Original Article

The effects of multiple controlled ovarian hyperstimulation over a 2-year period on ovarian reserve and reactivity: a retrospective clinical study

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Background: To evaluate the effects of controlled ovarian hyperstimulation (COH) on ovarian reserve function during *in vitro* fertilization and embryo transfer (IVF-ET).

Methods: From August 2018 to August 2020, the medical records of patients who received IVF-ET in the Department of Reproductive Medicine, Beijing Gynaecology and Obstetrics Hospital, Capital Medical University were analyzed retrospectively. Among them, 372 patients received 2 cycles of COH, 54 patients received 3 cycles, and 13 patients received 4 cycles. The levels of follicle-stimulating hormone (FSH), the number of antral follicles, levels of anti-Müllerian hormone (AMH), the total amount of gonadotropin (GN), the time of ovulation induction, the number of eggs obtained, the number of available embryos, and the number of high-quality embryos cycles were compared in different treatment.

Results: The age of female patients did not significantly affect the levels of AMH or FSH during menstruation, nor the number of antral follicles before ovulation induction ($P>0.05$). However, with an increase in age, an increase in the number of controlled COH cycles was observed. In patients who underwent 2 COH cycles, the number of high-quality embryos in the second cycle increased significantly compared to the first cycle ($P<0.05$). However, there were no significant differences in the ovulation induction time, the number of eggs, the GN dosage, and the number of available embryos ($P>0.05$). In patients with 3 treatment cycles, the GN dose used in the third cycle was significantly lower than that used in the first cycle ($P<0.05$). There were no significant differences in the ovulation induction time, the number of eggs obtained, and the quality of embryos ($P>0.05$). In patients with 4 treatment cycles, significant differences were observed in the ovulation induction time between the first and the fourth controlled COH cycle ($P<0.05$). However, no significant differences were detected in GN dosage, ovulation induction time, the number of eggs obtained, the number of available embryos, and the quality of embryos ($P>0.05$).

Conclusions: Ovarian reserve function was not significantly affected in patients with up to 4 ovarian stimulation cycles.

Keywords: *In vitro* fertilization-embryo transfer (IVF-ET); controlled ovarian hyperstimulation (COH); ovarian reserve function

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Introduction

The use of assisted reproductive technology emerged nearly 40 years ago. *In vitro* fertilization and embryo transfer (IVF-ET) is a technology that uses assisted reproductive technology to transfer embryo combined with *in vitro* fertilization into the patient to achieve pregnancy. At present, the clinical pregnancy rate of a single IVF-ET event is 40–60%, and the live birth rate can reach 20–30%. However, there are still many infertile couples who cannot achieve clinical pregnancy after one round of IVF-ET (1). These patients usually receive multiple controlled ovarian hyperstimulation (COH) treatments to improve the probability of pregnancy. However, the effects of multiple COH treatments and multiple ovarian punctures (necessary for follicle collection) on ovarian reserve function remains controversial (2,3). Some researchers believe that repeated COH does not affect the ovarian reserve function, and by adjusting the treatment plan, high-quality embryo formation rates and clinical pregnancy rates are significantly increased after repeated COH treatments. Some scholars believe the use of high dose FSH may accelerate the consumption of follicle reserves and reduce ovarian reactivity (4).

In this study, the clinical data of patients who received multiple COH treatments were retrospectively analyzed to further clarify the changes in ovarian reserve function and its reactivity after multiple COH iterations. This information will provide a scientific basis for assessing the risks and benefits of repeated COH on female ovarian function.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/apm-21-330).

Methods

Study population

Medical records were collected from patients who received IVF/intracytoplasmic sperm injection (ICSI)-ET after 2–4 cycles of COH between January 2018 and August 2020.

The inclusion criteria were as follows: (I) female patients aged 20–40 years; (II) causes of infertility included fallopian tube factors, ovulation disorders, endometriosis, or female and/or male factors; and (III) the interval time of each COH treatment cycle was more than 3 months. The exclusion criteria were as follows: (I) the levels of follicle-stimulating hormone (FSH) were greater than 10 IU/L; (II) the number of antral follicles was less than 5; (III) the levels of anti-Müllerian hormone (AMH) were less than 0.8 ng/mL; and (IV) the interval time between the first COH and the last COH exceeded 2 years.

A total of 964 patient cycles of COH with complete data were included in this retrospective study. Among them, 375 patients were treated for 2 cycles of COH, 54 patients had 3 cycles, and 13 patients underwent 4 cycles. Self-control comparison was performed for all patients (*Figure 1*).

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by institutional ethics board of Beijing Gynecology and Obstetrics Hospital, Capital Medical University (No.: 2016-KY-085-01). Individual consent for this retrospective analysis was waived.

Research design

Therapeutic regimen

The ovarian stimulation plan for each patient was determined according to the levels of FSH and AMH, and the number of antral follicles. The patient's response to medication at the previous ovulation induction was also considered. The ovarian stimulation plans included the gonadotropin-releasing hormone agonist plan (abbreviated as the agonist plan), the gonadotropin-releasing hormone antagonist plan (abbreviated as the antagonist plan), the luteal ovulation induction plan, and the micro-stimulation plan. When the diameter of 3 or more follicles measured 17 mm or greater, or when 2 or more follicles measured 18 mm or greater, the eggs were harvested by puncture under the guidance of transvaginal ultrasound after 36 hours using 250 mg recombinant human chorionic gonadotropin injection (Elzer, Serono Europe) or 0.2 mg triptorelin injection (Dabija, Huiling Pharmaceutical Co. Ltd.). The fertilization method was conventional IVF fertilization or ICSI fertilization.

Evaluation of embryo quality

After 16–18 hours of *in vitro* fertilization, the egg was examined under the dissecting microscope. Fertilized eggs with double pronucleus (2PN) were determined as normal fertilization. The quality of embryos in the cleavage stage was evaluated by observing the number and morphology of cleavage cells 3 days after collecting the eggs. The evaluation criteria for high-quality embryos were as follows: at least 8-cell embryos 3 days after harvesting the eggs; blastocysts observed 5 days after collecting the eggs; and the fragmentation of cleavage balls was less than 10%. The

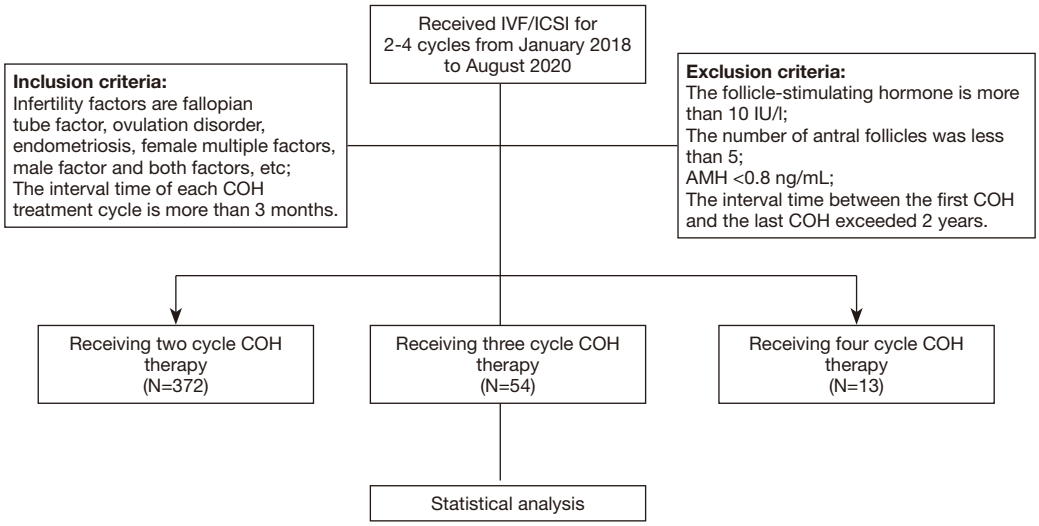

Figure 1 Flow diagram of the study design. AMH, anti-Müllerian hormone; COH, controlled ovarian hyperstimulation.

numbers of available embryos and high-quality embryos were recorded.

Observation indexes

Basal FSH, the number of antral follicles, levels of AMH, GN dosage, ovulation induction time, the number of eggs obtained, the number of available embryos, and the number of high-quality embryos were assessed.

Statistical analysis

Data were expressed as mean ± standard deviation for continuous variables and as proportions for categorical variables. The Student's *t*-test was used for pairwise comparison. Statistical analyses were performed using SPSS24.0 software. A P value <0.05 was considered statistically significant.

Results

A comparison of ovarian reserve function and reactivity in patients receiving 2 cycles of controlled COH therapy

A total of 372 patients received 2 cycles of COH therapy. There were no significant differences in the levels of AMH, basal FSH, and antral follicle numbers before the start of each cycle (P>0.05; *Table 1*).

Compared with the first COH cycle, the number of high-quality embryos in the second cycle increased significantly (P<0.05), but there were no significant differences in the ovulation induction time, the number of eggs, the GN dosage, and the number of available embryos (P>0.05; *Table 2*).

A comparison of ovarian reserve function and reactivity in patients receiving 3 cycles of controlled COH therapy

A total of 54 patients received 3 cycles of COH therapy. There were no significant differences in the levels of AMH, basal FSH levels, and antral follicle numbers before each cycle of ovulation induction (P>0.05; *Table 3*).

Compared with the first COH cycle, the amount of GN in the third cycle was significantly lower (P<0.05). However, there were no significant differences in the ovulation induction time, the number of eggs obtained, and the quality of embryos (P>0.05; *Table 4*).

A comparison of ovarian reserve function and reactivity in patients receiving four cycles of controlled COH therapy

A total of 13 patients received 4 cycles of COH therapy, and no significant differences were found in the levels of AMH, basal FSH levels, and antral follicle numbers before each cycle of ovulation induction (P>0.05; *Table 5*).

Significant differences were detected in the ovulation induction time between the first and the fourth cycles (P<0.05). However, there were no significant differences in GN dosage, ovulation induction time, the number of eggs obtained, the number of available embryos, and the quality

Table 1 A comparison of ovarian reserve in women receiving two cycles of controlled ovarian hyperstimulation

Group	Age (year)	FSH (mIU/mL)	AMH (ng/nL)	AFC
Cycle 1	32.14±5.21	7.26±1.89	6.23±5.87	14.29±6.87
Cycle 2	32.65±3.59	8±1.08	4.11±2.12	13.71±5.29

FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; AFC, antral follicle number.

Table 2 A comparison of ovarian reactivity in women receiving two cycles of controlled ovarian hyperstimulation

Group	GN usage (IU)	Promotion time (days)	Number of eggs obtained	Number of embryos available	Number of high-quality embryos
Cycle 1	3,407±1,319.7	12±2.65	10.57±7.52	2.71±1.38	1.29±1.89
Cycle 2	3,437.5±1,342.83	12±1.26	13.57±7.98	5.14±3.18	3.29±3.2*

Comparison with cycle 1 *P<0.05. GN, gonadotropin.

Table 3 A comparison of ovarian reserve in women receiving three cycles of controlled ovarian hyperstimulation

Group	Age (year)	FSH (mIU/mL)	AMH (ng/nL)	AFC
Cycle 1	34±2.55	7.34±2.21	4.42±2.85	10.4±3.38
Cycle 3	35±1.63	7.92±2.06	3.28±2.01	7.87±4.52*

Comparison with cycle 1 *P<0.05. FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; AFC, antral follicle number.

Table 4 A comparison of ovarian reactivity in women receiving three cycles of controlled ovarian hyperstimulation

Group	GN usage (IU)	Promotion time (day)	Number of eggs obtained	Number of embryos available	Number of high-quality embryos
Cycle 1	3,285±1,572.68	12.2±4.6	9.4±7.6	4.6±4.22	0.2±0.45
Cycle 3	2,205±283.62*	11±3.24	8.6±4.39	2.6±1.34	0.6±0.89

Comparison with cycle 1 *P<0.05. GN, gonadotropin.

Table 5 A comparison of ovarian reserve in women receiving four cycles of controlled ovarian hyperstimulation

Group	Age (year)	FSH (mIU/mL)	AMH (ng/nL)	AFC
Cycle 1	35±3.46	7.52±1.44	4.92±5.85	12.67±9.81
Cycle 4	37±2.53	8.31±2.2	4.01±5.59	10.33±7.5

FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; AFC, antral follicle number.

embryos (P>0.05; *Table 6*).

Discussion

Ovarian reserve function refers to the ability of ovarian cortical follicles to grow and develop and into mature oocytes, which is manifested by the number of follicles and the quality of oocytes existing in the ovary (5). At present, the most commonly used indicators for evaluating ovarian reserve function include age, AMH levels, basal hormone endocrine levels including FSH, luteinizing hormone (LH) and estradiol (E2), and basal antral follicle numbers (6).

During COH, the extra-physiological dose of GN can promote the growth and development of other follicles, avoid the occurrence of follicular atresia, and stimulate multiple oocytes. Some studies have shown that FSH

Table 6 A comparison of ovarian reactivity in women receiving four cycles of controlled ovarian hyperstimulation

Group	GN usage (IU)	Promotion time (day)	Number of eggs obtained	Number of embryos available	Number of high-quality embryos
Cycle 1	1,825±499.37	8.67±1.15	7.33±3.51	3±1	1.33±1.53
Cycle 4	2,100±75	11±2.65*	5.67±2.08	3±1.73	2.1±1.12

Comparison with cycle 1 *P<0.05. GN, gonadotropin.

stimulation at super-physiological doses may promote the growth of the remaining follicles in the follicular pool and contribute to the recruited antral follicles, thus accelerating the depletion of the follicular pool (7).

In addition, it has been reported that repeated vaginal puncture of the ovary may lead to the release of ovarian autoantigens, resulting in a decrease in the number of follicles in the follicular pool (8). Other studies have shown that transvaginal follicular puncture may damage ovarian capillaries and peripheral ovarian tissues. This has a cumulative negative effect with each increasing number of punctures. Other reports have suggested that GN will change the physiological choice of a single dominant follicle, but will not accelerate the recruitment of follicles from the next cycle, indicating that repeated COH has no adverse effects on ovarian function (3).

Infertile women aged 20–40 years were selected for this study. The results demonstrated that there were no significant changes in the basal FSH and AMH levels between each cycle of COH in patients who received 2–4 cycles of COH. This suggesting that COH, up to 4 cycles, did not lead to any significant decrease in ovarian reserve function. However, in patients receiving 2–4 cycles of COH therapy, AMH gradually decreased with each ovarian stimulation cycles, and basal FSH gradually increased. The number of antral follicles also gradually decreased with the increase of ovarian stimulation cycles. Although there was no statistical significance in the above differences, with the increase of each COH cycle, the ovarian reserve function gradually decreased. It remains to be determined whether this phenomenon is caused by the increase in the age of the patients.

Ovarian reactivity refers to the response of the ovary to GN during COH, and this is measured by GN dosage, ovulation induction time, number of eggs, and other indicators (9). At present, reports examining the effects of repeated COH on ovarian reactivity remain controversial.

Some researchers found that 25% of patients who used the antagonist regimen for repeated COH had increased GN dosage and decreased ovarian reactivity, while 75% of patients had no obvious changes in ovarian reactivity (10). However, our current study showed that the GN dosage in the third cycle was lower than that in the first cycle among women who had undergone COH for 3 cycles. This may be related to the downward trend of the number of follicles in the basal sinus after 2 cycles of COH treatment, and this may have led to more doctors adopting micro-stimulation or less GN to promote ovulation. Among women who had undergone 4 cycles of COH, the ovulation induction time in the fourth cycle was longer than that in the first cycle. This may be related to a decrease in ovarian reactivity.

It has been reported the antral follicle numbers in repeated COH cycles are significantly higher than that observed during the first cycle, and the number of eggs obtained, usable embryos, and high-quality embryos in repeated cycles are significantly higher than that in the first cycle (11). This study demonstrated that no significant changes were observed between any of the 4 cycles of COH in terms of the number of oocytes, usable embryos, and high-quality embryos obtained. However, the number of high-quality embryos tended to increase in the second, third, and fourth cycles. Previous reports have shown that adding growth hormone before and during ovulation promotion can improve the utilization rate of oocytes and improve the quality of embryos in COH treatment (12). In this study, 121 cases were pre-treated with growth hormone in the second cycle. The increase in the number of high-quality embryos may be related to the increased use of growth hormone, adjustment of the medication plan by doctors, and the small number of samples.

In IVF treatment, most patients will receive large doses of GN stimulation and be subjected to repeated surgery for egg harvesting. Whether these treatments have a negative effect on ovarian reserve and responsiveness remains controversial (7,13,14).

The results in this present study demonstrated that up to 4 cycles of COH treatment and egg harvesting did not significantly affect the ovarian reserve function. However,

4 cycles of COH may lead to a degree of decline in ovarian responsiveness. The second COH cycle resulted in an increased number of high-quality embryos, while the third and fourth COH cycles also showed an increasing trend in the number of high-quality embryos. This may be related to the adjustment of the protocol and the appropriate application of growth hormone.

Further research into COH is warranted to optimize the number of high-quality embryos and therefore the number of successful pregnancies.

In summary, up to 4 cycles of COH treatment is safe, and reproductive physicians need to conduct a comprehensive evaluation of individual patients and select reasonable row-promoting modes, drug doses, and pre-treatment modes to obtain a satisfactory outcome.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/apm-21-330

Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-21-330

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by institutional ethics board of Beijing Gynecology and Obstetrics Hospital, Capital Medical University (No.: 2016-KY-085-01). Individual consent for this retrospective analysis was waived.

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