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BMJ Open

Effect of folic acid supplementation on diminished ovarian reserve: study protocol of a single-center, open-label, randomized, placebo-controlled clinical trial

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Keywords:	CLINICAL PHARMACOLOGY, Reproductive medicine < GYNAECOLOGY, Subfertility < GYNAECOLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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4 **Effect of folic acid supplementation on diminished ovarian**
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6 **reserve: study protocol of a single-center, open-label,**
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8 **randomized, placebo-controlled clinical trial**
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Abstract

Introduction: The incidence of diminished ovarian reserve (DOR), a common gynecological 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 hypothesized that folic acid may improve the DOR.

Methods and analysis: A single-center, open-label, randomized, placebo-controlled clinical trial is designed. We plan to recruit 140 women with DOR at ages of 30 to 35 years. All participants will be randomized 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 endpoint is antral follicle count, and the secondary endpoints 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 February 10, 2021 (approval number: NPSY202002042). Written informed consent was obtained from all participants prior to randomization, 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.

Clinical trial registration number: ChiCTR2100047410.

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5 **Keywords:** Diminished ovarian reserve; Folic acid; Antral follicle count;
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7 Anti-müllerian hormone; Inhibin B
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11 **Strengths and limitations of this study**
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16 1. This is a single-center, open-label, randomized, placebo-controlled clinical
17 trial.
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19 2. This is the first clinical trial to examine the effect of folic acid on diminished
20 ovarian reserve.
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22 3. This is an open-label trial, which may affect the study results.
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Introduction

Diminished ovarian reserve (DOR) is a common gynecological disorder characterized 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 [1]. DOR is considered as a warning sign of ovarian disorders [2]. If early diagnosis and treatment is not given, the ovarian function will continue to diminish until premature ovarian failure [3], leading to the loss of timing for fertility [4]. 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 [5]. The exact pathogenesis of DOR remains unclear, and currently, there is no effective treatment for DOR [6]. This disorder has become a hot topic and difficulty in gynecological reproductive endocrinology [1]. 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 [7]. 5-methyltetrahydrofolate (5-MTHF), the active form of folic acid, functions as a methyl-group donor to re-methylate homocysteine to methionine [8]. Folic acid deficiency may indirectly lead to elevated plasma homocysteine concentrations and reduced S-adenosyl methionine (SAM) [8]. These folic acid-dependent functions mainly include DNA synthesis, modification and repair, control of gene expression and cell division [9,10]. Folic acid metabolism is reported to affect the whole period of pregnancy [11]. Folic acid supplementation has shown effective to remarkably reduce the prevalence of neural tube defects [12,13], reduce the incidence of abortion and mediate ovarian functions [14].

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4 Precision prediction of ovarian reserve is of great significance for DOR
5 patients. Currently, anti-Müllerian hormone (AMH), inhibin B and antral follicle
6 count (AFC) are common markers used to predict ovarian reserve [15]. Both
7 AMH and inhibin B, secreted by the ovary, are members of the transforming
8 growth factor (TGF)- β superfamily, which may serve as markers for prediction
9 of ovarian reserve [16,17]. AMH is secreted by preantral ovarian follicles and
10 small antral ovarian follicles, and its level is strongly correlated with the follicle
11 number in the ovary [18]. In addition, AMH presents a mild fluctuation during
12 the menstrual cycle in relative to estradiol, follicle-stimulating hormone (FSH)
13 and luteinizing hormone (LH) [19]; however, it is strongly associated with AFC
14 and age, which is accepted as one of the most important marker for
15 assessment of ovarian reserve functions [20]. AFC is a count of ovarian antral
16 follicles by B-mode ultrasonography, which is non-invasive, easy to perform
17 and effective to assess the reserve of each ovary [21]. As the best parameter
18 that reflects the number of ovarian reserve, AFC is commonly used for clinical
19 assessment of female infertility at childbearing ages and ovarian reserve
20 among women undergoing assisted reproductive technology (ART) [22].
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39 However, there have been no reports pertaining to folic acid supplementation
40 for improvements of DOR until now. This clinical trial is therefore designed with
41 aims to evaluate the efficacy of folic acid supplementation for patients with
42 DOR through measuring ovarian reserve markers, including AFC, serum AMH
43 and inhibin B, so as to provide novel regimens for the clinical treatment of
44 DOR.
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52 **Materials and methods**

53 54 55 56 **Design, setting and participants**

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60 This is a single-center, open-label, randomized, placebo-controlled clinical

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4 trial, and we plan to recruit infertile women with DOR admitted to the
5 Department of Gynecology, Nanping First Hospital Affiliated to Fujian Medical
6 University (Nanping, China) during the period from January through December
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10 2022.

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

15 16 17 18 19 **Inclusion criteria**

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23 1. Bilateral AFC < 6 follicles, AMH < 1 ng/mL, or FSH > 10 mIU/mL;
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25 2. Infertile women at ages of 30 to 35 years.

26 27 28 29 **Exclusion criteria**

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33 1. A history of previous ovarian or pelvic surgery, or a medical history of
34 chemotherapy or radiotherapy;
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36 2. Comorbidities with viral infections (hepatitis), tumors, autoimmune
37 diseases, endocrine disorders, metabolic disorders or acute inflammation;
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39 3. Patients with definite diagnosis of inherited diseases or a family history of
40 inherited diseases;
- 41
42 4. Comorbidities with pregnancy, hyperprolactinemia, polycystic ovary
43 syndrome (PCOS), endometriosis or positive signs in gynecological
44 examinations;
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46 5. A history of administration of folic acid or hormone, or presence of
47 drug-induced amenorrhea during the past 3 months.

48 49 50 51 52 53 54 55 56 **Recruitment and randomization**

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59 All eligible women with DOR will be invited to participate in this clinical trial.
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4 The investigators will explain the study protocol of this clinical trial to potential
5 participants. After signing the informed consent, all participants will be enrolled
6 and randomized to the folic acid group and the placebo group (Figure 1). A
7 computer-generated randomization list, which is created by an independent
8 statistician that is blinded to the trial and will not participate in the recruitment
9 of the study subjects, will be employed with a 1:1 allocation. All participants are
10 blinded to the list. The randomization list will be managed using sequentially
11 numbered identical study-drug containers, and these containers will be marked
12 uniformly by two investigators who will not participate in the recruitment of the
13 study subjects.
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25 **Interventions**

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

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40 The primary outcome is AFC, and we will compare the changes of AFC
41 between before *in vitro* fertilization (IVF) treatment and 6 months after folic
42 acid supplementation in both the folic acid group and the placebo group.
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48 The secondary outcomes include:

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50 1. Ovarian reserve markers. We plan to measure the changes of FSH and
51 AMH levels following folic acid supplementation.
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54 2. Clinical pregnancy, which is defined a positive urine human chorionic
55 gonadotropin (hCG) test and a positive serum β -hCG test 14 and 16 days
56 after embryo transfer. If persistent hCG tests are detected without
57 menstruation seen, transvaginal B-mode ultrasonography on 56 days after
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4 the last day of the menstrual period identifies a gestational sac in the
5 uterus, and embryo buds and primitive fetal heart beat are observed in the
6 gestational sac, indicating successful clinical pregnancy.
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10 3. Safety. We plan to observe drug-related adverse events, abnormal
11 laboratory examinations and incidence of severe adverse events.
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14 15 **Calculation of sample size**

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19 The prevalence of DOR is estimated to be approximately 10% among women
20 seeking fertility treatments. To allow a 25% increase in the ovarian reserve in
21 the folic acid group, a total of 128 participants are required (power 80% and α
22 error: 5%). Considering that 10% participants are withdrawn or lost to
23 follow-up, 140 subjects will be recruited in each group.
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30 31 **Data analysis**

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35 1. Effectiveness analysis. All measurement data are described with mean,
36 standard deviation, median, minimum, maximum and 95% confidential
37 interval (*CI*), and all categorical data are expressed as frequency and
38 proportion.
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42 2. Safety analysis. Drug-related adverse events, abnormal laboratory
43 examinations and incidence of severe adverse events are descriptively
44 analyzed.
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50 All statistical analyses are performed using the statistical software SPSS
51 version 21.0 (IBM; Chicago, IL, USA), and a *P* value < 0.05 is considered
52 statistically significant.
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58 **Study period**

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4 This clinical trial will start from July, 2021, and will continue for 18 months. It is
5 anticipated that the last participant will be recruited on December, 2022.
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9 **Patient enrollment**

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13 After written informed consent is obtained from all subjects that meet the
14 inclusion and exclusion criteria, all eligible subjects will be enrolled, and the
15 investigators will start the treatment.
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19 **Data collection**

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

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

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59 We thank Dr. Ting Wu for assisting the registration of our clinical trial in the
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4 website. Sincere thanks are also expressed to all participants included in this
5
6 study.
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8 9 **Contributors**

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13 ZHJ and ZCW conceived and designed the study. KLJ, YW, LZ, DLL, JXW,
14
15 BJW and ZCW will perform the study. KJL will collect and analyze all
16
17 experimental data. KJL and YW provided the first version of the manuscript.
18
19 YW, LZ, DLL, JXW, BJW and ZCW provided critical comments on the original
20
21 manuscript. ZHJ revised and finalized the manuscript. All authors read and
22
23 approved the final version of the manuscript.
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25

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34
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38 39 **Competing interests**

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42 The authors declare no conflict of interests.
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45 46 **Patient and public involvement**

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50 Patients and/or the public were not involved in the design, or conduct, or
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52 reporting, or dissemination plans of this research.
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55 56 **Patient consent for publication**

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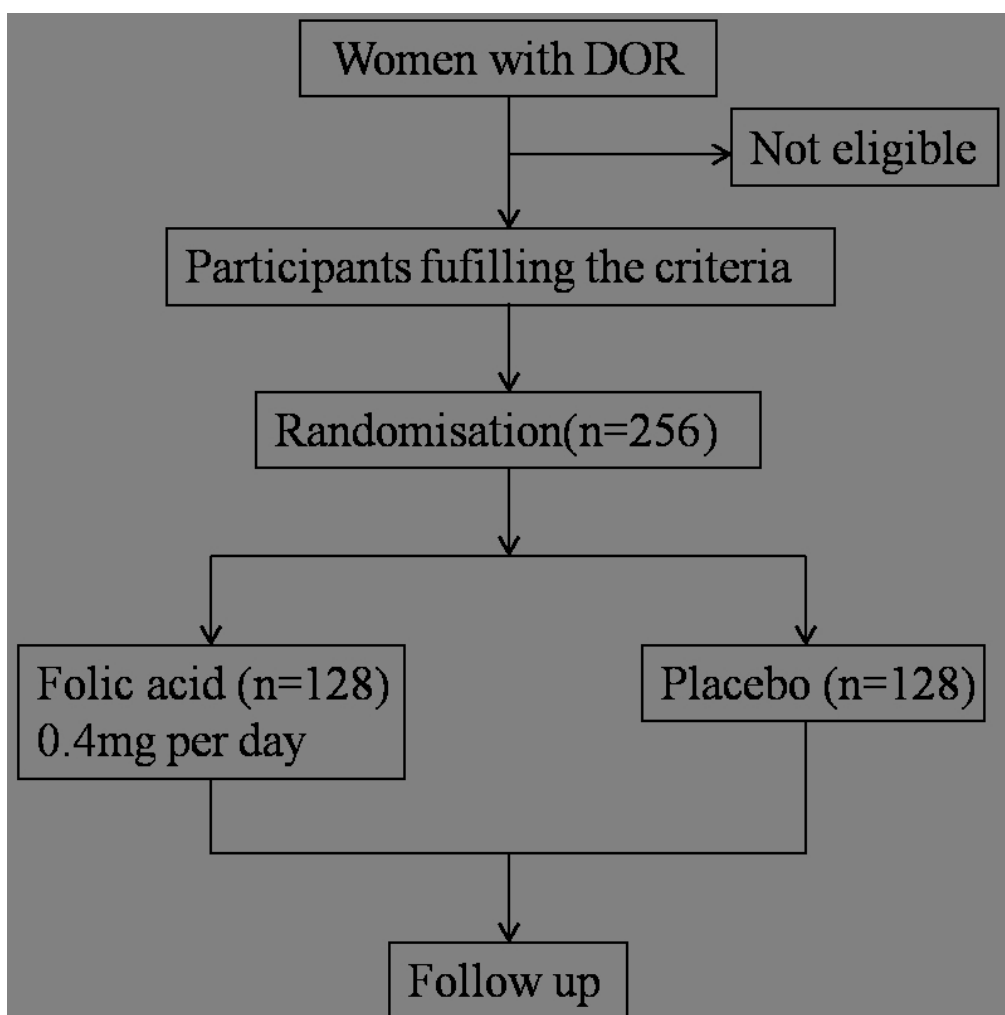
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Figure Legend

Figure 1. Flowchart of the study design

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Effect of folic acid supplementation on diminished ovarian reserve: study protocol of a single-center, open-label, randomized, placebo-controlled clinical trial

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Reproductive medicine
Keywords:	CLINICAL PHARMACOLOGY, Reproductive medicine < GYNAECOLOGY, Subfertility < GYNAECOLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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37 17 # These authors contributed equally to this study.
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4 30 **Abstract**

5 31 **Introduction:** The prevalence of diminished ovarian reserve (DOR), a
6 common gynecological disorder, is approximately 10% across the world.
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8 Failure in early diagnosis and treatment may result in continuous decreases in
9 ovarian function and the resultant loss in an opportunity of pregnancy, which
10 greatly affects the happiness of the women's family and women's physical and
11 mental health. Nevertheless, there has been no effective treatment for such a
12 disorder until now. Folic acid, a member of the vitamin B family, is involved in
13 one-carbon cycle and methylation regulation. It has been found that folic acid
14 affects the whole period of pregnancy, and folic acid supplementation has
15 shown effective to remarkably reduce the incidence of fetal neural tube defects
16 and decrease plasma homocysteic acid levels, thereby resulting in a decline in
17 the incidence of abortion. In addition, folic acid is reported to mediate ovarian
18 functions. It is therefore hypothesized that folic acid may improve DOR.

19 32 **Methods and analysis:** A single-center, open-label, randomized,
20 placebo-controlled clinical trial is designed. We plan to recruit 140 women with
21 DOR at ages of 30 to 35 years. All participants will be randomized into the folic
22 acid group and placebo group, and each subject will be given a tablet with the
23 same appearance daily for 6 months. The primary outcome is antral follicle
24 count, and the secondary outcomes are ovarian reserve markers, ovarian
25 low-dose stimulation responses and safety.

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27 Committee of Nanping First Hospital Affiliated to Fujian Medical University on
28 February 10, 2021 (approval number: NPSY202002042). Written informed
29 consent was obtained from all participants prior to randomization, following a
30 detailed description of the purpose of the study. The results of this clinical trial
31 will be presented at scientific conferences and submitted to a peer-reviewed
32 journal.

33 34 **Clinical trial registration number:** ChiCTR2100047410.
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61 **Keywords:** Diminished ovarian reserve; Folic acid; Antral follicle count;
62 Anti-müllerian hormone; Inhibin B

63

64 **Strengths and limitations of this study**

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- 66 1. This is a single-center, open-label, randomized, placebo-controlled clinical
67 trial, which will provide insights into the understanding of the effect of folic
68 acid supplementation on diminished ovarian reserve.
- 69 2. Random numbers are generated by computers, and either participants or
70 investigators are blind to the treatment.
- 71 3. This is the largest randomized controlled trial to investigate effect of folic
72 acid supplementation on diminished ovarian reserve until now, which will
73 recruit 140 women with diminished ovarian reserve.
- 74 4. Although there are diverse definitions of diminished ovarian reserve, the
75 diagnostic indicators employed for the inclusion criteria in this trial are the
76 most common and most frequently recommended measures.

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90 Introduction

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92 Diminished ovarian reserve (DOR) is a common gynecological disorder
93 characterized by poor fertility outcomes, which is caused by a reduced
94 capacity of the ovaries to produce eggs and a decline in the number of oocytes
95 [1]. DOR is considered as a warning sign of ovarian disorders [2]. If early
96 diagnosis and treatment is not given, the ovarian function will continue to
97 diminish until premature ovarian failure [3], leading to the loss of timing for
98 fertility [4]. Worsening environmental pollution, alteration of life styles and
99 delayed childbearing result in an increasing incidence of DOR, and the global
100 prevalence of DOR is estimated to be 10% in women seeking fertility treatment
101 [5]. The exact pathogenesis of DOR remains unclear, and currently, there is no
102 effective treatment for DOR [6]. This disorder has become a hot topic and
103 difficulty in gynecological reproductive endocrinology [1]. A search for novel
104 treatments is therefore of urgent need to improve DOR and increase the
105 opportunity of pregnancy among infertile women.

106

107 Folic acid, a member of the vitamin B family, is an essential nutrient for
108 humans, which is involved in one-carbon cycle and methylation regulation [7].
109 5-methyltetrahydrofolate (5-MTFH), the active form of folic acid, functions as a
110 methyl-group donor to re-methylate homocysteine to methionine [8]. Folic acid
111 deficiency may indirectly lead to elevated plasma homocysteic acid
112 concentrations and reduced S-adenosyl methionine (SAM) [8]. These folic
113 acid-dependent functions mainly include DNA synthesis, modification and
114 repair, control of gene expression and cell division [9,10]. Folic acid
115 metabolism is reported to affect the whole period of pregnancy [11]. Folic acid
116 supplementation has shown effective to remarkably reduce the prevalence of
117 neural tube defects [12,13], reduce the incidence of abortion and mediate
118 ovarian functions [14].

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4 120 Precision prediction of ovarian reserve is of great significance for DOR
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6 121 patients. Currently, anti-Müllerian hormone (AMH), inhibin B and antral follicle
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8 122 count (AFC) are common markers used to predict ovarian reserve [15]. Both
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10 123 AMH and inhibin B, secreted by the ovary, are members of the transforming
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12 124 growth factor (TGF)- β superfamily, which may serve as markers for prediction
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14 125 of ovarian reserve [16,17]. AMH is secreted by preantral ovarian follicles and
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16 126 small antral ovarian follicles, and its level is strongly correlated with follicle
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18 127 number in ovary [18]. In addition, AMH presents a mild fluctuation during the
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20 128 menstrual cycle in relative to estradiol, follicle-stimulating hormone (FSH) and
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22 129 luteinizing hormone (LH) [19]; however, it is strongly associated with AFC and
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24 130 age, which is accepted as one of the most important marker for assessment of
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26 131 ovarian reserve functions [20]. AFC is a count of ovarian antral follicles by
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28 132 B-mode ultrasonography, which is non-invasive, easy to perform and effective
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30 133 to assess the reserve of each ovary [21]. As the best parameter that reflects
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32 134 the number of ovarian reserve, AFC is commonly used for clinical assessment
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34 135 of female infertility at childbearing ages and ovarian reserve among women
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36 136 undergoing assisted reproductive technology (ART) [22].

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39 138 However, there have been no reports pertaining to folic acid supplementation
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41 139 for improving DOR until now. This clinical trial is therefore designed with aims
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43 140 to evaluate the efficacy of folic acid supplementation for patients with DOR
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45 141 through measuring ovarian reserve markers, including AFC, serum AMH and
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47 142 inhibin B, so as to provide novel regimens for the clinical treatment of DOR.

143 144 **Materials and methods**

145 146 **Design, setting and participants**

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148 This is a single-center, open-label, randomized, placebo-controlled clinical
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150 trial, and we plan to recruit infertile women with DOR admitted to the

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4 150 Department of Gynecology, Nanping First Hospital Affiliated to Fujian Medical
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6 151 University (Nanping, China) during the period from July 2021 through July
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8 152 2022. In this study, DOR is defined as having at least two of the following
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10 153 criteria: (1) AMH < 1 ng/mL; (2) AFC < 5 follicles; (3) FSH > 10 IU/L on day 2 or
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12 154 3 of the menstrual cycle; and (4) a previous poor ovarian response (3 and less
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14 155 oocytes with a normal stimulation protocol).
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17 157 Subjects that meet the following inclusion and exclusion criteria will be enrolled
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19 158 in this clinical trial.
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22 23 160 **Inclusion criteria**

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- 26 162 1. Bilateral AFC < 5 follicles, AMH < 1 ng/mL, or FSH > 10 IU/L;
 - 27 163 2. Infertile women at ages of 30 to 35 years;
 - 28 164 3. Subjects are informed of the study purpose and are willing to sign the
29 165 informed consent.
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37 167 **Exclusion criteria**

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- 40 169 1. A history of previous ovarian or pelvic surgery, or a medical history of
41 170 chemotherapy or radiotherapy;
 - 42 171 2. Comorbidities with viral infections (hepatitis), tumors, autoimmune
43 172 diseases, endocrine disorders, metabolic disorders or acute inflammation;
 - 44 173 3. Patients with definite diagnosis of inherited diseases or a family history of
45 174 inherited diseases;
 - 46 175 4. Comorbidities with pregnancy, hyperprolactinemia, polycystic ovary
47 176 syndrome (PCOS), endometriosis or positive signs in gynecological
48 177 examinations;
 - 49 178 5. A history of administration of folic acid or hormone, or presence of
50 179 drug-induced amenorrhea during the past 3 months.

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181 **Recruitment and randomization**

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183 All eligible women with DOR will be invited to participate in this clinical trial.

184 The investigators will explain the study protocol of this clinical trial to potential

185 participants. After signing the informed consent, all participants will be enrolled

186 and randomized to the folic acid group and the placebo group (Figure 1). A

187 computer-generated randomization list, which is created by an independent

188 statistician that is blinded to the trial and will not participate in the recruitment

189 of the study subjects, will be employed with a 1:1 allocation. All participants are

190 blinded to the list. The randomization list will be managed using sequentially

191 numbered identical study-drug containers, and these containers will be marked

192 uniformly by two investigators who will not participate in the recruitment of the

193 study subjects.

194

195 **Interventions**

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197 All subjects in the folic acid group will be given supplementation of folic acid at

198 a daily dose of 0.4 mg for 6 months, while participants in the placebo group will

199 be given placebo for 6 months.

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201 **Outcomes**

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203 The primary outcome is AFC, and we will compare the changes of AFC

204 between before *in vitro* fertilization (IVF) treatment and 6 months after folic

205 acid supplementation in both the folic acid group and the placebo group.

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207 The secondary outcomes include:

208 1. Ovarian reserve markers. We plan to measure the changes of FSH, AMH

209 and inhibin B levels following folic acid supplementation.

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4 210 2. Clinical pregnancy, which is defined a positive urine human chorionic
5 211 gonadotropin (hCG) test and a positive serum β -hCG test 14 and 16 days
6 212 after embryo transfer. If persistent hCG tests are detected without
7 213 menstruation seen, transvaginal B-mode ultrasonography on 56 days after
8 214 the last day of the menstrual period identifies a gestational sac in the
9 215 uterus, and embryo buds and primitive fetal heart beat are observed in the
10 216 gestational sac, indicating successful clinical pregnancy.
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17 217 3. Safety. We plan to observe drug-related adverse events, abnormal
18 218 laboratory examinations and incidence of severe adverse events.
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23 220 **Calculation of sample size**

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27 222 The prevalence of DOR is estimated to be approximately 10% among women
28 223 seeking fertility treatments. To allow a 25% increase in the ovarian reserve in
29 224 the folic acid group, a total of 128 participants are required (power 80% and α
30 225 error: 5%). Considering that 10% participants are withdrawn or lost to
31 226 follow-up, 140 subjects will be recruited in each group.
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38 228 **Data analysis**

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42 230 1. Effectiveness analysis. All measurement data are described with mean,
43 231 standard deviation, median, minimum, maximum and 95% confidential
44 232 interval (CI), and all categorical data are expressed as frequency and
45 233 proportion.
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50 234 2. Safety analysis. Drug-related adverse events, abnormal laboratory
51 235 examinations and incidence of severe adverse events are descriptively
52 236 analyzed.
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58 238 All statistical analyses are performed using the statistical software SPSS
59 239 version 21.0 (IBM; Chicago, IL, USA), and a P value < 0.05 is considered

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4 240 statistically significant.

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7 242 **Study period**

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11 244 This clinical trial will start from July, 2021, and will continue for 12 months. It is
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13 245 anticipated that the last participant will be recruited in July, 2022.

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17 247 **Patient enrollment**

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21 249 After written informed consent is obtained from all subjects that meet the
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23 250 inclusion and exclusion criteria, all eligible subjects will be enrolled, and the
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25 251 investigators will start the treatment.

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29 253 **Data collection**

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33 255 All data will be saved as a password-protected file and uploaded to an
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35 256 access-restricted database, and all data will be only given to the principle
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37 257 investigators and statisticians. In addition, all drug-related toxicity, treatment
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39 258 cessation or termination, and deviation in the treatment regimen will be
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41 259 recorded.

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45 261 **Ethics and dissemination:**

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48
49 263 This study was approved by the Ethics Review Committee of Nanping First
50
51 264 Hospital Affiliated to Fujian Medical University on February 10, 2021 (approval
52
53 265 number: NPSY202002042), and registered in Chinese Clinical Trial Registry
54
55 266 (registration number: ChiCTR2100047410). Written informed consent was
56
57 267 obtained from all participants prior to randomization, following a detailed
58
59 268 description of the purpose of the study. The results of this clinical trial will be
60
269 presented at scientific conferences and submitted to a peer-reviewed journal.

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5 271 **Acknowledgements**

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8
9 273 We thank Dr. Ting Wu for assisting the registration of our clinical trial in the
10 274 website. Sincere thanks are also expressed to all participants included in this
11 275 study.
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17 277 **Contributors**

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21 279 ZHJ and ZCW conceived and designed the study. KLJ, YW, LZ, DLL, JXW,
22 280 BJW and ZCW will perform the study. KJL will collect and analyze all
23 281 experimental data. KJL and YW provided the first version of the manuscript.
24 282 YW, LZ, DLL, JXW, BJW and ZCW provided critical comments on the original
25 283 manuscript. ZHJ revised and finalized the manuscript. All authors read and
26 284 approved the final version of the manuscript.
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34
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37 287

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41 290 2019QH1247).
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45 291

46 292 **Competing interests**

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48 293

49
50 294 The authors declare no conflict of interests.
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54 296 **Patient and public involvement**

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58 298 Patients and/or the public were not involved in the design, or conduct, or
59 299 reporting, or dissemination plans of this research.
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301 **Patient consent for publication**

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303 N/A

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4 390 **Figure Legend**

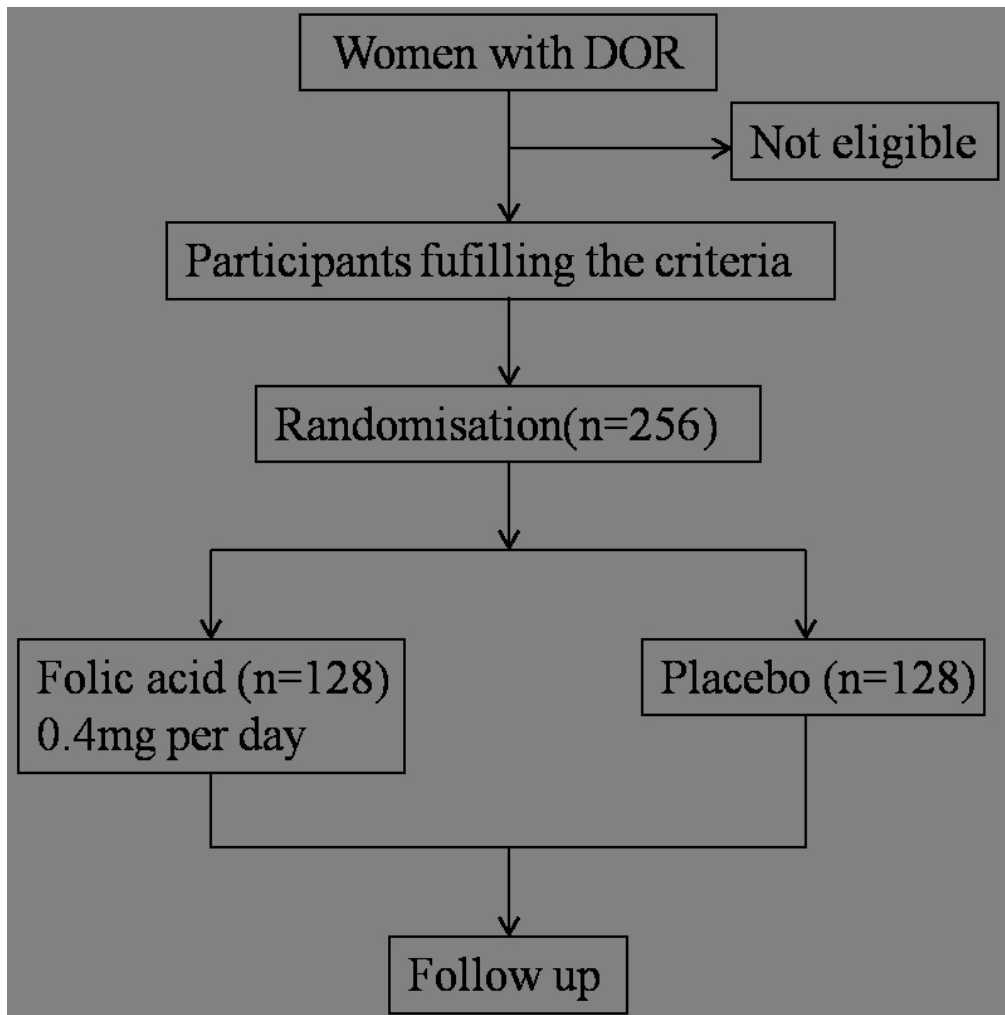
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8 392 Figure 1. Flowchart of the study design

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For peer review only

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page and Line No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, lines 1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 9, lines 263-266
	2b	All items from the World Health Organization Trial Registration Data Set	Page 2, line 59
Protocol version	3	Date and version identifier	Page 9, lines 263-266
Funding	4	Sources and types of financial, material, and other support	Page 10, lines 286-290
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 10, lines 279-284
	5b	Name and contact information for the trial sponsor	Page 1, lines 22-27
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 10, lines 279-284
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Page 10, lines 279-284
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 4/5, lines 92-118

1				
2		6b	Explanation for choice of comparators	Page 5, lines
3				120-136
4				
5	Objectives	7	Specific objectives or hypotheses	Page 5, lines
6				139-142
7				
8	Trial design	8	Description of trial design including type of trial (eg, parallel	Page 5/6, lines
9			group, crossover, factorial, single group), allocation ratio,	148-149
10			and framework (eg, superiority, equivalence, noninferiority,	
11			exploratory)	
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic	Page 4/5, lines
17			hospital) and list of countries where data will be collected.	149-151
18			Reference to where list of study sites can be obtained	
19				
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21	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable,	Page 5, lines
22			eligibility criteria for study centres and individuals who will	162-179
23			perform the interventions (eg, surgeons, psychotherapists)	
24				
25	Interventions	11a	Interventions for each group with sufficient detail to allow	Page 7, lines
26			replication, including how and when they will be	197-199
27			administered	
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30		11b	Criteria for discontinuing or modifying allocated interventions	N/A
31			for a given trial participant (eg, drug dose change in	
32			response to harms, participant request, or	
33			improving/worsening disease)	
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36		11c	Strategies to improve adherence to intervention protocols,	N/A
37			and any procedures for monitoring adherence (eg, drug	
38			tablet return, laboratory tests)	
39				
40		11d	Relevant concomitant care and interventions that are	N/A
41			permitted or prohibited during the trial	
42				
43	Outcomes	12	Primary, secondary, and other outcomes, including the	Page 7/8, lines
44			specific measurement variable (eg, systolic blood pressure),	203-218
45			analysis metric (eg, change from baseline, final value, time	
46			to event), method of aggregation (eg, median, proportion),	
47			and time point for each outcome. Explanation of the clinical	
48			relevance of chosen efficacy and harm outcomes is strongly	
49			recommended	
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52	Participant	13	Time schedule of enrolment, interventions (including any	Page 7, lines
53	timeline		run-ins and washouts), assessments, and visits for	183-193, Figure
54			participants. A schematic diagram is highly recommended	1
55			(see Figure)	
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2	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 8, lines 222-226
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7	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
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10 **Methods: Assignment of interventions (for controlled trials)**

11 Allocation:

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14	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 7, lines 183-193
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24	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 7, lines 183-193
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30	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 7, lines 183-193
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33	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 7, lines 183-193
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38		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 7, lines 183-193
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42 **Methods: Data collection, management, and analysis**

43				
44	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 8/9, lines 230-240
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53		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
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2	Data	19	Plans for data entry, coding, security, and storage, including	N/A
3	management		any related processes to promote data quality (eg, double	
4			data entry; range checks for data values). Reference to	
5			where details of data management procedures can be found,	
6			if not in the protocol	
7				
8	Statistical	20a	Statistical methods for analysing primary and secondary	Page 8/9, lines
9	methods		outcomes. Reference to where other details of the statistical	230-240
10			analysis plan can be found, if not in the protocol	
11				
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13		20b	Methods for any additional analyses (eg, subgroup and	N/A
14			adjusted analyses)	
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16		20c	Definition of analysis population relating to protocol non-	N/A
17			adherence (eg, as randomised analysis), and any statistical	
18			methods to handle missing data (eg, multiple imputation)	
19				
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21	Methods: Monitoring			
22				
23	Data monitoring	21a	Composition of data monitoring committee (DMC); summary	N/A
24			of its role and reporting structure; statement of whether it is	
25			independent from the sponsor and competing interests; and	
26			reference to where further details about its charter can be	
27			found, if not in the protocol. Alternatively, an explanation of	
28			why a DMC is not needed	
29				
30				
31		21b	Description of any interim analyses and stopping guidelines,	N/A
32			including who will have access to these interim results and	
33			make the final decision to terminate the trial	
34				
35	Harms	22	Plans for collecting, assessing, reporting, and managing	N/A
36			solicited and spontaneously reported adverse events and	
37			other unintended effects of trial interventions or trial conduct	
38				
39				
40	Auditing	23	Frequency and procedures for auditing trial conduct, if any,	N/A
41			and whether the process will be independent from	
42			investigators and the sponsor	
43				
44				
45	Ethics and dissemination			
46				
47	Research ethics	24	Plans for seeking research ethics committee/institutional	Page 9, lines
48	approval		review board (REC/IRB) approval	263-268
49				
50	Protocol	25	Plans for communicating important protocol modifications	N/A
51	amendments		(eg, changes to eligibility criteria, outcomes, analyses) to	
52			relevant parties (eg, investigators, REC/IRBs, trial	
53			participants, trial registries, journals, regulators)	
54				
55	Consent or assent	26a	Who will obtain informed consent or assent from potential	Page 9, lines
56			trial participants or authorised surrogates, and how (see Item	263-268
57			32)	
58				
59				
60				

1			
2		26b	Additional consent provisions for collection and use of
3			participant data and biological specimens in ancillary
4			studies, if applicable
5			
6	Confidentiality	27	How personal information about potential and enrolled
7			participants will be collected, shared, and maintained in
8			order to protect confidentiality before, during, and after the
9			trial
10			
11			
12	Declaration of	28	Financial and other competing interests for principal
13	interests		investigators for the overall trial and each study site
14			
15	Access to data	29	Statement of who will have access to the final trial dataset,
16			and disclosure of contractual agreements that limit such
17			access for investigators
18			
19	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
20	post-trial care		compensation to those who suffer harm from trial
21			participation
22			
23			
24	Dissemination	31a	Plans for investigators and sponsor to communicate trial
25	policy		results to participants, healthcare professionals, the public,
26			and other relevant groups (eg, via publication, reporting in
27			results databases, or other data sharing arrangements),
28			including any publication restrictions
29			
30			
31		31b	Authorship eligibility guidelines and any intended use of
32			professional writers
33			
34		31c	Plans, if any, for granting public access to the full protocol,
35			participant-level dataset, and statistical code
36			
37			
38	Appendices		
39			
40	Informed consent	32	Model consent form and other related documentation given
41	materials		to participants and authorised surrogates
42			
43	Biological	33	Plans for collection, laboratory evaluation, and storage of
44	specimens		biological specimens for genetic or molecular analysis in the
45			current trial and for future use in ancillary studies, if
46			applicable
47			

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