PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

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

Authors

Xie, Yanqiu; Li, Ping; Deng, Weifen; Fan, Qi; Sun, Peng; Kang, Jiajing; Wang, Kun; Shi, Yuhua

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Pavone, Mary Ellen**
Affiliation	**Northwestern University Feinberg School of Medicine**
Date	**05-Mar-2024**
COI	**none**

Thank you for the opportunity to review this article which describes a protocol for evaluating short term safety and efficacy of the use of letrozole in fertility preservation cycles. One of the main outcomes is OHSS. However, given the very small sample size and the fact that OHSS occurs in about 1% of IVF cycles, I am not sure if this would really be a meaningful endpoint.

more specifics:

1. page 2 lines 45-46: “and the evidence of its efficacy and safety from the perspective study is insufficient.” This sentence should be reworded for clarity.

2. abstract methods and analysis : should mention that all patients will be diagnosed with breast cancer

3. exclusion criteria: please elaborate on why patients requiring “preimplantation genetic screening” are excluded. Also, why would preimplantation genetic screening be required as opposed to couples opting to do it?

4. Will there be any longer term follow up of these patients beyond the study time frame? One of the bigger concerns is how the very short term elevated E2 levels would impact overall cancer recurrence and mortality.

Reviewer	**2**
Name	**Datta, A K**
Affiliation	**CREATE Fertility**
Date	**22-Jun-2024**
COI	**No competing interest**

This is not the first randomised trial on this topic, but we need more well designed RCTs.

The topic is of high clinical importance. However, the proposed study protocol could be improved.

* Although recent evidence indicates ovarian stimulation without letrozole or tamoxifen may not increase the risk of recurrence of breast cancer, there is still doubt and uncertainties. Specific references need to be cited to to substantiate the use of ovarian stimulation without supposedly protective letrozole against breast cancer.

* Authors described the plan of follow-up to note any relapse of breast cancer. So, this can be an important secondary outcome that may add value to this trial.

* In case of embryo-freezing, details of male factor (whether significant semen abnormalities are also present) should be stated to assess any impact of sperm on the embryo development and quality.

* stimulation dose is an important determinant of the oocyte and embryo yield, therefore exact process of calculating the dose, stimulation medication and whether the same will be applied in both the study arms need to de detailed. On what situation 2.5 mg dose and when 5 mg dose of letrozole will be used?

* What are the criteria for GnRH agonist trigger and HCG trigger?

* It's better to make it clear that a random- start protocol will not be used.

* "Sex hormone" is a very vague, unscientific term. Please specify exactly what hormone levels will be tested.

* The purpose of a study cannot be to test a study question in a specific population/ country. Except in multi-national studies, there will always be a possibility of geographic/ ethnic variations which requires validation by replicating the study in other countries. As such, by mention the study is meant for answering the study query in only Chinese population, it is restricting its wider value.

VERSION 1 - AUTHOR RESPONSE

Dear Reviewers:

We are truly grateful for your kind consideration of our manuscript. Based on the comments and suggestions of the reviewers, we have made corresponding modifications

on the original manuscript. All changes made to the text are highlighted. We hope the new manuscript will be accepted for publication. Below you will find our point-by-point responses to the reviewers' comments:

Reviewer 1

1. One of the main outcomes is OHSS. However, given the very small sample size and the fact that OHSS occurs in about 1% of IVF cycles, I am not sure if this would really be a meaningful endpoint.

Reply: Thank you for your comments. In this study, the primary outcome is the number of mature oocytes, and the secondary outcomes include the number of high-quality embryos and the incidence of OHSS. The study has limited power for assessing the secondary outcomes because the sample size was determined based on the primary outcome. Since our target population is a group with normal ovarian function, and OHSS is one of the important complications during ovarian stimulation, it is necessary to monitor the incidence of OHSS.

2. Page 2 lines 45-46: "and the evidence of its efficacy and safety from the perspective study is insufficient." This sentence should be reworded for clarity.

Reply: Thank you. We have revised the sentence to: "the evidence of oocyte retrieval during ovarian stimulation and short-term safety from the perspective study is insufficient" in the abstract.

3. Abstract methods and analysis : should mention that all patients will be diagnosed with breast cancer

Reply: Thank you. We have added "64 eligible patients diagnosed with breast cancer " in the abstract section.

4. Exclusion criteria: please elaborate on why patients requiring "preimplantation genetic screening" are excluded. Also, why would preimplantation genetic screening be required as opposed to couples opting to do it?

Reply: In this study, our primary outcome is the number of mature oocytes, with secondary outcomes including the number of high-quality embryos. Breast cancer patients requiring PGT may carry hereditary germline mutations, such as *BRCA1/2*, which could negatively impact the quality of oocytes and embryos.

5. Will there be any longer term follow up of these patients beyond the study time frame? One of the bigger concerns is how the very short term elevated E2 levels would impact overall cancer recurrence and mortality.

Reply: Yes, long-term follow-up is necessary in this study. Beyond the study time frame, we will enroll these patients in a cohort study with 5 to 10 years of follow-up, focusing on overall cancer recurrence and mortality to better understand the effect of short-term estradiol elevation during ovarian stimulation.

Reviewer 2

1. Although recent evidence indicates ovarian stimulation without letrozole or tamoxifen may not increase the risk of recurrence of breast cancer, there is still doubt and uncertainties. Specific references need to be cited to to substantiate the use of ovarian stimulation without supposedly protective letrozole against breast cancer.

Reply: Thank you for your comments. A meta-analysis (Ref 6) concluded that performing COH before or ART after anticancer treatment in young women with breast cancer did not negatively affect breast cancer recurrence, mortality, or event-free survival. Additionally, both a retrospective study (Ref 16) and a prospective study (Ref 19) found no significant difference in the safety of the traditional COH protocol versus the letrozole protocol in breast cancer patients. We have added more detail about these studies in the Introduction.

2. Authors described the plan of follow-up to note any relapse of breast cancer. So, this can be an important secondary outcome that may add value to this trial.

Reply: Thank you. We have added the recurrence rate of breast cancer as a secondary outcome.

3. In case of embryo-freezing, details of male factor (whether significant semen abnormalities are also present) should be stated to assess any impact of sperm on the embryo development and quality.

Reply: Thank you. Our primary outcome is the number of mature oocytes. For patients diagnosed with severe oligozoospermia, hypospermia, or teratospermia, we will analyze their oocyte outcomes but not embryo outcomes. We have updated the analysis plan in the Data Collection and Management section.

4. Stimulation dose is an important determinant of the oocyte and embryo yield, therefore exact process of calculating the dose, stimulation medication and whether the same will be applied in both the study arms need to de detailed. On what situation 2.5 mg dose and when 5 mg dose of letrozole will be used?

Reply: Thank you. That's a good suggestion. We have specified the letrozole dose in the manuscript. Patients weighing less than 40 kg will receive 2.5 mg daily, while others will receive 5 mg daily.

5. What are the criteria for GnRH agonist trigger and HCG trigger?

Reply: Thank you. The administration of letrozole during COH is the variable between the two groups. We will follow routine standard clinical procedures to improve the feasibility of the study and simulate clinical realities as much as possible. GnRH-a triggers will be used for patients at risk of OHSS, while HCG triggers will be used for the general population.

6. It's better to make it clear that a random- start protocol will not be used.

Reply: Thank you. In the revised version, we specified that the antagonist COH protocol (letrozole antagonist COH or conventional antagonist COH) will be used in the Interventions section.

7. "Sex hormone" is a very vague, unscientific term. Please specify exactly what hormone levels will be tested.

Reply: Thank you. We have added more details, specifying the hormones to be tested: follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), progesterone (P), prolactin (PRL), and testosterone (T).

8. The purpose of a study cannot be to test a study question in a specific population/ country. Except in multi-national studies, there will always be a possibility of geographic/ ethnic variations which requires validation by replicating the study in other countries. As such, by mention the study is meant for answering the study query in only Chinese population, it is restricting its wider value.

Reply: Thank you for your comments. We have removed the description restricting the study to the Chinese population to avoid misunderstanding. The results of our study will have clinical implications globally.

VERSION 2 - REVIEW

Reviewer	**2**
Name	**Datta, A K**
Affiliation	**CREATE Fertility**
Date	**04-Nov-2024**
COI	

The authors have addressed the comments made by the reviewers. Although there are few published RCT on this topic, adding more data would strengthen the evidence.

However, the following matters may need further clarification:

1. There are few RCTs on this topic have already been published. How this trial is different from the published RCTs needs highlighting in the introduction.

2. In the power calculation section, it was not clear what outcome is taken to decide the sample size- is it the numer of eggs or the number of mature eggs (or embryos)? What outcome the previous study used?

3. How the authors be sure that the recurrence of breast cancer in next 2 years tiime is linked with the stimulation protocol used, and not due to the stage or histological type of the tumour or not due to lack of response to chemotherapy/ radiotherapy?

4. At the start of this trial, it is unknown how many eligible patient will opt of egg freezing and how many go for embryo freezing. What step the investigators will take if they get only very few embryo freezing cycles? Or if there is a large difference in the embryo freezing cycles between the protocols? Obviously, the results on fertilisation rate, failed fertilisation rate, high grade embryos etc will lose credential if only a minority of patients opt for creating embryos. Particularly when only 32 patients are targeted to be recruited in each arm. It needs to be stated how the trial will proceed in this situation- should the trial keep recruiting patients until a descent sample size for embryo freezing is achieved.

5. The severity of preexisting morbidity may also affect ovaran stimulation and egg quality. How this aspect be adjusted for?

VERSION 2 - AUTHOR RESPONSE

1. Reviewer Comments:

The authors have addressed the comments made by the reviewers. Although there are few published RCT on this topic, adding more data would strengthen the evidence. However, the following matters may need further clarification:

(1) There are few RCTs on this topic have already been published. How this trial is different from the published RCTs needs highlighting in the introduction.

Reply: Thank you for this important comment. Previous prospective studies have focused on the number of retrieved oocytes or disease-free survival, while retrospective studies have indicated lower oocyte maturation rates in L-COH. Therefore, it is essential to investigate the efficacy of L-COH, with the number of mature oocytes as the primary outcome. We have added this information to the "Introduction" section.

(2) In the power calculation section, it was not clear what outcome is taken to decide the sample size- is it the numer of eggs or the number of mature eggs (or embryos)? What outcome the previous study used?

Reply: Our primary outcome is the number of mature eggs. The sample size was calculated based on a previous study, which we have now cited in the manuscript.

(3) How the authors be sure that the recurrence of breast cancer in next 2 years time is linked with the stimulation protocol used, and not due to the stage or histological type of the tumour or not due to lack of response to chemotherapy/ radiotherapy?

Reply: Cancer recurrence is complex, and establishing a causal link between ovarian stimulation protocol and recurrence is challenging. However, since the main variable between groups is the ovarian stimulation protocol, we will analyze the relevance. Additionally, we will collect detailed information on breast cancer, including stage, histological type, and history of chemotherapy, radiotherapy, targeted therapy, or endocrine therapy.

(4) At the start of this trial, it is unknown how many eligible patient will opt of egg freezing and how many go for embryo freezing. What step the investigators will take if they get only very few embryo freezing cycles? Or if there is a large difference in the embryo freezing cycles between the protocols? Obviously, the results on fertilisation rate, failed fertilisation rate, high grade embryos etc will lose credential if only a minority of patients opt for creating embryos. Particularly when only 32 patients are targeted to be recruited in each arm. It needs to be stated how the trial will proceed in this situation- should the trial keep recruiting patients until a descent sample size for embryo freezing is achieved.

Reply: In our study, single patients or those whose partners are unable to provide sperm will have their mature oocytes vitrified, while the remaining participants will have oocytes or embryos vitrified. All participants will undergo COH with either the letrozole antagonist COH or conventional antagonist protocol. The proportion of embryo vitrification may indeed be lower than anticipated, which is one reason why we have selected the number of mature oocytes as the primary outcome, rather than embryo-related outcomes. We anticipate that most participants will be aged 30-40 and married,

increasing the likelihood of embryo vitrification. If the embryo vitrification rate falls below 50%, we will consider expanding the sample size.

(5) The severity of preexisting morbidity may also affect ovarian stimulation and egg quality. How this aspect be adjusted for?

Reply: Participants are generally referred by breast oncologists, who assess their medical condition before recommending fertility preservation. If a patient's condition is severe or at an advanced stage, fertility preservation will not be advised.

(6) If you have selected 'Yes' above, please provide details of any competing interests.: Nil

Reply: The authors declare that they have no competing interests.