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Controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: study protocol for a randomized controlled trial

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ABSTRACT

Introduction

Multimodal anticancer therapies greatly damage the fertility of breast cancer patients, which raises urgent demand for fertility preservation. The standard options for fertility preservation are oocyte and embryo cryopreservation; both require controlled ovarian hyperstimulation (COH). However, there are safety concerns regarding breast cancer relapse due to the elevated serum estradiol level during COH. Serum estradiol levels can be effectively decreased with the highly specific aromatase inhibitor letrozole. Letrozole is still uncommonly used during COH for fertility preservation that has only been reported in a small number of studies including populations in Europe and America, and the evidence of its efficacy and safety from the perspective study is insufficient. In addition, high-quality randomized controlled trials (RCTs) in China comparing letrozole COH and non-letrozole COH are lacking. This study will compare the efficacy and safety of two protocols for preserving fertility in patients with breast cancer.

Methods and analysis

This is an open-label, multicentre RCT being conducted in four Chinese reproductive medical centres. 64 eligible patients will be randomly assigned (1:1) to the letrozole or non-letrozole group during their COH cycles. The primary outcome is the number of mature

oocytes. The secondary outcomes are the number of high-quality embryos and the incidence of ovarian hyperstimulation syndrome(OHSS).

Ethics and dissemination

Ethical approval was obtained from the Ethics Review Committee of Guangdong Provincial People's Hospital (KY-Q-2023-840-02). Written informed consent will be obtained from each participant. Findings will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publications.

Trial registration number ChiCTR2300078625

Keywords: breast tumours, randomized controlled trial, reproductive medicine

Strengths and limitations

⇒ This is a multicentre, randomised controlled clinical trial comparing letrozole COH and non-letrozole COH protocols for fertility preservation in breast cancer patients.

⇒ Participants will be followed up to two years after fertility preservation, allowing the comparison of the long-term safety of the two protocols.

⇒ The trial will be an important ancillary study to investigate the safe COH protocol for fertility preservation with breast cancer patients in the Chinese population.

⇒ Neither study participants nor investigators will be blinded, which could potentially introduce bias.

⇒ The sample size was determined based on the primary outcome, and the study has limited power concerning the assessment of the secondary outcomes.

INTRODUCTION

Breast cancer is the most common malignancy in women, with 2,261,419 new cases worldwide and 416,371 new cases in China in 2020.[1] It affects women at least a decade earlier in China than in Europe and America,[2,3] and the percentage of young patients under the age of 40 is greatly larger, accounting for approximately 15% of cases while only 5% in US.[4] Over the past several decades, long-term survival has been significantly improved due to the promotion of breast cancer screening and the development of comprehensive anticancer treatments. The 5-year disease-free survival rate exceeds 90% for patients in early-stage.[3] However, multimodal gonadotoxic treatment has the potential to cause

premature ovarian insufficiency, which reduces fertility significantly. Even worse, patients' younger onset and postponed childbearing further exacerbate their reproductive issues. The optimal course of action is to preserve fertility beforehand, and the established standard options are oocyte and embryo cryopreservation, both of which require controlled ovarian hyperstimulation (COH).[5] There is still a great controversy regarding the COH protocols for breast cancer patients.

Despite encouraging results about the safety of estrogen exposure during pregnancy and following ART in breast cancer survivors, conventional COH is associated with a substantial rise in estradiol levels, which raises some safety concerns, particularly in hormone-responsive tumors.[6-9] As a result, an alternate COH protocol involving the addition of a selective estrogen receptor modulator or an aromatase inhibitor has been introduced to maintain low estradiol levels.[10] Letrozole is a third-generation, highly selective aromatase inhibitor that reduces estrogen levels in the blood by inhibiting the conversion of testosterone to estrogen. This alleviates the negative feedback on the hypothalamus and pituitary gland. The pituitary gland is essentially prompted to generate follicle-stimulating hormone, which facilitates follicle growth.[11]

For breast cancer, letrozole can be used as an endocrine therapy and as a medication to induce follicle growth. The administration of letrozole per os in COH significantly reduced the serum estradiol levels.[7,8,12] The previous studies revealed that the letrozole antagonist COH (L-COH) enhanced ovarian responsiveness and increased the number of retrieved oocytes, mature oocytes, and available embryos as compared to the non-letrozole COH (conventional antagonist COH, C-COH).[13-15] Even though the number of retrieved oocytes was similar in both groups, a retrospective investigation concluded that the oocyte maturation rates were lower in L-COH.[16] However, a European prospective study found no difference in the number of retrieved oocytes and banked embryos between L-COH and C-COH.[17]

There is still debate over the efficacy of L-COH in terms of the number of mature oocytes and high-quality embryos, and there are few published findings on the risk of ovarian hyperstimulation syndrome (OHSS) and recurrence in breast cancer.[17,18] Furthermore, compared to the Western population, the earlier onset age and higher proportion of young patients in China highlight the urgent need for Chinese prospective studies regarding fertility preservation in breast cancer.[2-4,19] We intend to evaluate the efficacy and safety of the L-COH in breast cancer patients undergoing fertility preservation in this multi-centre randomized controlled trial. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, will be compared to that of the C-COH.

METHODS AND ANALYSIS

Study design

This multicentre RCT aims to evaluate the efficacy and safety of COH with or without letrozole for preserving fertility in breast cancer patients. In their IVF cycles, women will be randomized (1:1) to the L-COH or C-COH group. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, will be monitored and analyzed. This trial will adhere to the Standard Protocol Items: Interventional Trial Recommendations. The enrollment, interventions, and evaluation schedule are shown in Figure 1, the study flow chart is displayed in Figure 2, and the SPIRIT checklist is presented in Additional File 1.[20]

Study setting

Participants will be enrolled at four hospitals including Guangdong Provincial People's Hospital, the Second Hospital of Hebei Medical University, Shenzhen Hengsheng Hospital and Women and Children's Hospital, School of Medicine, Xiamen University. An independent data and safety monitoring board (DSMB) consisting of clinical, statistical, and ethical experts will periodically oversee the trial's progress and results.

Inclusion criteria

Eligible patients need to meet all of the following inclusion criteria:

1. Women with breast cancer.
2. Women aged ≤40 years.
3. Anti-müllerian hormone(AMH)level ≥1.1ng/ml.

Exclusion criteria

Women will be excluded if they meet any of the following exclusion criteria:

1. Chromosome abnormality (except chromosomal polymorphism) of either partner
2. A preimplantation genetic screening is required
3. Women with severe liver and kidney dysfunction or diabetes mellitus.

Recruitment

Fertility preservation will be recommended for all breast cancer patients who have fertility needs

in the future. The eligible patients will be informed about the study's specifics by the researchers, who will also allow them enough time to decide whether or not to take part in the trial. Women who agree to participate will be required to sign an informed consent form after the assessment. Women who choose not to take part in this experiment will receive routine clinical procedures. Each woman can only be enrolled once. The standardized case report forms are used to capture current medication status and previous medical history, including a detailed history of breast cancer. Patients' names are substituted with their phonetic initials to protect their privacy. A transvaginal ultrasound scan and a physical examination (height, body weight, waistline, hipline, blood pressure) are carried out. The serum basal sex hormones and anti-müllerian hormone will be measured. Besides, the results of routine tests will be recorded, such as blood routine, coagulation function, renal function, liver function, and electrolyte examinations. In addition, the tumour markers, including CEA, CA153, CA125, and CA199, will be measured before fertility preservation. Serum and follicular fluid will be collected and stored for future use in ancillary studies. The research protocol, version 1.2, was completed on November 20, 2023.

Randomization and Blinding

The computer-generated randomization scheme will be generated by an independent statistician in a 1:1 ratio between the two groups (the L-COH and C-COH groups). An independent statistician and a computer professional will upload the random list to the central randomization system, an interactive web response system while keeping it concealed from the clinical researchers. The researchers can only obtain the assigned groups for the eligible patients by logging into the central randomization system with unique identifications and passwords once a patient is recruited.

This study is an open-label trial. Participants, investigators, data collectors and analysts will not be blinded to the group assignment.

Interventions

Controlled ovarian hyperstimulation

Eligible patients are assigned at random to either the L-COH or C-COH group. The patients in the C-COH group will start receiving daily injections of gonadotropin (Gn) within 3 days of menstruation if the follicles are smaller than 10 mm and serum estrogen and progesterone levels are at the basic levels. Gn dosage is determined by age, antral follicle count, and basal sex hormone levels. Once the dominant follicle's mean diameter reaches 12-14 mm, or after 5–6 days of Gn administration, a

short-acting gonadotropin-releasing hormone antagonist (GnRH-ant) will be administered daily until the trigger day. Serum sex hormones and transvaginal ultrasonography are used to monitor follicle development. When at least two follicles reach 18mm in mean diameter, human chorionic gonadotropin (HCG) or gonadotropin-releasing hormone agonist (GnRHa) will be injected for final oocyte triggering. Oocyte retrieval is performed 34-36 hours later.

In addition to receiving daily injections of Gn, patients in the L-COH group will also receive oral letrozole 2.5 or 5.0 mg until the trigger day. The C-COH protocol is followed for the remaining steps.

Oocyte vitrification and embryo vitrification

The maturity of the oocytes will be evaluated by the embryologists. The patients who are single or whose partners are unable to obtain sperm will have their mature eggs vitrified, and the remaining patients will have their oocytes inseminated. After keeping the fertilized embryos cultured for three to seven days, the embryologists will vitrify the cleavage embryos and blastocysts that are available. The number of mature oocytes, high-quality embryos and available embryos will be recorded.

Criteria for cycle cancellation

The COH cycle will be cancelled if any of the following criteria are satisfied.

1. No oocytes retrieved.
2. Oocytes not fertilized or fertilized abnormally.
3. No embryos available.
4. Other private considerations.

OHSS evaluation

If the patient exhibits OHSS-related symptoms following oocyte retrieval, such as abdominal distension, nausea, vomiting, chest tightness, shortness of breath, oliguria, and so on, we should evaluate the OHSS grade and document the onset and duration of the symptoms. Patients with mild OHSS need close observation of their symptoms in the outpatient department. Patients with moderate OHSS require the routine tests and ultrasound scan. Hospitalization is usually necessary for patients with severe OHSS, and treatment plans involving particular interventions, such as intravenous infusion, albumin infusion, aspirin, and thoracic and abdominal drainage, must be documented. Each OHSS patient will receive ongoing care until their symptoms disappear.

Follow-up after fertility preservation

One year after fertility preservation, we need to monitor the breast cancer patient's medical record, taking note of the surgery time, surgery approach, pathology, TNM stage, and clinical classification. We should document the precise treatment regimen if the patient needs adjuvant therapy, such as chemotherapy, radiotherapy, endocrine therapy, or targeted therapy. It should also be noted if they have relapsed or not.

Two years after fertility preservation, we will monitor these individuals to see if they need targeted therapy or endocrine medication, and to see if they relapse. We will also inquire about their pregnancy plans at the same time.

Outcomes

The primary outcome is the number of mature oocytes. The secondary outcomes include the number of high-quality embryos and the incidence of OHSS.

Mature oocytes are those in the MII stage. High-quality embryos are defined as cleavage embryos graded 2 or higher according to the Peter scoring system and blastocysts graded 4BB or higher according to the Gardner scoring system. The OHSS is classified as mild, moderate, and severe categories according to the Golan criteria.

Statistical considerations

Sample size

According to the previously published retrospective study, we assumed the overall standard deviation was 3.8 and the expected difference was 0.7 between the two groups with the cut-off value of non-inferiority of 4.0. Thus, the estimated sample size is 64 cases with 32 cases in each group considering a power of 80%, an alpha error of 0.05, and a 10% follow-up drop-out rate.

Statistical analysis

Data analysis will be conducted using the Statistical Package for the Social Sciences (version 24.0; SPSS Inc., USA). The trial will adhere to the intention-to-treat principle. Every test will have two tails, and a statistically significant result is defined as a P value less than 0.05. The Shapiro-Wilk test will be used to test whether the continuous variable is normally distributed. For normally distributed data, we will use the Student's t-test to examine the differences between groups. However, for non-normally distributed data, we will use the

Wilcoxon rank-sum test. The mean ± standard deviation will be utilized for presenting continuous variables. Frequencies and percentages will be utilized for characterizing categorical variables, and Fisher's exact tests or the Chi-square test will be used to examine the group differences.

Data collection and management

The data will mainly derive from medical records and telephone follow-up records following fertility preservation. We will use an electronic data capture (EDC) system to record and deposit the study data. A three-level data quality control will be performed to make sure the input data is accurate. The first step will be real-time logical and range checking built into the EDC. The second step will involve the validation of the original data by clinical researchers and remote data monitoring by EDC data managers, and then the data entry staff will be responsible for the entry and correction of study data after receiving professional training. Careful data checks can help to detect more complex and uncommon errors. The third step will involve site visits by the DSMB to supervise the data. Errors found will be marked, and staff responsible for data entry will receive notifications to confirm and fix the errors. Only authorized researchers will have access to consent forms, screening and identification records, and other participant-identifiable data, which will all be stored in site files.

Women will be questioned about adverse events at each visit. Any adverse medical problems that occur during the study period are referred to as adverse events, regardless of whether or not they are related to the intervention. Every adverse event will be noted and sent to the DSMB. Furthermore, DSMB, which is independent of the sponsor and competing interests, will have the final decision on whether to terminate the trial.

Patient and public involvement

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

(word count: 2124)

ETHICS AND DISSEMINATION

This research has been approved by the Ethics Review Committee of Guangdong Provincial People's Hospital (KY-Q-2023-840-02). Each patient will be asked to sign a written informed consent prior to the interventions. The trial was registered on December 14, 2023, with the Chinese Clinical

Trial Registry (ChiCTR2300078625). This research permits trial-related monitoring, audits and regulatory inspections. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication.

Trial status The trial is under recruitment and no patients has been enrolled at the time of manuscript submission.

Additional file Additional file 1: SPIRIT checklist.

Abbreviations AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; C-COH: conventional antagonist COH; DSMB: data and safety monitoring board; EDC: electronic data capture; FP: fertility preservation; Gn: gonadotropin; GnRHa: gonadotropin-releasing hormone agonist; GnRH-ant: gonadotropin-releasing hormone antagonist; HCG: human chorionic gonadotropin; L-COH: letrozole antagonist COH; OHSS: ovarian hyperstimulation syndrome; RCT: randomized controlled trial.

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Author contributions YX, PL, WD, YS were involved in the study concept and design and in drafting of the manuscript. QF, JK and PS contributed to the study design and critical revision of the manuscript. All authors contributed to the revision of the manuscript. All authors read and approved the final manuscript.

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Data statement The datasets generated during the current study are available from the corresponding author upon reasonable request. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication after finishing all visits.

Competing interests The authors declare that they have no competing interests.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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Figures

Schedule		Recruitment and consent	COH	Oocyte retrieval	Oocyte vitrification	Insemination and embyo culture	Embryo vitrification	3-30 days after oocyte retrieval	One year after FP	Two years after FP
Enrollment	Eligible screen	√								
	Informed consent	√								
	Medical history	√								
	Physical examination	√								
	AMH	√								
	Basal sex hormones	√	√					√		
	Ultrasound scan	√	√					√		
	Routine tests	√						√		
	Tumor markers	√								
	Randomization	√								
Interventions	Letrozole COH		√	√						
	Non-letrozole COH		√	√						
Evaluation	Oocyte outcome				√					
	Embryo outcome					√	√			
	OHSS evaluation							√		
	Breast cancer history								√	√

Figure 1: SPIRIT diagram for schedule of enrollment, interventions, and evaluation.

Routine tests include the examination of blood routine, coagulation function, renal function, hepatic function, and electrolytes. Tumour markers include CEA, CA153, CA125, and CA199.

AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; FP: fertility preservation; OHSS: ovarian hyperstimulation syndrome.

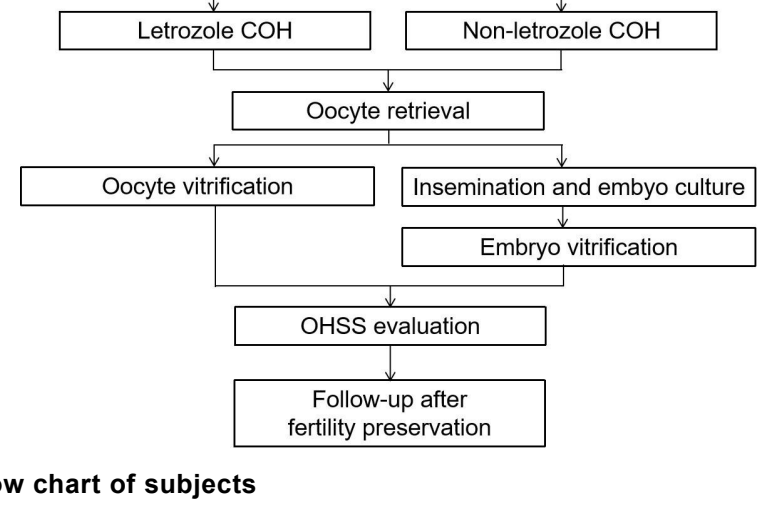

Figure 2: Flow chart of subjects

COH: controlled ovarian hyperstimulation; OHSS: ovarian hyperstimulation syndrome.

Schedule		Recruitment and consent	COH	Oocyte retrieval	Oocyte vitrification	Insemination and embyo culture	Embryo vitrification	3-30 days after oocyte retrieval	One year after FP	Two years after FP
Enrollment	Eligible screen	√								
	Informed consent	√								
	Medical history	√								
	Physical examination	√								
	AMH	√								
	Basal sex hormones	√	√					√		
	Ultrasound scan	√	√					√		
	Routine tests	√						√		
	Tumor markers	√								
	Randomization	√								
Interventions	Letrozole COH		√	√						
	Non-letrozole COH		√	√						
Evaluation	Oocyte outcome				√					
	Embryo outcome					√	√			
	OHSS evaluation							√		
	Breast cancer history								√	√

Figure 1: SPIRIT diagram for schedule of enrollment, interventions, and evaluation.
Routine tests include the examination of blood routine, coagulation function, renal function, hepatic function, and electrolytes. Tumour markers include CEA, CA153, CA125, and CA199.
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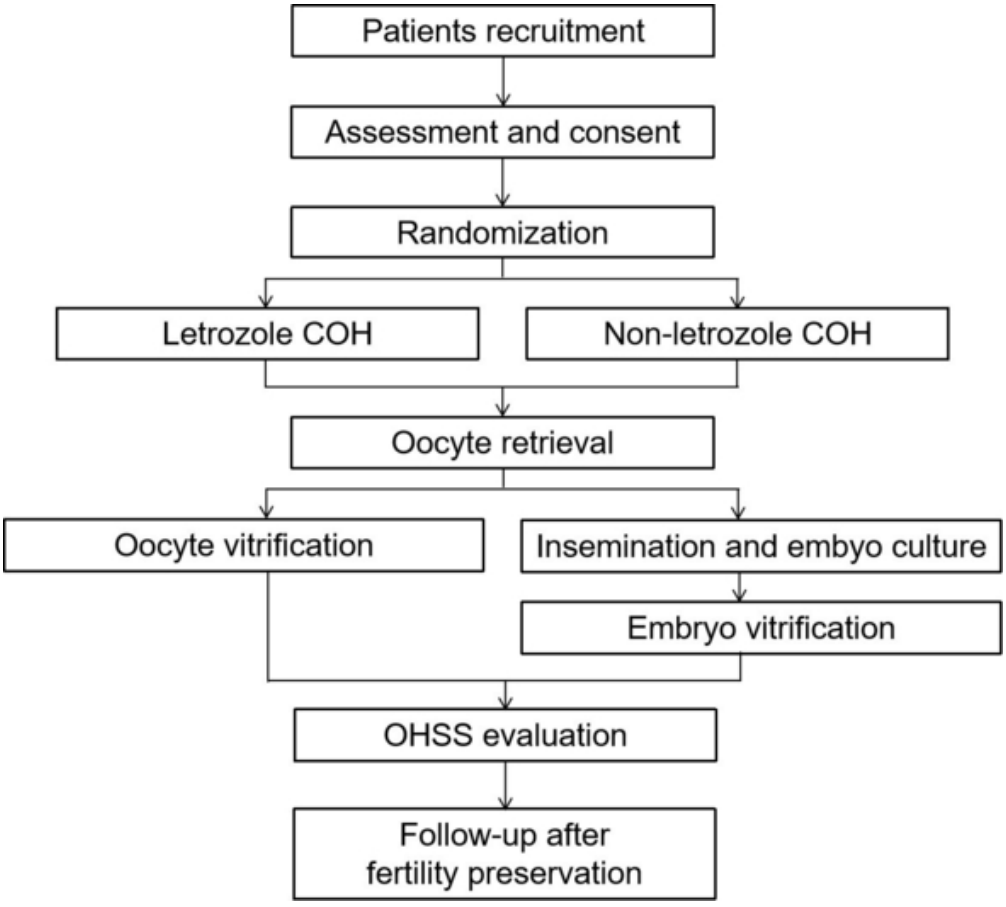

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COH: controlled ovarian hyperstimulation; OHSS: ovarian hyperstimulation syndrome.

49x44mm (300 x 300 DPI)

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Controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: study protocol for a randomized controlled trial

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ABSTRACT

Introduction

Multimodal anticancer therapies greatly damage the fertility of breast cancer patients, which raises urgent demand for fertility preservation. The standard options for fertility preservation are oocyte and embryo cryopreservation; both require controlled ovarian hyperstimulation (COH). However, there are safety concerns regarding breast cancer relapse due to the elevated serum estradiol level during COH. Serum estradiol levels can be effectively decreased with the highly specific aromatase inhibitor letrozole. Letrozole is still uncommonly used during COH for fertility preservation that has only been reported in a small number of studies, and the evidence of oocyte retrieval during ovarian stimulation and short-term safety from the perspective study is insufficient. As a result, this study will compare the efficacy of ovarian stimulation and short-term safety of letrozole COH and non-letrozole COH protocols for preserving fertility in patients with breast cancer.

Methods and analysis

This is an open-label, multicentre RCT being conducted in four Chinese reproductive medical centres. 64 eligible patients diagnosed with breast cancer will be randomly assigned (1:1) to the letrozole or non-letrozole group during their COH cycles.The primary outcome is the number of mature oocytes. The secondary outcomes are the number of high-quality embryos,,the incidence of ovarian hyperstimulation syndrome(OHSS) and the recurrence rate of breast cancer.

Ethics and dissemination

Ethical approval was obtained from the Ethics Review Committee of Guangdong Provincial People's Hospital (KY-Q-2023-840-02). Written informed consent will be obtained from each participant. Findings will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publications.

Trial registration number ChiCTR2300078625

Keywords: breast tumours, randomized controlled trial, reproductive medicine

Strengths and limitations

⇒ This is a multicentre, randomised controlled clinical trial comparing letrozole COH and non-letrozole COH protocols for fertility preservation in breast cancer patients.

⇒ Participants will be followed up to two years after fertility preservation, allowing the comparison of the long-term safety of the two protocols.

⇒ The trial will be an important ancillary study to investigate the safe COH protocol for fertility preservation with breast cancer patients.

⇒ Neither study participants nor investigators will be blinded, which could potentially introduce bias.

⇒ The sample size was determined based on the primary outcome, and the study has limited power concerning the assessment of the secondary outcomes.

INTRODUCTION

Breast cancer is one of the most common malignancies in women, with 2,308,897 new cases worldwide and 357,200 new cases in China in 2022.[1] It affects women at least a decade earlier in China than in Europe and America,[2,3] and the percentage of young patients under the age of 40 is greatly larger,

accounting for approximately 15% of cases while only 5% in US.[4] Over the past several decades, long-term survival has been significantly improved due to the promotion of breast cancer screening and the development of comprehensive anticancer treatments. The 5-year disease-free survival rate exceeds 90% for patients in early-stage.[3] However, multimodal gonadotoxic treatment has the potential to cause premature ovarian insufficiency, which reduces fertility significantly. Even worse, patients' younger onset and postponed childbearing further exacerbate their reproductive issues. The optimal course of action is to preserve fertility beforehand, and the established standard options are oocyte and embryo cryopreservation, both of which require controlled ovarian hyperstimulation (COH).[5] There is still a great controversy regarding the COH protocols for breast cancer patients.

A meta-analysis concluded that performing COH before, or ART following anticancer treatment in young women with breast cancer did not associate with detrimental prognostic effect in terms of breast cancer recurrence, mortality or event-free survival.[6] Despite encouraging results about the safety of estrogen exposure during pregnancy and following ART in breast cancer survivors, conventional COH is associated with a substantial rise in estradiol levels, which raises some safety concerns, particularly in hormone-responsive tumors.[7-10] As a result, an alternate COH protocol involving the addition of a selective estrogen receptor modulator or an aromatase inhibitor has been introduced to maintain low estradiol levels.[11] Letrozole is a third-generation, highly selective aromatase inhibitor that reduces estrogen levels in the blood by inhibiting the conversion of testosterone to estrogen. This alleviates the negative feedback on the hypothalamus and pituitary gland. The pituitary gland is essentially prompted to generate follicle-stimulating hormone, which facilitates follicle growth.[12]

For breast cancer, letrozole can be used as an endocrine therapy and as a medication to induce follicle growth. The administration of letrozole per os in COH significantly reduced the serum estradiol levels.[8,9,13] The previous studies revealed that the letrozole antagonist COH (L-COH) enhanced ovarian responsiveness and increased the number of retrieved oocytes, mature oocytes, and available embryos as compared to the non-letrozole COH (conventional antagonist COH, C-COH).[14-16] Even though the number of retrieved oocytes was similar in both groups, a retrospective investigation concluded that the oocyte maturation rates were lower in L-COH.[17] However, a European prospective study found no difference in the number of retrieved oocytes and banked embryos between L-COH and C-COH.[18]

There is still debate over the efficacy of L-COH in terms of the number of mature oocytes and high-quality embryos, and there are few published findings on the risk of ovarian hyperstimulation syndrome

(OHSS) and recurrence in breast cancer.[18,19] Two studies found there was no significant difference about the safety of traditional COH protocol and letrozole protocol among breast cancer patients.[16,19] Furthermore, compared to the Western population, the earlier onset age and higher proportion of young patients in China highlight the urgent need for prospective studies regarding fertility preservation in breast cancer.[2-4,20] We intend to evaluate the efficacy and safety of the L-COH in breast cancer patients undergoing fertility preservation in this multi-centre randomized controlled trial. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, and the recurrence rate of breast cancer will be compared to that of the C-COH.

METHODS AND ANALYSIS

Study design

This multicentre RCT aims to evaluate the efficacy and safety of COH with or without letrozole for preserving fertility in breast cancer patients. In their IVF cycles, women will be randomized (1:1) to the L-COH or C-COH group. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, will be monitored and analyzed. This trial will adhere to the Standard Protocol Items: Interventional Trial Recommendations. The enrollment, interventions, and evaluation schedule are shown in Figure 1, the study flow chart is displayed in Figure 2, and the SPIRIT checklist is presented in Additional File 1.[21]

Study setting

Participants will be enrolled at four hospitals including Guangdong Provincial People's Hospital, the Second Hospital of Hebei Medical University, Shenzhen Hengsheng Hospital and Women and Children's Hospital, School of Medicine, Xiamen University. An independent data and safety monitoring board (DSMB) consisting of clinical, statistical, and ethical experts will periodically oversee the trial's progress and results.

Inclusion criteria

Eligible patients need to meet all of the following inclusion criteria:

1. Women with breast cancer.

2. Women aged ≤40 years.

3. Anti-müllerian hormone(AMH)level ≥1.1ng/ml.

Exclusion criteria

Women will be excluded if they meet any of the following exclusion criteria:

1. Chromosome abnormality (except chromosomal polymorphism) of either partner
2. A preimplantation genetic screening is required
3. Women with severe liver and kidney dysfunction or diabetes mellitus.

Recruitment

Fertility preservation will be recommended for all breast cancer patients who have fertility needs in the future. The eligible patients will be informed about the study's specifics by the researchers, who will also allow them enough time to decide whether or not to take part in the trial. Women who agree to participate will be required to sign an informed consent form after the assessment. Women who choose not to take part in this experiment will receive routine clinical procedures. Each woman can only be enrolled once. The standardized case report forms are used to capture current medication status and previous medical history, including a detailed history of breast cancer. Patients' names are substituted with their phonetic initials to protect their privacy. A transvaginal ultrasound scan and a physical examination (height, body weight, waistline, hipline, blood pressure) are carried out. The serum basal sex hormones including follicle stimulating hormone(FSH), luteinizing hormone(LH), estradiol(E_2), progesterone(P), prolactin(PRL), testosterone(T) and anti-müllerian hormone(AMH) will be measured. Besides, the results of routine tests will be recorded, such as blood routine, coagulation function, renal function, liver function, and electrolyte examinations. In addition, the tumour markers, including CEA, CA153, CA125, and CA199, will be measured before fertility preservation. Serum and follicular fluid will be collected and stored for future use in ancillary studies. The research protocol, version 1.2, was completed on November 20, 2023.

Randomization and Blinding

The computer-generated randomization scheme will be generated by an independent statistician in a 1:1 ratio between the two groups (the L-COH and C-COH groups). An independent statistician and a computer professional will upload the random list to the central randomization system, an interactive web response system while keeping it concealed from the clinical researchers. The researchers can only obtain the assigned groups for the eligible patients by logging into the central randomization system with unique identifications and passwords once a patient is recruited.

This study is an open-label trial. Participants, investigators, data collectors and analysts will not be blinded to the group assignment.

Interventions

Controlled ovarian hyperstimulation

Eligible patients are assigned at random to either the letrozole antagonist COH (L-COH) or conventional antagonist COH(C-COH) group. The patients in the C-COH group will start receiving daily injections of gonadotropin (Gn) within 3 days of menstruation if the follicles are smaller than 10 mm and serum estrogen and progesterone levels are at the basic levels. Gn dosage is determined by age, antral follicle count, and basal sex hormone levels including FSH, LH and E_2. Once the dominant follicle's mean diameter reaches 12-14 mm, or after 5–6 days of Gn administration, a short-acting gonadotropin-releasing hormone antagonist (GnRH-ant) will be administered daily until the trigger day. Serum sex hormones including FSH, LH, P and E_2 and transvaginal ultrasonography are used to monitor follicle development. When at least two follicles reach 18mm in mean diameter, human chorionic gonadotropin (HCG) or gonadotropin-releasing hormone agonist (GnRHa) will be injected for final oocyte triggering. Oocyte retrieval is performed 34-36 hours later.

In addition to receiving daily injections of Gn, patients in the L-COH group will also receive oral letrozole 2.5 or 5.0 mg until the trigger day. Patients weighing less than 40 kg will receive 2.5 mg daily, while others will receive 5 mg daily.The C-COH protocol is followed for the remaining steps.

Oocyte vitrification and embryo vitrification

The maturity of the oocytes will be evaluated by the embryologists. The patients who are single or whose partners are unable to obtain sperm will have their mature eggs vitrified, and the remaining patients will have their oocytes inseminated. After keeping the fertilized embryos cultured for three to seven days, the embryologists will vitrify the cleavage embryos and blastocysts that are available. The number of mature oocytes, high-quality embryos and available embryos will be recorded.

Criteria for cycle cancellation

The COH cycle will be cancelled if any of the following criteria are satisfied.

1. No oocytes retrieved.

2. Oocytes not fertilized or fertilized abnormally.

3. No embryos available.

4. Other private considerations.

OHSS evaluation

If the patient exhibits OHSS-related symptoms following oocyte retrieval, such as abdominal distension, nausea, vomiting, chest tightness, shortness of breath, oliguria, and so on, we should evaluate the OHSS grade and document the onset and duration of the symptoms. Patients with mild OHSS need close observation of their symptoms in the outpatient department. Patients with moderate OHSS require the routine tests and ultrasound scan. Hospitalization is usually necessary for patients with severe OHSS, and treatment plans involving particular interventions, such as intravenous infusion, albumin infusion, aspirin, and thoracic and abdominal drainage, must be documented. Each OHSS patient will receive ongoing care until their symptoms disappear.

Follow-up after fertility preservation

One year after fertility preservation, we need to monitor the breast cancer patient's medical record, taking note of the surgery time, surgery approach, pathology, TNM stage, and clinical classification. We should document the precise treatment regimen if the patient needs adjuvant therapy, such as chemotherapy, radiotherapy, endocrine therapy, or targeted therapy. It should also be noted if they have relapsed or not.

Two years after fertility preservation, we will monitor these individuals to see if they need targeted therapy or endocrine medication, and to see if they relapse. We will also inquire about their pregnancy plans at the same time.

Outcomes

The primary outcome is the number of mature oocytes. The secondary outcomes include the number of high-quality embryos,the incidence of OHSS and the recurrence rate of breast cancer.

Mature oocytes are those in the MII stage. High-quality embryos are defined as cleavage embryos graded 2 or higher according to the Peter scoring system and blastocysts graded 4BB or higher according to the Gardner scoring system. The OHSS is classified as mild, moderate, and severe categories according to the Golan criteria.

Statistical considerations

Sample size

According to the previously published retrospective study, we assumed the overall standard deviation was 3.8 and the expected difference was 0.7 between the two groups with the cut-off value of non-inferiority of 4.0. Thus, the estimated sample size is 64 cases with 32 cases in each group considering a power of 80%, an alpha error of 0.05, and a 10% follow-up drop-out rate.

Statistical analysis

Data analysis will be conducted using the Statistical Package for the Social Sciences (version 24.0; SPSS Inc., USA). The trial will adhere to the intention-to-treat principle. Every test will have two tails, and a statistically significant result is defined as a P value less than 0.05. The Shapiro-Wilk test will be used to test whether the continuous variable is normally distributed. For normally distributed data, we will use the Student's t-test to examine the differences between groups. However, for non-normally distributed data, we will use the Wilcoxon rank-sum test. The mean ± standard deviation will be utilized for presenting continuous variables. Frequencies and percentages will be utilized for characterizing categorical variables, and Fisher's exact tests or the Chi-square test will be used to examine the group differences.

Data collection and management

The data will mainly derive from medical records and telephone follow-up records following fertility preservation. We will use an electronic data capture (EDC) system to record and deposit the study data. A three-level data quality control will be performed to make sure the input data is accurate. The first step will be real-time logical and range checking built into the EDC. The second step will involve the validation of the original data by clinical researchers and remote data monitoring by EDC data managers, and then the data entry staff will be responsible for the entry and correction of study data after receiving professional training. Careful data checks can help to detect more complex and uncommon errors. The third step will involve site visits by the DSMB to supervise the data. Errors found will be marked, and staff responsible for data entry will receive notifications to confirm and fix the errors. Only authorized researchers will have access to consent forms, screening and identification records, and other participant-identifiable data, which will all be stored in site files.

For those husbands diagnosed with severe oligozoospermia, hypospermia and teratospermia, we will analyse their oocyte outcome but not embryo outcome. Women will be questioned about

adverse events at each visit. Any adverse medical problems that occur during the study period are referred to as adverse events, regardless of whether or not they are related to the intervention. Every adverse event will be noted and sent to the DSMB. Furthermore, DSMB, which is independent of the sponsor and competing interests, will have the final decision on whether to terminate the trial.

Patient and public involvement

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

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ETHICS AND DISSEMINATION

This research has been approved by the Ethics Review Committee of Guangdong Provincial People's Hospital (KY-Q-2023-840-02). Each patient will be asked to sign a written informed consent prior to the interventions. The trial was registered on December 14, 2023, with the Chinese Clinical Trial Registry (ChiCTR2300078625). This research permits trial-related monitoring, audits and regulatory inspections. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication.

Trial status The trial is under recruitment and no patients has been enrolled at the time of manuscript submission.

Additional file Additional file 1: SPIRIT checklist.

Abbreviations AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; C-COH: conventional antagonist COH; DSMB: data and safety monitoring board; EDC: electronic data capture; FP: fertility preservation; Gn: gonadotropin; GnRHa: gonadotropin-releasing hormone agonist; GnRH-ant: gonadotropin-releasing hormone antagonist; HCG: human chorionic gonadotropin; L-COH: letrozole antagonist COH; OHSS: ovarian hyperstimulation syndrome; RCT: randomized controlled trial.

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Author contributions and statements Yanqiu Xie, Ping Li, Weifen Deng, Kun Wang, Yuhua Shi were involved in the study concept and design and in drafting of the manuscript. Qi Fan, Jiajing Kang, Peng Sun and Kun Wang contributed to the study design and critical revision of the manuscript. All authors contributed to the revision of the manuscript. All authors read and approved the final manuscript. Yuhua Shi is responsible for the overall content [as guarantor].

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Data statement The datasets generated during the current study are available from the corresponding author upon reasonable request. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication after finishing all visits.

Competing interests The authors declare that they have no competing interests.

Patient consent for publication Not applicable.

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Figures

Figure 1: SPIRIT diagram for schedule of enrollment, interventions, and evaluation.

Routine tests include the examination of blood routine, coagulation function, renal function, hepatic function, and electrolytes. Tumour markers include CEA, CA153, CA125, and CA199.
AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; FP: fertility preservation; OHSS: ovarian hyperstimulation syndrome.

Figure 2: Flow chart of subjects

COH: controlled ovarian hyperstimulation; OHSS: ovarian hyperstimulation syndrome.

Schedule		Recruitment and consent	COH	Oocyte retrieval	Oocyte vitrification	Insemination and embyo culture	Embryo vitrification	3-30 days after oocyte retrieval	One year after FP	Two years after FP
Enrollment	Eligible screen	√								
	Informed consent	√								
	Medical history	√								
	Physical examination	√								
	AMH	√								
	Basal sex hormones	√	√					√		
	Ultrasound scan	√	√					√		
	Routine tests	√						√		
	Tumor markers	√								
	Randomization	√								
Interventions	Letrozole COH		√	√						
	Non-letrozole COH		√	√						
Evaluation	Oocyte outcome				√					
	Embryo outcome					√	√			
	OHSS evaluation							√		
	Breast cancer history								√	√

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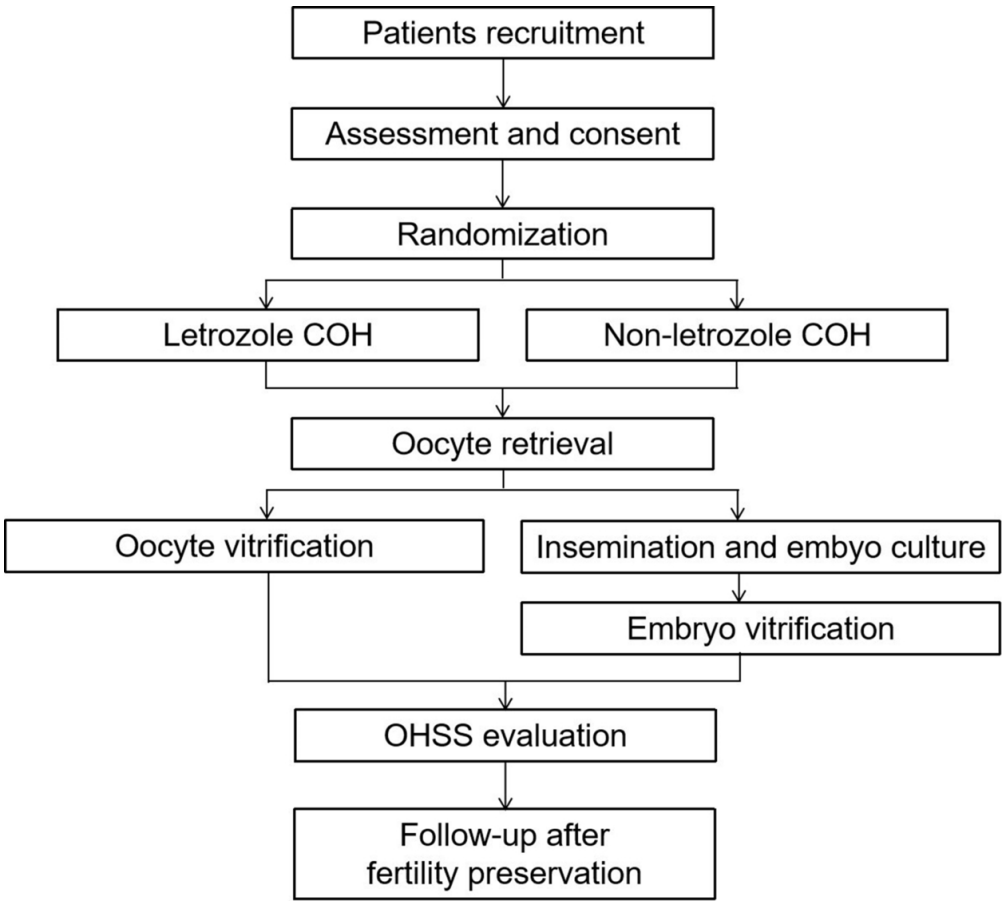

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Controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: study protocol for a randomized controlled trial

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Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Oncology, Obstetrics and gynaecology
Keywords:	Breast tumours < ONCOLOGY, Randomized Controlled Trial, Reproductive medicine < GYNAECOLOGY

Controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: study protocol for a randomized controlled trial

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ABSTRACT

Introduction

Multimodal anticancer therapies greatly damage the fertility of breast cancer patients, which raises urgent demand for fertility preservation. The standard options for fertility preservation are oocyte and embryo cryopreservation; both require controlled ovarian hyperstimulation (COH). However, there are safety concerns regarding breast cancer relapse due to the elevated serum estradiol level during COH. Serum estradiol levels can be effectively decreased with the highly specific aromatase inhibitor letrozole. Letrozole is still uncommonly used during COH for fertility preservation that has only been reported in a small number of studies, and the evidence of oocyte retrieval during ovarian stimulation and short-term safety from the perspective study is insufficient. As a result, this study will compare the efficacy of ovarian stimulation and short-term safety of letrozole COH and non-letrozole COH protocols for preserving fertility in patients with breast cancer.

Methods and analysis

This is an open-label, multicentre RCT being conducted in four Chinese reproductive medical centres. 64 eligible patients diagnosed with breast cancer will be randomly assigned (1:1) to the letrozole or non-letrozole group during their COH cycles.The primary outcome is the number of mature oocytes. The secondary outcomes are the number of high-quality embryos,,the incidence of ovarian hyperstimulation syndrome(OHSS) and the recurrence rate of breast cancer.

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⇒ Participants will be followed up to two years after fertility preservation, allowing the comparison of the long-term safety of the two protocols.

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accounting for approximately 15% of cases while only 5% in US.[4] Over the past several decades, long-term survival has been significantly improved due to the promotion of breast cancer screening and the development of comprehensive anticancer treatments. The 5-year disease-free survival rate exceeds 90% for patients in early-stage.[3] However, multimodal gonadotoxic treatment has the potential to cause premature ovarian insufficiency, which reduces fertility significantly. Even worse, patients' younger onset and postponed childbearing further exacerbate their reproductive issues. The optimal course of action is to preserve fertility beforehand, and the established standard options are oocyte and embryo cryopreservation, both of which require controlled ovarian hyperstimulation (COH).[5] There is still a great controversy regarding the COH protocols for breast cancer patients.

A meta-analysis concluded that performing COH before, or ART following anticancer treatment in young women with breast cancer did not associate with detrimental prognostic effect in terms of breast cancer recurrence, mortality or event-free survival.[6] Despite encouraging results about the safety of estrogen exposure during pregnancy and following ART in breast cancer survivors, conventional COH is associated with a substantial rise in estradiol levels, which raises some safety concerns, particularly in hormone-responsive tumors.[7-10] As a result, an alternate COH protocol involving the addition of a selective estrogen receptor modulator or an aromatase inhibitor has been introduced to maintain low estradiol levels.[11] Letrozole is a third-generation, highly selective aromatase inhibitor that reduces estrogen levels in the blood by inhibiting the conversion of testosterone to estrogen. This alleviates the negative feedback on the hypothalamus and pituitary gland. The pituitary gland is essentially prompted to generate follicle-stimulating hormone, which facilitates follicle growth.[12]

For breast cancer, letrozole can be used as an endocrine therapy and as a medication to induce follicle growth. The administration of letrozole per os in COH significantly reduced the serum estradiol levels.[8,9,13] The previous studies revealed that the letrozole antagonist COH (L-COH) enhanced ovarian responsiveness and increased the number of retrieved oocytes, mature oocytes, and available embryos as compared to the non-letrozole COH (conventional antagonist COH, C-COH).[14-16] Even though the number of retrieved oocytes was similar in both groups, a retrospective investigation concluded that the oocyte maturation rates were lower in L-COH.[17] However, a European prospective study found no difference in the number of retrieved oocytes and banked embryos between L-COH and C-COH.[18]

Two studies found there was no significant difference about the safety of traditional COH protocol and letrozole protocol among breast cancer patients.[16,19] Previous prospective studies have focused on

the number of retrieved oocytes or disease-free survival,[18,19] while retrospective studies have indicated lower oocyte maturation rates in L-COH.[17] Therefore, it is essential to investigate the efficacy of L-COH, with the number of mature oocytes as the primary outcome. Furthermore, compared to the Western population, the earlier onset age and higher proportion of young patients in China highlight the urgent need for prospective studies regarding fertility preservation in breast cancer.[2-4,20] We intend to evaluate the efficacy and safety of the L-COH in breast cancer patients undergoing fertility preservation in this multi-centre randomized controlled trial. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, and the recurrence rate of breast cancer will be compared to that of the C-COH.

METHODS AND ANALYSIS

Study design

This multicentre RCT aims to evaluate the efficacy and safety of COH with or without letrozole for preserving fertility in breast cancer patients. In their IVF cycles, women will be randomized (1:1) to the L-COH or C-COH group. The number of mature oocytes and high-quality embryos, as well as the incidence of OHSS, will be monitored and analyzed. This trial will adhere to the Standard Protocol Items: Interventional Trial Recommendations. The study period spans from December 2023 to December 2028. The enrollment, interventions, and evaluation schedule are shown in Figure 1, the study flow chart is displayed in Figure 2, and the SPIRIT checklist is presented in Additional File 1.[21]

Study setting

Participants will be enrolled at four hospitals including Guangdong Provincial People's Hospital, the Second Hospital of Hebei Medical University, Shenzhen Hengsheng Hospital and Women and Children's Hospital, School of Medicine, Xiamen University. An independent data and safety monitoring board (DSMB) consisting of clinical, statistical, and ethical experts will periodically oversee the trial's progress and results.

Inclusion criteria

Eligible patients need to meet all of the following inclusion criteria:

1. Women with breast cancer.

2. Women aged ≤40 years.

3. Anti-müllerian hormone(AMH)level ≥1.1ng/ml.

Exclusion criteria

Women will be excluded if they meet any of the following exclusion criteria:

1. Chromosome abnormality (except chromosomal polymorphism) of either partner

2. A preimplantation genetic screening is required

3. Women with severe liver and kidney dysfunction or diabetes mellitus.

Recruitment

Fertility preservation will be recommended for all breast cancer patients who have fertility needs in the future. The eligible patients will be informed about the study's specifics by the researchers, who will also allow them enough time to decide whether or not to take part in the trial. Women who agree to participate will be required to sign an informed consent form after the assessment. Signing informed consent involves several important steps to ensure that participants fully understand the study, its potential risks and benefits, their rights as participants, and have an opportunity to ask questions. These steps include an introduction to the study, a discussion of key information, and, finally, signing the consent form. The research team's role is to support the participant in making an informed, voluntary decision without pressure or coercion. Women who choose not to take part in this experiment will receive routine clinical procedures. Each woman can only be enrolled once. The standardized case report forms are used to capture current medication status and previous medical history, including a detailed history of breast cancer. Patients' names are substituted with their phonetic initials to protect their privacy. A transvaginal ultrasound scan and a physical examination (height, body weight, waistline, hipline, blood pressure) are carried out. The serum basal sex hormones including follicle stimulating hormone(FSH), luteinizing hormone(LH), estradiol(E_2), progesterone(P), prolactin(PRL), testosterone(T) and anti-müllerian hormone(AMH) will be measured. Besides, the results of routine tests will be recorded, such as blood routine, coagulation function, renal function, liver function, and electrolyte examinations. In addition, the tumour markers, including CEA, CA153, CA125, and CA199, will be measured before fertility preservation. Serum and follicular fluid will be collected and stored for future use in ancillary studies. The research protocol, version 1.2, was completed on November 20, 2023.

Randomization and Blinding

The computer-generated randomization scheme will be generated by an independent statistician in

a 1:1 ratio between the two groups (the L-COH and C-COH groups). An independent statistician and a computer professional will upload the random list to the central randomization system, an interactive web response system while keeping it concealed from the clinical researchers. The researchers can only obtain the assigned groups for the eligible patients by logging into the central randomization system with unique identifications and passwords once a patient is recruited.

This study is an open-label trial. Participants, investigators, data collectors and analysts will not be blinded to the group assignment.

Interventions

Controlled ovarian hyperstimulation

Eligible patients are assigned at random to either the letrozole antagonist COH (L-COH) or conventional antagonist COH(C-COH) group. The patients in the C-COH group will start receiving daily injections of gonadotropin (Gn) within 3 days of menstruation if the follicles are smaller than 10 mm and serum estrogen and progesterone levels are at the basic levels. Gn dosage is determined by age, antral follicle count, and basal sex hormone levels including FSH, LH and E_2. Once the dominant follicle's mean diameter reaches 12-14 mm, or after 5–6 days of Gn administration, a short-acting gonadotropin-releasing hormone antagonist (GnRH-ant) will be administered daily until the trigger day. Serum sex hormones including FSH, LH, P and E_2 and transvaginal ultrasonography are used to monitor follicle development. When at least two follicles reach 18mm in mean diameter, human chorionic gonadotropin (HCG) or gonadotropin-releasing hormone agonist (GnRHa) will be injected for final oocyte triggering. Oocyte retrieval is performed 34-36 hours later.

In addition to receiving daily injections of Gn, patients in the L-COH group will also receive oral letrozole 2.5 or 5.0 mg until the trigger day. Patients weighing less than 40 kg will receive 2.5 mg daily, while others will receive 5 mg daily.The C-COH protocol is followed for the remaining steps.

Oocyte vitrification and embryo vitrification

The maturity of the oocytes will be evaluated by the embryologists. The patients who are single or whose partners are unable to obtain sperm will have their mature eggs vitrified, and the remaining patients will have their oocytes inseminated. After keeping the fertilized embryos cultured for three to seven days, the embryologists will vitrify the cleavage embryos and blastocysts that are available. The number of mature oocytes, high-quality embryos and available embryos will be recorded.

Criteria for cycle cancellation

The COH cycle will be cancelled if any of the following criteria are satisfied.

1. No oocytes retrieved.

2. Oocytes not fertilized or fertilized abnormally.

3. No embryos available.

4. Other private considerations.

OHSS evaluation

If the patient exhibits OHSS-related symptoms following oocyte retrieval, such as abdominal distension, nausea, vomiting, chest tightness, shortness of breath, oliguria, and so on, we should evaluate the OHSS grade and document the onset and duration of the symptoms. Patients with mild OHSS need close observation of their symptoms in the outpatient department. Patients with moderate OHSS require the routine tests and ultrasound scan. Hospitalization is usually necessary for patients with severe OHSS, and treatment plans involving particular interventions, such as intravenous infusion, albumin infusion, aspirin, and thoracic and abdominal drainage, must be documented. Each OHSS patient will receive ongoing care until their symptoms disappear.

Follow-up after fertility preservation

One year after fertility preservation, we need to monitor the breast cancer patient's medical record, taking note of the surgery time, surgery approach, pathology, TNM stage, and clinical classification. We should document the precise treatment regimen if the patient needs adjuvant therapy, such as chemotherapy, radiotherapy, endocrine therapy, or targeted therapy. It should also be noted if they have relapsed or not.

Two years after fertility preservation, we will monitor these individuals to see if they need targeted therapy or endocrine medication, and to see if they relapse. We will also inquire about their pregnancy plans at the same time.

Outcomes

The primary outcome is the number of mature oocytes. The secondary outcomes include the number of high-quality embryos,the incidence of OHSS and the recurrence rate of breast cancer.

Mature oocytes are those in the MII stage. High-quality embryos are defined as cleavage embryos graded 2 or higher according to the Peter scoring system and blastocysts graded 4BB or

higher according to the Gardner scoring system. The OHSS is classified as mild, moderate, and severe categories according to the Golan criteria.

Statistical considerations

Sample size

According to the previously published study,[18] the difference of the number of mature occytes between the two groups was 0.7. We assumed the overall standard deviation was 3.8 with the cut-off value of non-inferiority of 4.0. Thus, the estimated sample size is 64 cases with 32 cases in each group considering a power of 80%, an alpha error of 0.05, and a 10% follow-up drop-out rate.

Statistical analysis

Data analysis will be conducted using the Statistical Package for the Social Sciences (version 27.0; SPSS Inc., USA). The trial will adhere to the intention-to-treat principle. Every test will have two tails, and a statistically significant result is defined as a P value less than 0.05. The Shapiro-Wilk test will be used to test whether the continuous variable is normally distributed. For normally distributed data, we will use the Student's t-test to examine the differences between groups. However, for non-normally distributed data, we will use the Mann-Whitney U test. The mean ± standard deviation will be utilized for presenting continuous variables. Frequencies and percentages will be utilized for characterizing categorical variables, and Fisher's exact tests or the Chi-square test will be used to examine the group differences.

Data collection and management

The data will mainly derive from medical records and telephone follow-up records following fertility preservation. We will use an electronic data capture (EDC) system to record and deposit the study data. A three-level data quality control will be performed to make sure the input data is accurate. The first step will be real-time logical and range checking built into the EDC. The second step will involve the validation of the original data by clinical researchers and remote data monitoring by EDC data managers, and then the data entry staff will be responsible for the entry and correction of study data after receiving professional training. Careful data checks can help to detect more complex and uncommon errors. The third step will involve site visits by the DSMB to supervise the data. Errors found will be marked, and staff responsible for data entry will receive

notifications to confirm and fix the errors. Only authorized researchers will have access to consent forms, screening and identification records, and other participant-identifiable data, which will all be stored in site files.

For those husbands diagnosed with severe oligozoospermia, hypospermia and teratospermia, we will analyse their oocyte outcome but not embryo outcome. Women will be questioned about adverse events at each visit. Any adverse medical problems that occur during the study period are referred to as adverse events, regardless of whether or not they are related to the intervention. Every adverse event will be noted and sent to the DSMB. Furthermore, DSMB, which is independent of the sponsor and competing interests, will have the final decision on whether to terminate the trial.

Patient and public involvement

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

(word count: 2332)

ETHICS AND DISSEMINATION

This research has been approved by the Ethics Review Committee of Guangdong Provincial People's Hospital (KY-Q-2023-840-02). Each patient will be asked to sign a written informed consent prior to the interventions. The trial was registered on December 14, 2023, with the Chinese Clinical Trial Registry (ChiCTR2300078625). This research permits trial-related monitoring, audits and regulatory inspections. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication.

Trial status The trial is under recruitment and no patients has been enrolled at the time of manuscript submission.

Additional file Additional file 1: SPIRIT checklist.

Abbreviations AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; C-COH: conventional antagonist COH; DSMB: data and safety monitoring board; EDC: electronic data capture; FP: fertility preservation; Gn: gonadotropin; GnRHa: gonadotropin-releasing hormone agonist; GnRH-ant: gonadotropin-releasing hormone antagonist; HCG: human chorionic gonadotropin; L-COH: letrozole antagonist COH; OHSS: ovarian hyperstimulation syndrome; RCT: randomized controlled trial.

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Author contributions and statements Yanqiu Xie, Ping Li, Weifen Deng, Kun Wang, Yuhua Shi were involved in the study concept and design and in drafting of the manuscript. Qi Fan, Jiajing Kang, Peng Sun and Kun Wang contributed to the study design and critical revision of the manuscript. All authors contributed to the revision of the manuscript. All authors read and approved the final manuscript. Yuhua Shi is responsible for the overall content [as guarantor].

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Data statement The datasets generated during the current study are available from the corresponding author upon reasonable request. The results will be disseminated to patients, clinicians and commissioning groups through peer-reviewed publication after finishing all visits.

Competing interests The authors declare that they have no competing interests.

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Figures

Figure 1: SPIRIT diagram for schedule of enrollment, interventions, and evaluation.

Routine tests include the examination of blood routine, coagulation function, renal function, hepatic function, and electrolytes. Tumour markers include CEA, CA153, CA125, and CA199.
AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; FP: fertility preservation; OHSS: ovarian hyperstimulation syndrome.

Figure 2: Flow chart of subjects

COH: controlled ovarian hyperstimulation; OHSS: ovarian hyperstimulation syndrome.

Schedule		Recruitment and consent	COH	Oocyte retrieval	Oocyte vitrification	Insemination and embyo culture	Embryo vitrification	3-30 days after oocyte retrieval	One year after FP	Two years after FP
Enrollment	Eligible screen	√								
	Informed consent	√								
	Medical history	√								
	Physical examination	√								
	AMH	√								
	Basal sex hormones	√	√					√		
	Ultrasound scan	√	√					√		
	Routine tests	√						√		
	Tumor markers	√								
	Randomization	√								
Interventions	Letrozole COH		√	√						
	Non-letrozole COH		√	√						
Evaluation	Oocyte outcome				√					
	Embryo outcome					√	√			
	OHSS evaluation							√		
	Breast cancer history								√	√

Figure 1: SPIRIT diagram for schedule of enrollment, interventions, and evaluation. Routine tests include the examination of blood routine, coagulation function, renal function, hepatic function, and electrolytes. Tumour markers include CEA, CA153, CA125, and CA199.AMH: anti-müllerian hormone; COH: controlled ovarian hyperstimulation; FP: fertility preservation; OHSS: ovarian hyperstimulation syndrome.

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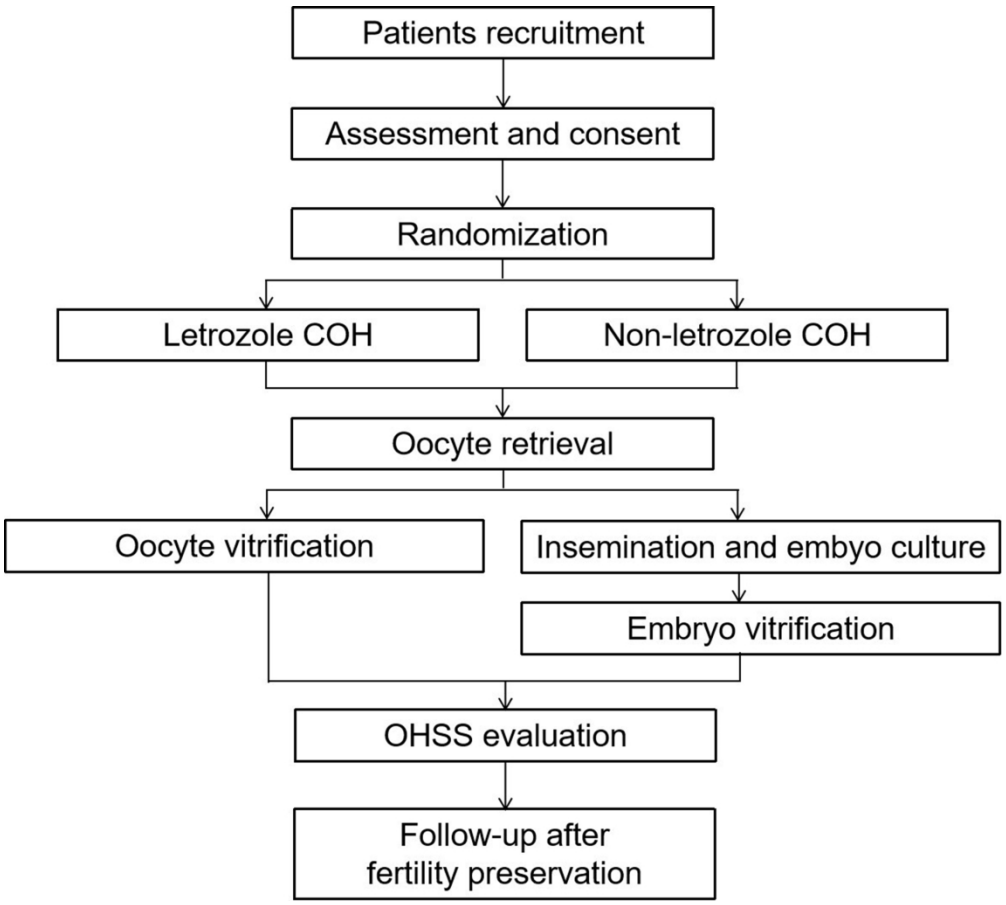

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