


# BMJ Open Effect of folic acid supplementation on diminished ovarian reserve: study protocol of a single-centre, open-label, randomised, placebo-controlled clinical trial

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## ABSTRACT

**Introduction** The prevalence of diminished ovarian reserve (DOR), a common gynaecological disorder, is approximately 10% across the world. Failure in early diagnosis and treatment may result in continuous decreases in ovarian function and the resultant loss in an opportunity of pregnancy, which greatly affects the happiness of the women's family and women's physical and mental health. Nevertheless, there has been no effective treatment for such a disorder until now. Folic acid, a member of the vitamin B family, is involved in one-carbon cycle and methylation regulation. It has been found that folic acid affects the whole period of pregnancy, and folic acid supplementation has shown effective to remarkably reduce the incidence of fetal neural tube defects and decrease plasma homocysteic acid levels, thereby resulting in a decline in the incidence of abortion. In addition, folic acid is reported to mediate ovarian functions. It is therefore hypothesised that folic acid may improve DOR.

**Methods and analysis** A single-centre, open-label, randomised, placebo-controlled clinical trial is designed. We plan to recruit 140 women with DOR at ages of 30–35 years. All participants will be randomised into the folic acid group and placebo group, and each subject will be given a tablet with the same appearance daily for 6 months. The primary outcome is antral follicle count, and the secondary outcomes are ovarian reserve markers, ovarian low-dose stimulation responses and safety.

**Ethics and dissemination** This study was approved by the Ethics Review Committee of Nanping First Hospital Affiliated to Fujian Medical University on 10 February 2021 (approval number: NPSY202002042). Written informed consent was obtained from all participants prior to randomisation, following a detailed description of the purpose of the study. The results of this clinical trial will be presented at scientific conferences and submitted to a peer-reviewed journal.

**Trial registration number** Chinese Clinical Trial Registry, ChiCTR2100047410.

## INTRODUCTION

Diminished ovarian reserve (DOR) is a common gynaecological disorder

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is a single-centre, open-label, randomised, placebo-controlled clinical trial, which will provide insights into the understanding of the effect of folic acid supplementation on diminished ovarian reserve.
- ⇒ Random numbers are generated by computers, and either participants or investigators are blind to the treatment.
- ⇒ This is the largest randomised controlled trial to investigate effect of folic acid supplementation on diminished ovarian reserve until now, which will recruit 140 women with diminished ovarian reserve.
- ⇒ Although there are diverse definitions of diminished ovarian reserve, the diagnostic indicators employed for the inclusion criteria in this trial are the most common and most frequently recommended measures.

characterised by poor fertility outcomes, which is caused by a reduced capacity of the ovaries to produce eggs and a decline in the number of oocytes.<sup>1</sup> DOR is considered as a warning sign of ovarian disorders.<sup>2</sup> If early diagnosis and treatment is not given, the ovarian function will continue to diminish until premature ovarian failure,<sup>3</sup> leading to the loss of timing for fertility.<sup>4</sup> Worsening environmental pollution, alteration of life styles and delayed childbearing result in an increasing incidence of DOR, and the global prevalence of DOR is estimated to be 10% in women seeking fertility treatment.<sup>5</sup> The exact pathogenesis of DOR remains unclear, and currently, there is no effective treatment for DOR.<sup>6</sup> This disorder has become a hot topic and difficulty in gynaecological reproductive endocrinology.<sup>1</sup> A search for novel treatments is therefore of urgent need to improve DOR

and increase the opportunity of pregnancy among infertile women.

Folic acid, a member of the vitamin B family, is an essential nutrient for humans, which is involved in one-carbon cycle and methylation regulation.<sup>7</sup> 5-Methyltetrahydrofolate, the active form of folic acid, functions as a methyl-group donor to remethylate homocysteine to methionine.<sup>8</sup> Folic acid deficiency may indirectly lead to elevated plasma homocysteic acid concentrations and reduced S-adenosyl methionine.<sup>8</sup> These folic acid-dependent functions mainly include DNA synthesis, modification and repair, control of gene expression and cell division.<sup>9 10</sup> Folic acid metabolism is reported to affect the whole period of pregnancy.<sup>11</sup> Folic acid supplementation has shown effective to remarkably reduce the prevalence of neural tube defects,<sup>12 13</sup> reduce the incidence of abortion and mediate ovarian functions.<sup>14</sup>

Precision prediction of ovarian reserve is of great significance for DOR patients. Currently, anti-Müllerian hormone (AMH), inhibin B and antral follicle count (AFC) are common markers used to predict ovarian reserve.<sup>15</sup> Both AMH and inhibin B, secreted by the ovary, are members of the transforming growth factor- $\beta$  superfamily, which may serve as markers for prediction of ovarian reserve.<sup>16 17</sup> AMH is secreted by preantral ovarian follicles and small antral ovarian follicles, and its level is strongly correlated with follicle number in ovary.<sup>18</sup> In addition, AMH presents a mild fluctuation during the menstrual cycle in relative to oestradiol, follicle-stimulating hormone (FSH) and luteinising hormone<sup>19</sup>; however, it is strongly associated with AFC and age, which is accepted as one of the most important marker for assessment of ovarian reserve functions.<sup>20</sup> AFC is a count of ovarian antral follicles by B-mode ultrasonography, which is non-invasive, easy to perform and effective to assess the reserve of each ovary.<sup>21</sup> As the best parameter that reflects the number of ovarian reserve, AFC is commonly used for clinical assessment of female infertility at childbearing ages and ovarian reserve among women undergoing assisted reproductive technology.<sup>22</sup>

However, there have been no reports pertaining to folic acid supplementation for improving DOR until now. This clinical trial is therefore designed with aims to evaluate the efficacy of folic acid supplementation for patients with DOR through measuring ovarian reserve markers, including AFC, serum AMH and inhibin B, so as to provide novel regimens for the clinical treatment of DOR.

## MATERIALS AND METHODS

### Design, setting and participants

This is a single-centre, open-label, randomised, placebo-controlled clinical trial, and we plan to recruit infertile women with DOR admitted to the department of gynaecology, Nanping First Hospital Affiliated to Fujian Medical University (Nanping, China) during the period from July 2021 through July 2022. In this study, DOR is

defined as having at least two of the following criteria: (1) AMH <1 ng/mL, (2) AFC <5 follicles, (3) FSH >10 IU/L on day 2 or 3 of the menstrual cycle and (4) a previous poor ovarian response (three and less oocytes with a normal stimulation protocol).

Subjects that meet the following inclusion and exclusion criteria will be enrolled in this clinical trial.

### Inclusion criteria

1. Bilateral AFC <5 follicles, AMH <1 ng/mL or FSH >10 IU/L.
2. Infertile women at ages of 30–35 years.
3. Subjects are informed of the study purpose and are willing to sign the informed consent.

### Exclusion criteria

1. A history of ovarian or pelvic surgery, or a medical history of chemotherapy or radiotherapy.
2. Comorbidities with viral infections (hepatitis), tumours, autoimmune diseases, endocrine disorders, metabolic disorders or acute inflammation.
3. Patients with definite diagnosis of inherited diseases or a family history of inherited diseases.
4. Comorbidities with pregnancy, hyperprolactinaemia, polycystic ovary syndrome, endometriosis or positive signs in gynaecological examinations.
5. A history of administration of folic acid or hormone, or presence of drug-induced amenorrhoea during the past 3 months.

### Recruitment and randomisation

All eligible women with DOR will be invited to participate in this clinical trial. The investigators will explain the study protocol of this clinical trial to potential participants. After signing the informed consent, all participants will be enrolled and randomised to the folic acid group and the placebo group (figure 1). A computer-generated randomisation list, which is created by an independent statistician that is blinded to the trial and will not participate in the recruitment of the study subjects, will be employed with a 1:1 allocation. All participants are blinded to the list. The randomisation list will be managed using sequentially numbered identical study-drug containers, and these containers will be marked uniformly by two investigators who will not participate in the recruitment of the study subjects.

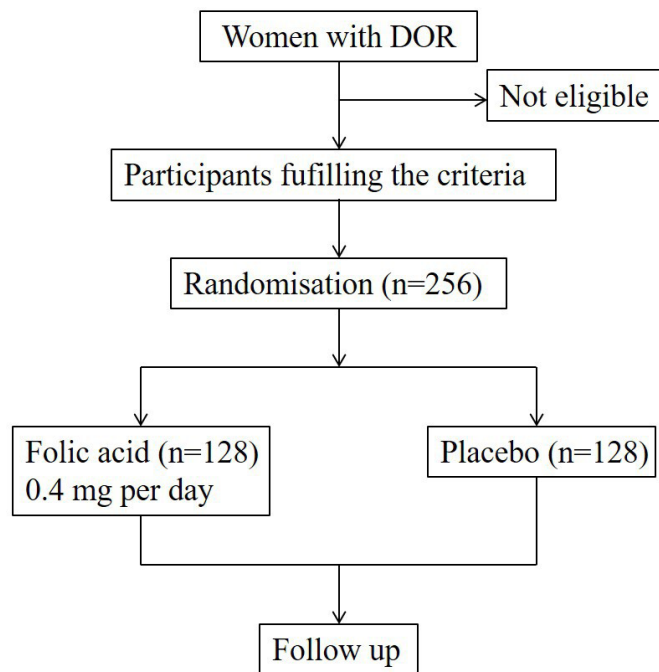
### Interventions

All subjects in the folic acid group will be given supplementation of folic acid at a daily dose of 0.4 mg for 6 months, while participants in the placebo group will be given placebo for 6 months.

### Outcomes

The primary outcome is AFC, and we will compare the changes of AFC between before in vitro fertilisation treatment and 6 months after folic acid supplementation in both the folic acid group and the placebo group.

The secondary outcomes include:



**Figure 1** Flowchart of the study design. DOR, diminished ovarian reserve.

1. Ovarian reserve markers. We plan to measure the changes of FSH, AMH and inhibin B levels following folic acid supplementation.
2. Clinical pregnancy, which is defined a positive urine human chorionic gonadotropin (hCG) test and a positive serum  $\beta$ -hCG test 14 and 16 days after embryo transfer. If persistent hCG tests are detected without menstruation seen, transvaginal B-mode ultrasonography on 56 days after the last day of the menstrual period identifies a gestational sac in the uterus, and embryo buds and primitive fetal heart beat are observed in the gestational sac, indicating successful clinical pregnancy.
3. Safety. We plan to observe drug-related adverse events, abnormal laboratory examinations and incidence of severe adverse events.

### Calculation of sample size

The prevalence of DOR is estimated to be approximately 10% among women seeking fertility treatments. To allow a 25% increase in the ovarian reserve in the folic acid group, a total of 128 participants are required (power 80% and  $\alpha$  error: 5%). Considering that 10% participants are withdrawn or lost to follow-up, 140 subjects will be recruited in each group.

### Data analysis

1. Effectiveness analysis. All measurement data are described with mean, SD, median, minimum, maximum and 95% confidential interval, and all categorical data are expressed as frequency and proportion.
2. Safety analysis. Drug-related adverse events, abnormal laboratory examinations and incidence of severe adverse events are descriptively analysed.

All statistical analyses are performed using the statistical software SPSS V.21.0 (IBM; Chicago, IL, USA), and a  $p$  value  $< 0.05$  is considered statistically significant.

### Study period

This clinical trial will start from July 2021 and will continue for 12 months. It is anticipated that the last participant will be recruited in July 2022.

### Patient enrolment

After written informed consent is obtained from all subjects that meet the inclusion and exclusion criteria, all eligible subjects will be enrolled, and the investigators will start the treatment.

### Data collection

All data will be saved as a password-protected file and uploaded to an access-restricted database, and all data will be only given to the principle investigators and statisticians. In addition, all drug-related toxicity, treatment cessation or termination, and deviation in the treatment regimen will be recorded.

### ETHICS AND DISSEMINATION

This study was approved by the Ethics Review Committee of Nanping First Hospital Affiliated to Fujian Medical University on 10 February 2021 (approval number: NPSY202002042), and registered in Chinese Clinical Trial Registry (registration number: ChiCTR2100047410). Written informed consent was obtained from all participants prior to randomisation, following a detailed description of the purpose of the study. The results of this clinical trial will be presented at scientific conferences and submitted to a peer-reviewed journal.

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**Contributors** ZJ and ZW conceived and designed the study. KL, YW, LZ, DL, JW, BW and ZW will perform the study. KL will collect and analyse all experimental data. KL and YW provided the first version of the manuscript. YW, LZ, DL, JW, BW and ZW provided critical comments on the original manuscript. ZJ revised and finalised the manuscript. All authors read and approved the final version of the manuscript.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

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