrieved, the quality of the oocytes and embryos is often poor.[11] Although a middle FORT value leads to better pregnancy outcomes in patients with PCOS,[12] whether the FORT can be used to predict the outcome

of pregnancy is unclear. In this study, a large number of patients were studied to investigate the relationship between the FORT and pregnancy outcomes. Our findings could provide insight into treatment for OHSS for better clinical outcomes.

Methods

Study design

This was a retrospective cohort study that was performed in China between January 2012 and June 2017.

Subjects

A total of 841 patients with PCOS who were treated with IVF-ET cycles between January 2012 and June 2017 in Liaocheng People's Hospital were selected as the study subjects. All patients were treated with IVF-ET for the first time and included on the basis of diagnostic criteria recommended by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (2003).[13] Patients were diagnosed with PCOS if they had two of the following three conditions: (1) oligo- and/or anovulation; (2) clinical and/or biochemical signs of hyperandrogenism; and (3) polycystic ovaries. Patients were excluded for the following reasons: if they had a history of ovarian surgery or pelvic surgery within 6 months, or they had major damage of ovarian function due to radiotherapy or chemotherapy; contraindications for gonadotropin (Gn) treatment; the presence of endometriosis, adenomyosis, hydrosalpinx, uterine cavity abnormalities, thyroid dysfunction, congenital adrenal hyperplasia, or Cushing syndrome; and patients with cancer who were secreting androgen. All patients were subjected to the standardized long agonist protocol. Patient-related data were retrieved from the hospital's electronic database system. The study was approved by the Research Ethics Committee of Liaocheng People's Hospital (reference number: LPR2018-21, dated October 10, 2018) and written consent was received from every participant.

Superovulation program

The standard superovulation program was used. Patients were asked to take one Dianette tablet (ethinyl estradiol and cyproterone acetate tablet; Lloyds Pharmacy, Coventry, UK) from the third day of previous menstruation and were injected daily intramuscularly with Diphereline (1.25 mg) and 150 U recombinant FSH (Gonal F; Merck, Serono, Switzerland) on the 17th day of Dianette administration. When the follicles grew to 18 μm in diameter and the number was $\geq$ two or they grew to 17 μm in diameter and the number was $\geq$ three, 6000 U human chorionic gonadotropin (HCG) (Ovidrel, Merck) was injected intramuscularly and the oocytes were isolated. Morphological observation and fresh embryo transfer were performed 3 days after oocyte retrieval. Embryos were judged according to the number of embryonic cells, size, morphology, and the percentage of fragmentation. Embryos with two prokaryotic nuclei sources, $< 20\%$ debris, and seven to nine cells were judged to be high quality. If possible, one to two embryos were transferred and the remaining embryos were cryopreserved. If the patient was found to be at high risk for OHSS, the transfer was cancelled and all high-quality embryos were cryopreserved for later transfer.

Assessments

The AFC was assessed by a transvaginal ultrasound scan (color ultrasonic Doppler scanner; Polytron Technologies, Shenzhen, China). Serum FSH and estradiol (E_2)

levels were measured using a microparticle chemiluminescence immunoassay with a chemiluminescence analyzer (Unicel DXI800; Beckman Coulter, Miami, FL, USA) according to manufacturer's instructions. Clinical pregnancy was defined as the presence of a gestational sac in the uterine cavity 4 weeks after embryo transfer. OHSS was classified as previously described.[14]

Statistical analysis

The sample size was calculated to compare three proportions. Clinical pregnancy rates of 35% among women in IVF cycles were described in American Society for Reproductive Medicine reports. We expected to detect a 10% increase in the clinical pregnancy rate. A unilateral test was calculated, and 153 women per group were necessary to obtain a power of 80% at a significance level of 0.05. The variables were tested for their normality and all of them were normal. One-way analysis of variance was used to compare continuous variables. Pearson's chi-squared test was used to compare categorical variables. All statistical analyses were performed using SPSS19.0 (IBM, Armonk, NY, USA). Values of $P < 0.05$ were considered statistically significant.

Results

Baseline status

The median FORT was 52%. Therefore, the patients were divided into high (>58%, n = 246), middle (58%–46%, n = 375), and low (<46%, n = 220) FORT groups. The baseline information of patients is shown in Table 1. No significant differences were found in age, duration of infertility, body mass index (BMI), basal FSH levels, basal E_2 levels, and basal AFC among the groups.

Superovulation outcomes

During the superovulation process, the mean amount of Gn used, Gn stimulation days, and the number of transplanted embryos were not different among the groups (Table 2). E_2 levels on the day of HCG injection were significantly different among the high, middle, and low FORT groups ($P < 0.05$), with the highest levels in the high FORT group. The number of retrieved oocytes was significantly lower in the low FORT group compared with the other groups (both $P < 0.05$). The preovulatory follicle count (PFC) was significantly higher in the high FORT group than in the middle and low FORT groups (both $P < 0.05$).

Clinical outcomes

The mean percentage of high-quality embryos was significantly higher in the high FORT group than in the low FORT group ($P < 0.05$). The clinical pregnancy rate was also significantly higher in the high FORT group than in the low FORT group ($P < 0.05$), and showed an increasing trend as the FORT increased. The incidence of moderate to severe OHSS was significantly lower in the middle FORT group compared with the high and low FORT groups (both $P < 0.05$, Table 3).

Discussion

PCOS is a common gynecological disease and some patients with PCOS are infertile. For these patients, IVF-ET is an assisted reproductive option, where superovulation is the main step. Studies have shown that the outcomes of superovulation are dependent on age, and basal FSH and AMH levels.[15–18] However, Hsu et al.[19] found that the AFC may be used to predict ovarian response, but not embryo quality or pregnancy. A recent study with 1156 patients showed that during the first IVF

Table 1. Baseline characteristics in the low, middle, and high follicular output rate groups.

Variables	High follicular output rate	Middle follicular output rate	Low follicular output rate
Age (years)	29.4 ± 3.2	29.1 ± 2.9	29.2 ± 2.2
Infertility (years)	2.9 ± 1.1	2.9 ± 1.2	2.7 ± 1.0
Body mass index (kg/m^2)	23.1 ± 1.3	23.2 ± 1.3	22.9 ± 1.9
Baseline follicle-stimulating hormone (mIU/mL)	6.24 ± 0.88	6.29 ± 0.87	6.32 ± 0.82
Baseline estradiol (pg/mL)	51.24 ± 6.46	52.31 ± 5.97	51.18 ± 5.62
Antral follicle count	19.3 ± 3.7	18.6 ± 3.3	18.2 ± 2.9

Values are mean ± standard deviation.

Table 2. Controlled ovarian hyperstimulation data.

Variables	High follicular output rate	Middle follicular output rate	Low follicular output rate
Serum estradiol on the day of human chorionic gonadotropin injection (pg/mL)	3780.5 ± 692.9a	3599.9 ± 714.1b	3375.7 ± 678.9c
Pre-ovulatory follicle count	13.5 ± 1.5a	9.6 ± 1.9b	7.1 ± 1.7b
Gonadotropin (U)	2250.0 ± 122.7	2251.7 ± 180.5	2258.9 ± 154.4
Gonadotropin duration (days)	9.3 ± 0.96	9.5 ± 1.10	9.6 ± 0.96
Number of oocytes retrieved	17.5 ± 2.8a	16.1 ± 2.6a	14.8 ± 1.8b
Number of embryos transferred	1.78 ± 0.5	1.79 ± 0.4	1.84 ± 0.5

Values are mean ± standard deviation. Means with different letters in the same row are significantly different ($P < 0.05$).

Table 3. *In vitro* fertilization-embryo transfer outcomes.

Variables	High follicular output rate	Middle follicular output rate	Low follicular output rate
High-quality embryo (%)	51.4 ± 4.4a	48.5 ± 4.7a	45.2 ± 5.4b
Clinical pregnancy rate in the *in vitro* fertilization-embryo transfer cycle (%)	68.3 (153/223)a	64.3 (210/326)a	38.1 (70/183)b
Incidence of moderate to severe ovarian hyperstimulation syndrome (%)	20.6 (49/246)a	9 (34/375)b	17.8 (39/220)c

Values are mean ± standard deviation or numbers. Means or numbers with different letters in the same row are significantly different ($P < 0.05$).

cycle, baseline AMH and serum AFC levels were modestly associated with ovarian response and age, but may not provide additional value on top of women's age.[20] The FORT is considered as a better alternative predictor.[8,10] However, little is known regarding the relationship between the FORT and clinical outcome and incidence of OHSS in patients with PCOS. Because patients with PCOS account for a relatively large proportion of infertile patients, there is a need for better

understanding of the relationship between the FORT and clinical outcomes and incidence of OHSS for better treatment.

The patients in this study all had PCOS and their AFC was higher than that in non-PCOS patients. Therefore, the standard long superovulation scheme was used with the same triggering dose to induce a uniform ovarian response. During superovulation, the ovarian response was more remarkable in patients with PCOS than in non-PCOS patients. As a result, the number of retrieved oocytes and E_2 levels on the day of HCG injection were higher than those in non-PCOS patients. When the patients with PCOS were divided into three groups based on the FORT, their baseline status, including age, sex, duration of infertility, body mass index, AFC, and baseline FSH and E_2 levels, were similar among the groups. They all had a similar amount of total Gn used and similar simulation days. These measurements are related to the ovarian response. Therefore, the similarity of these measurements among the groups indicated that the clinical outcomes could be compared and be attributed to the FORT. E_2 levels on the day of HCG injection and PFC decreased as the FORT decreased (Table 2), which resulted in reduced mature and retrieved oocytes. Because E_2 is secreted by granulosa cells in mature follicles, more mature follicles would have better function in granulosa cells and more E_2 secretion. The change in the number of retrieved oocytes was similar to that of E_2 levels on the day of HCG injection. Because maturity of follicles directly affects the quality of oocytes, more mature follicles would lead to better oocytes, and subsequently better embryo and higher pregnancy rates. Our study showed that with decreasing FORTs, the number of retrieved oocytes, high-quality embryos, and the clinical pregnancy rate decreased (Table 3). This finding is similar to that obtained in a previous study.[21] Patients in the high FORT group had a better ovarian response to Gn, which led to better clinical outcomes. This finding is in consistent with Rehana et al.[22] and Zhang et al.'s[23] studies. Rehana et al.[22] found that the FORT may be used to predict ovarian potential, as well as the number of dividing embryos and the clinical pregnancy rate Zhang et al.[23] showed that patients with a high FORT had better clinical outcomes in the IVF cycles.

Our study also showed that patients in the high and low FORT groups had a higher incidence of OHSS compared with patients in the middle FORT group. The cause of OHSS is not fully clear and may be attributed to genetic factors.[24] One of the high risk factors for occurrence of OHSS is PCOS.[25] The patients in this study all had PCOS, and their incidence of OHSS was obviously higher than that in non-PCOS patients. OHHS is also related to VEGF. VEGF is a signaling protein that promotes growth of new blood vessels and outflow of fluid into the extracellular space, resulting in inter-tissue effusion and a number of clinical complications. Patients with a high FORT have more mature follicles and granulosa cells around the egg. As such, more VEGF can be produced when HCG is injected, and VEGF binds to VEGF receptor 2 in vascular endothelial cells. This results in increased vascular permeability, exudation of intravascular fluid to the extravascular tissue, and a number of clinical manifestations. In patients with a low FORT, the PFC is relatively low. However, after retrieval of mature oocytes, there are still many immature oocytes in which granulosa cells might develop after injection of HCG to secrete VEGF, causing increased vascular permeability and occurrence of OHSS. However, in patients with a middle FORT, the number of mature follicles is moderate, and the amount of VEGF secreted by the granulosa cells might not be too high. During isolation of oocytes,

most of the mature oocytes are retrieved, leaving few immature oocytes to secrete VEGF. This might result in less occurrence of moderate to severe OHSS.

In this study, a relatively large number of samples were analyzed. However, this was a retrospective study, and patients were selected from a sub-population in China, which limits its representativeness. To further validate our results, larger studies, preferably randomized, controlled trials, are required. Nevertheless, our results support previous studies[10,11,14] in Chinese cohorts, and will be helpful for improving the efficacy of IVF/intracytoplasmic sperm injection, especially in patients with PCOS.

Conclusions

The FORT can be used to predict ovarian response and clinical outcomes in patients with PCOS. For patients with a high or low FORT, precaution should be taken for the occurrence of OHSS. Because the FORT is determined on the day of HCG injection, when ovulation has ended, an earlier and more accurate predictor for ovarian response and clinical outcomes for better treatment of patients is desirable.

List of abbreviations

- FORT: follicular output rate
- PCOS: polycystic ovary syndrome
- IVF-ET: *in vitro* fertilization-embryo transfer
- PFC: pre-ovulatory follicle count
- AFC: antral follicle count
- HCG: human chorionic gonadotropin
- Gn: gonadotropin
- FSH: follicle-stimulating hormone
- AMH: anti-Müllerian hormone
- E_2: estradiol
- OHSS: ovarian hyperstimulation syndrome
- VEGF: vascular endothelial growth factor

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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