BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057689
Article Type:	Protocol
Date Submitted by the Author:	23-Sep-2021
Complete List of Authors:	Liao, Kaijun; First Hospital of Nanping City Wang, Yan; First Hospital of Nanping City Zheng, Ling; First Hospital of Nanping City Lu, Dongli; First Hospital of Nanping City Wu, Jiaxin; First Hospital of Nanping City Wu, Binjin; First Hospital of Nanping City Wu, Zhaochun; Nanping First Hospital Affiliated to Fujian Medical University, Pharmacy Jiang, Zhenghua; Nanping First Hospital affiliated to Fujian Medical University
Keywords:	CLINICAL PHARMACOLOGY, Reproductive medicine < GYNAECOLOGY, Subfertility < GYNAECOLOGY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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

Kaijun Liao[1#], Yan Wang[2#], Ling Zheng[1], Dongli Lu[1], Jiaxin Wu[1], Binjin Wu[1], Zhaohcun Wu[1*], Zhenghua Jiang[1*]

1 Department of Pharmacy, Nanping First Hospital Affiliated to Fujian Medical University, Nanping City, Fujian Province, 353033, China;
2 Department of Gynecology and Obstetrics, Nanping First Hospital Affiliated to Fujian Medical University, Nanping City, Fujian Province, 353033, China.

# These authors contributed equally to this study.

* Corresponding authors.
Department of Pharmacy, Nanping First Hospital Affiliated to Fujian Medical University, No. 317 Zhongshan Road, Yanping District, Nanping City, Fujian Province, 353033, China.
Tel/Fax: 86-599-8608039;
E-mail: 53018640@qq.com (Zhaochun Wu) or 798563491@qq.com (Zhenghua Jiang).

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.

Keywords: Diminished ovarian reserve; Folic acid; Antral follicle count; Anti-müllerian hormone; Inhibin B

Strengths and limitations of this study

1. This is a single-center, open-label, randomized, placebo-controlled clinical trial.
2. This is the first clinical trial to examine the effect of folic acid on diminished ovarian reserve.
3. This is an open-label trial, which may affect the study results.

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-MTFH), the active form of folic acid, functions as a methyl-group donor to re-methylate homocysteine to methionine [8]. Focid acid deficiency may indirectly lead to elevated plasma homocysteic acid 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].

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 [15]. Both AMH and inhibin B, secreted by the ovary, are members of the transforming growth factor (TGF)-β superfamily, which may serve as markers for prediction of ovarian reserve [16,17]. AMH is secreted by preantral ovarian follicles and small antral ovarian follicles, and its level is strongly correlated with the follicle number in the ovary [18]. In addition, AMH presents a mild fluctuation during the menstrual cycle in relative to estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [19]; 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 [20]. 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 [21]. 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 (ART) [22].

However, there have been no reports pertaining to folic acid supplementation for improvements of 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-center, open-label, randomized, placebo-controlled clinical

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

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

Inclusion criteria

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

Exclusion criteria

1. A history of previous ovarian or pelvic surgery, or a medical history of chemotherapy or radiotherapy;
2. Comorbidities with viral infections (hepatitis), tumors, 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, hyperprolactinemia, polycystic ovary syndrome (PCOS), endometriosis or positive signs in gynecological examinations;
5. A history of administration of folic acid or hormone, or presence of drug-induced amenorrhea during the past 3 months.

Recruitment and randomization

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 randomized to the folic acid group and the placebo group (Figure 1). A computer-generated randomization 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 randomization 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* fertilization (IVF) treatment and 6 months after folic acid supplementation in both the folic acid group and the placebo group.

The secondary outcomes include:

1. Ovarian reserve markers. We plan to measure the changes of FSH and AMH levels following folic acid supplementation.
2. Clinical pregnancy, which is defined a positive urine human chorionic gonadotropin (hCG) test and a positive serum β-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 α 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, standard deviation, median, minimum, maximum and 95% confidential interval (*CI*), 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 analyzed.

All statistical analyses are performed using the statistical software SPSS version 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 18 months. It is anticipated that the last participant will be recruited on December, 2022.

Patient enrollment

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 February 10, 2021 (approval number: NPSY202002042), and registered in Chinese Clinical Trial Registry (registration number: ChiCTR2100047410). 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.

Acknowledgements

We thank Dr. Ting Wu for assisting the registration of our clinical trial in the

website. Sincere thanks are also expressed to all participants included in this study.

Contributors

ZHJ and ZCW conceived and designed the study. KLJ, YW, LZ, DLL, JXW, BJW and ZCW will perform the study. KJL will collect and analyze all experimental data. KJL and YW provided the first version of the manuscript. YW, LZ, DLL, JXW, BJW and ZCW provided critical comments on the original manuscript. ZHJ revised and finalized the manuscript. All authors read and approved the final version of the manuscript.

Funding

This study was supported the grants from the Startup Fund for Scientific Research, Fujian Medical University (grant numbers: 2019QH1223 and 2019QH1247).

Competing interests

The authors declare no conflict of interests.

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

N/A

References

1. Ata B, Seyhan A, Seli E. Diminished ovarian reserve versus ovarian aging: overlaps and differences. Curr Opin Obstet Gynecol. 2019; 31(3): 139-147.
2. Barros F, Carvalho F, Barros A, Dória S. Premature ovarian insufficiency: clinical orientations for genetic testing and genetic counseling. Porto Biomed J. 2020; 5(3): e62.
3. Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder--a plea for universal definitions. J Assist Reprod Genet. 2015; 32(12): 1709-1712.
4. Cohen J, Mounsambote L, Prier P, Mathieu d'ARGENT E, Selleret L, Chabbert-Buffet N, Delarouziere V, Levy R, Darai E, Antoine JM. Outcomes of first IVF/ICSI in young women with diminished ovarian reserve. Minerva Ginecol. 2017; 69(4): 315-321.
5. Wald KA, Shahine LK, Lamb JD, Marshall LA, Hickok LR. High incidence of diminished ovarian reserve in young unexplained recurrent pregnancy loss patients. Gynecol Endocrinol. 2020; 36(12): 1079-1081.
6. Cedars MI. Diminished ovarian reserve. How do you diagnosis it? What does it mean? Semin Reprod Med. 2013; 31(6): 391-392.
7. Likis F. Folic Acid. J Midwifery Womens Health. 2016; 61(6): 797-798.
8. Bailey LB, Gregory JF 3rd. Folate metabolism and requirements. J Nutr. 1999; 129(4): 779-782.
9. Kim KC, Friso S, Choi SW. DNA methylation, an epigenetic mechanism connecting folate to healthy embryonic development and aging. J Nutr Biochem. 2009; 20(12): 917-926.
10. Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. FASEB J. 2010; 24(11): 4167-4174.

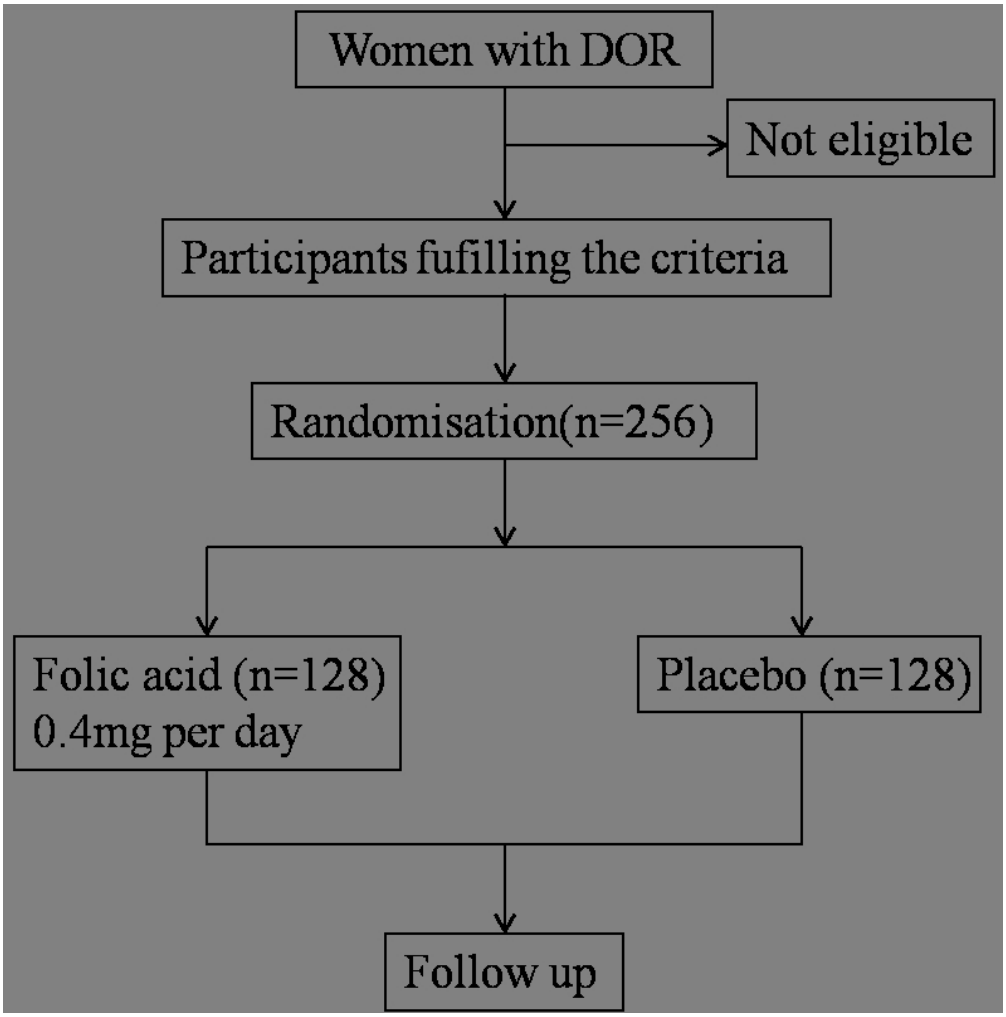
11. Ferrazzi E, Tiso G, Di Martino D. Folic acid versus 5- methyl tetrahydrofolate supplementation in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2020; 253: 312-319.
12. Viswanathan M, Treiman KA, Kish-Doto J, Middleton JC, Coker-Schwimmer EJ, Nicholson WK. Folic acid supplementation for the prevention of neural tube defects: An updated evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2017; 317(2): 190-203.
13. van Gool JD, Hirche H, Lax H, De Schaepdrijver L. Folic acid and primary prevention of neural tube defects: A review. Reprod Toxicol. 2018; 80: 73-84.
14. Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL. Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update. 2007; 13(3): 225-238.
15. Yilmaz N, Uygur D, Inal H, Gorkem U, Cicek N, Mollamahmutoglu L. Dehydroepiandrosterone supplementation improves predictive markers for diminished ovarian reserve: serum AMH, inhibin B and antral follicle count. Eur J Obstet Gynecol Reprod Biol. 2013; 169(2): 257-260.
16. Moolhuijsen LME, Visser JA. Anti-müllerian hormone and ovarian reserve: update on assessing ovarian function. J Clin Endocrinol Metab. 2020; 105(11): 3361-3373.
17. Wen J, Huang K, Du X, Zhang H, Ding T, Zhang C, Ma W, Zhong Y, Qu W, Liu Y, Li Z, Deng S, Luo A, Jin Y, Zhang J, Wang S. Can inhibin B reflect ovarian reserve of healthy reproductive age women effectively? Front Endocrinol (Lausanne). 2021; 12: 626534.
18. Bedenk J, Vrtačnik-Bokal E, Virant-Klun I. The role of anti-Müllerian hormone (AMH) in ovarian disease and infertility. J Assist Reprod Genet. 2020; 37(1): 89-100.
19. Victoria M, Labrosse J, Krief F, Cédrin-Durnerin I, Comtet M, Grynberg M. Anti Müllerian Hormone: More than a biomarker of female reproductive

function. J Gynecol Obstet Hum Reprod. 2019; 48(1): 19-24.

20. Hawkins Bressler L, Steiner A. Anti-Müllerian hormone as a predictor of reproductive potential. Curr Opin Endocrinol Diabetes Obes. 2018; 25(6): 385-390.
21. Coelho Neto MA, Ludwin A, Borrell A, Benacerraf B, Dewailly D, da Silva Costa F, Condous G, Alcazar JL, Jokubkiene L, Guerriero S, Van den Bosch T, Martins WP. Counting ovarian antral follicles by ultrasound: a practical guide. Ultrasound Obstet Gynecol. 2018; 51(1): 10-20.
22. Scheffer GJ, Broekmans FJ, Looman CW, Blankenstein M, Fauser BC, teJong FH, teVelde ER. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. Hum Reprod. 2003; 18(4): 700-706.

Figure Legend

Figure 1. Flowchart of the study design



342x344mm (59 x 59 DPI)

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057689.R1
Article Type:	Protocol
Date Submitted by the Author:	02-Apr-2022
Complete List of Authors:	Liao, Kaijun; First Hospital of Nanping City Wang, Yan; First Hospital of Nanping City Zheng, Ling; First Hospital of Nanping City Lu, Dongli; First Hospital of Nanping City Wu, Jiaxin; First Hospital of Nanping City Wu, Binjin; First Hospital of Nanping City Wu, Zhaochun; Nanping First Hospital Affiliated to Fujian Medical University, Pharmacy Jiang, Zhenghua; Nanping First Hospital affiliated to Fujian Medical University
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

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

Kaijun Liao[1#], Yan Wang[2#], Ling Zheng[1], Dongli Lu[1], Jiaxin Wu[1], Binjin Wu[1], Zhaochun Wu[1*], Zhenghua Jiang[1*]

1 Department of Pharmacy, Nanping First Hospital Affiliated to Fujian Medical University, Nanping City, Fujian Province, 353033, China;
2 Department of Gynecology and Obstetrics, Nanping First Hospital Affiliated to Fujian Medical University, Nanping City, Fujian Province, 353033, China.

# These authors contributed equally to this study.

* Corresponding authors.
Department of Pharmacy, Nanping First Hospital Affiliated to Fujian Medical University, No. 317 Zhongshan Road, Yanping District, Nanping City, Fujian Province, 353033, China.
Tel/Fax: 86-599-8608039;
E-mail: 53018640@qq.com (Zhaochun Wu) or 798563491@qq.com (Zhenghua Jiang).

Abstract

Introduction: The prevalence 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 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 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 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.

Keywords: Diminished ovarian reserve; Folic acid; Antral follicle count; Anti-müllerian hormone; Inhibin B

Strengths and limitations of this study

1. This is a single-center, open-label, randomized, placebo-controlled clinical trial, which will provide insights into the understanding of the effect of folic acid supplementation on diminished ovarian reserve.
2. Random numbers are generated by computers, and either participants or investigators are blind to the treatment.
3. This is the largest randomized controlled trial to investigate effect of folic acid supplementation on diminished ovarian reserve until now, which will recruit 140 women with diminished ovarian reserve.
4. 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.

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-MTFH), the active form of folic acid, functions as a methyl-group donor to re-methylate homocysteine to methionine [8]. Focid acid deficiency may indirectly lead to elevated plasma homocysteic acid 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].

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 [15]. Both AMH and inhibin B, secreted by the ovary, are members of the transforming growth factor (TGF)-β superfamily, which may serve as markers for prediction of ovarian reserve [16,17]. AMH is secreted by preantral ovarian follicles and small antral ovarian follicles, and its level is strongly correlated with follicle number in ovary [18]. In addition, AMH presents a mild fluctuation during the menstrual cycle in relative to estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [19]; 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 [20]. 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 [21]. 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 (ART) [22].

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-center, open-label, randomized, placebo-controlled clinical trial, and we plan to recruit infertile women with DOR admitted to the

Department of Gynecology, 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 (3 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 to 35 years;
3. Subjects are informed of the study purpose and are willing to sign the informed consent.

Exclusion criteria

1. A history of previous ovarian or pelvic surgery, or a medical history of chemotherapy or radiotherapy;
2. Comorbidities with viral infections (hepatitis), tumors, 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, hyperprolactinemia, polycystic ovary syndrome (PCOS), endometriosis or positive signs in gynecological examinations;
5. A history of administration of folic acid or hormone, or presence of drug-induced amenorrhea during the past 3 months.

Recruitment and randomization

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 randomized to the folic acid group and the placebo group (Figure 1). A computer-generated randomization 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 randomization 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* fertilization (IVF) treatment and 6 months after folic acid supplementation in both the folic acid group and the placebo group.

The secondary outcomes include:

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 β-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 α 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, standard deviation, median, minimum, maximum and 95% confidential interval (*CI*), 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 analyzed.

All statistical analyses are performed using the statistical software SPSS version 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 enrollment

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 February 10, 2021 (approval number: NPSY202002042), and registered in Chinese Clinical Trial Registry (registration number: ChiCTR2100047410). 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.

Acknowledgements

We thank Dr. Ting Wu for assisting the registration of our clinical trial in the website. Sincere thanks are also expressed to all participants included in this study.

Contributors

ZHJ and ZCW conceived and designed the study. KLJ, YW, LZ, DLL, JXW, BJW and ZCW will perform the study. KJL will collect and analyze all experimental data. KJL and YW provided the first version of the manuscript. YW, LZ, DLL, JXW, BJW and ZCW provided critical comments on the original manuscript. ZHJ revised and finalized the manuscript. All authors read and approved the final version of the manuscript.

Funding

This study was supported the grants from the Startup Fund for Scientific Research, Fujian Medical University (grant numbers: 2019QH1223 and 2019QH1247).

Competing interests

The authors declare no conflict of interests.

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

N/A

References

1. Ata B, Seyhan A, Seli E. Diminished ovarian reserve versus ovarian aging: overlaps and differences. Curr Opin Obstet Gynecol. 2019; 31(3): 139-147.
2. Barros F, Carvalho F, Barros A, Dória S. Premature ovarian insufficiency: clinical orientations for genetic testing and genetic counseling. Porto Biomed J. 2020; 5(3): e62.
3. Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder--a plea for universal definitions. J Assist Reprod Genet. 2015; 32(12): 1709-1712.
4. Cohen J, Mounsambote L, Prier P, Mathieu d'ARGENT E, Selleret L, Chabbert-Buffet N, Delarouziere V, Levy R, Darai E, Antoine JM. Outcomes of first IVF/ICSI in young women with diminished ovarian reserve. Minerva Ginecol. 2017; 69(4): 315-321.
5. Wald KA, Shahine LK, Lamb JD, Marshall LA, Hickok LR. High incidence of diminished ovarian reserve in young unexplained recurrent pregnancy loss patients. Gynecol Endocrinol. 2020; 36(12): 1079-1081.
6. Cedars MI. Diminished ovarian reserve. How do you diagnosis it? What does it mean? Semin Reprod Med. 2013; 31(6): 391-392.
7. Likis F. Folic Acid. J Midwifery Womens Health. 2016; 61(6): 797-798.
8. Bailey LB, Gregory JF 3rd. Folate metabolism and requirements. J Nutr. 1999; 129(4): 779-782.
9. Kim KC, Friso S, Choi SW. DNA methylation, an epigenetic mechanism connecting folate to healthy embryonic development and aging. J Nutr

Biochem. 2009; 20(12): 917-926.

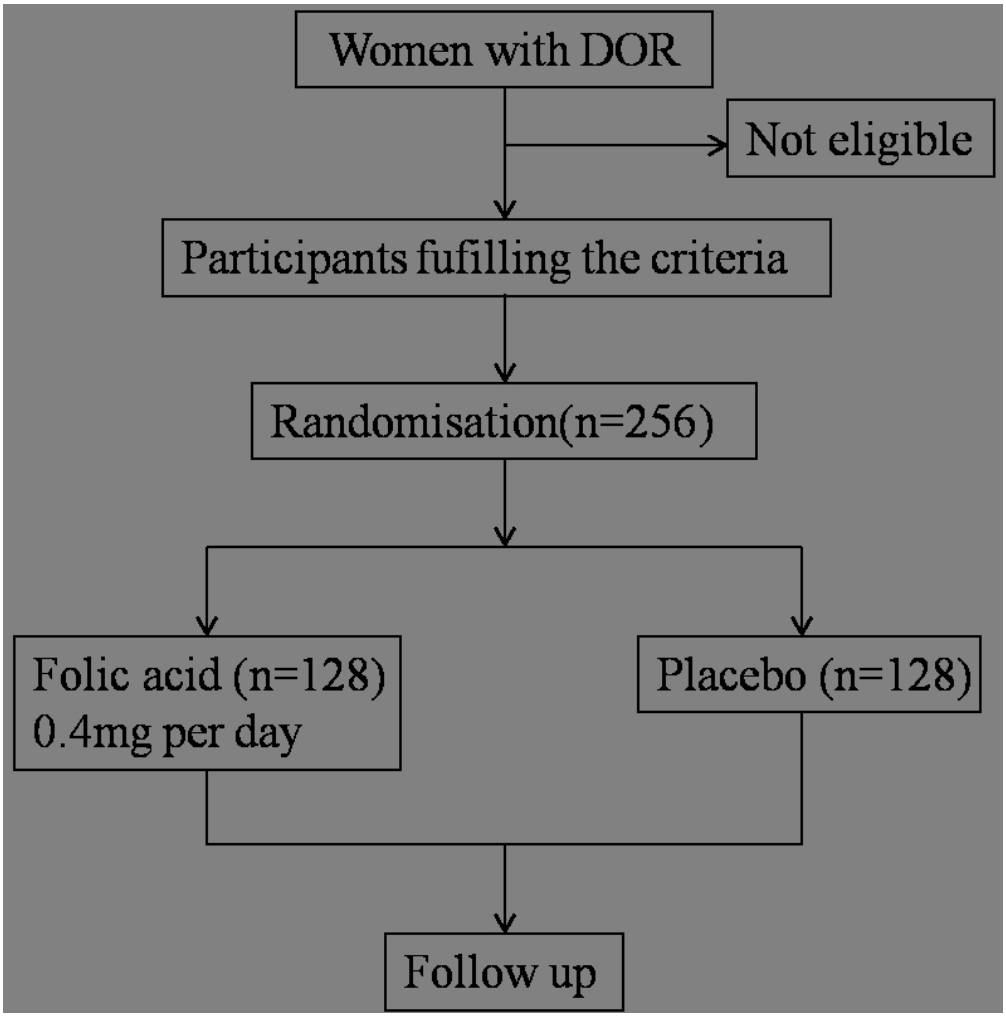
10. Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. FASEB J. 2010; 24(11): 4167-4174.
11. Ferrazzi E, Tiso G, Di Martino D. Folic acid versus 5- methyl tetrahydrofolate supplementation in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2020; 253: 312-319.
12. Viswanathan M, Treiman KA, Kish-Doto J, Middleton JC, Coker-Schwimmer EJ, Nicholson WK. Folic acid supplementation for the prevention of neural tube defects: An updated evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2017; 317(2): 190-203.
13. van Gool JD, Hirche H, Lax H, De Schaepdrijver L. Folic acid and primary prevention of neural tube defects: A review. Reprod Toxicol. 2018; 80: 73-84.
14. Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL. Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update. 2007; 13(3): 225-238.
15. Yilmaz N, Uygur D, Inal H, Gorkem U, Cicek N, Mollamahmutoglu L. Dehydroepiandrosterone supplementation improves predictive markers for diminished ovarian reserve: serum AMH, inhibin B and antral follicle count. Eur J Obstet Gynecol Reprod Biol. 2013; 169(2): 257-260.
16. Moolhuijsen LME, Visser JA. Anti-müllerian hormone and ovarian reserve: update on assessing ovarian function. J Clin Endocrinol Metab. 2020; 105(11): 3361-3373.
17. Wen J, Huang K, Du X, Zhang H, Ding T, Zhang C, Ma W, Zhong Y, Qu W, Liu Y, Li Z, Deng S, Luo A, Jin Y, Zhang J, Wang S. Can inhibin B reflect ovarian reserve of healthy reproductive age women effectively? Front Endocrinol (Lausanne). 2021; 12: 626534.
18. Bedenk J, Vrtačnik-Bokal E, Virant-Klun I. The role of anti-Müllerian

hormone (AMH) in ovarian disease and infertility. J Assist Reprod Genet. 2020; 37(1): 89-100.

19. Victoria M, Labrosse J, Krief F, Cédrin-Durnerin I, Comtet M, Grynberg M. Anti Müllerian Hormone: More than a biomarker of female reproductive function. J Gynecol Obstet Hum Reprod. 2019; 48(1): 19-24.
20. Hawkins Bressler L, Steiner A. Anti-Müllerian hormone as a predictor of reproductive potential. Curr Opin Endocrinol Diabetes Obes. 2018; 25(6): 385-390.
21. Coelho Neto MA, Ludwin A, Borrell A, Benacerraf B, Dewailly D, da Silva Costa F, Condous G, Alcazar JL, Jokubkiene L, Guerriero S, Van den Bosch T, Martins WP. Counting ovarian antral follicles by ultrasound: a practical guide. Ultrasound Obstet Gynecol. 2018; 51(1): 10-20.
22. Scheffer GJ, Broekmans FJ, Looman CW, Blankenstein M, Fauser BC, teJong FH, teVelde ER. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. Hum Reprod. 2003; 18(4): 700-706.

Figure Legend

Figure 1. Flowchart of the study design



342x344mm (59 x 59 DPI)

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

	6b	Explanation for choice of comparators	Page 5, lines 120-136
Objectives	7	Specific objectives or hypotheses	Page 5, lines 139-142
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 5/6, lines 148-149
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 4/5, lines 149-151
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 5, lines 162-179
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 7, lines 197-199
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 7/8, lines 203-218
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 7, lines 183-193, Figure 1

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
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
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
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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 7, lines 183-193
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
	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
Methods: Data collection, management, and analysis			
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
	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

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	N/A
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 8/9, lines 230-240
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 9, lines 263-268
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 9, lines 263-268

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Supplementary file
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 9, lines 255-259
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 10, line 294
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 10, lines 298-299
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supplementary file

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.