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Fertility preservation for women with breast cancer: a multicentre randomized controlled trial on various ovarian stimulation protocols

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STUDY QUESTION: Does ovarian stimulation with the addition of tamoxifen or letrozole affect the number of cumulus-oocyte complexes (COCs) retrieved compared to standard ovarian stimulation in women with breast cancer who undergo fertility preservation?

SUMMARY ANSWER: Alternative ovarian stimulation protocols with tamoxifen or letrozole did not affect the number of COCs retrieved at follicle aspiration in women with breast cancer.

WHAT IS KNOWN ALREADY: Alternative ovarian stimulation protocols have been introduced for women with breast cancer who opt for fertility preservation by means of banking of oocytes or embryos. How these ovarian stimulation protocols compare to standard ovarian stimulation in terms of COC yield is unknown.

STUDY DESIGN, SIZE, DURATION: This multicentre, open-label randomized controlled superiority trial was carried out in 10 hospitals in the Netherlands and 1 hospital in Belgium between January 2014 and December 2018. We randomly assigned women with breast cancer, aged 18–43 years, who opted for banking of oocytes or embryos to one of three study arms; ovarian stimulation plus tamoxifen, ovarian stimulation plus letrozole or standard ovarian stimulation. Standard ovarian stimulation included GnRH antagonist, recombinant FSH and GnRH agonist trigger. Randomization was performed with a web-based system in a 1:1:1 ratio, stratified for oral contraception usage at start of ovarian stimulation, positive estrogen receptor (ER) status and positive lymph nodes. Patients and caregivers were not blinded to the assigned treatment. The primary outcome was number of COCs retrieved at follicle aspiration.

PARTICIPANTS/MATERIALS, SETTING, METHODS: During the study period, 162 women were randomly assigned to one of three interventions. Fifty-four underwent ovarian stimulation plus tamoxifen, 53 ovarian stimulation plus letrozole and 55 standard ovarian stimulation. Analysis was according to intention-to-treat principle.

MAIN RESULTS AND THE ROLE OF CHANCE: No differences among groups were observed in the mean (±SD) number of COCs retrieved: 12.5 (10.4) after ovarian stimulation plus tamoxifen, 14.2 (9.4) after ovarian stimulation plus letrozole and 13.6 (11.6) after standard ovarian stimulation (mean difference −1.13, 95% CI −5.70 to 3.43 for tamoxifen versus standard ovarian stimulation and 0.58,

95% CI −4.03 to 5.20 for letrozole versus standard ovarian stimulation). After adjusting for oral contraception usage at the start of ovarian stimulation, positive ER status and positive lymph nodes, the mean difference was −1.11 (95% CI −5.58 to 3.35) after ovarian stimulation plus tamoxifen versus standard ovarian stimulation and 0.30 (95% CI −4.19 to 4.78) after ovarian stimulation plus letrozole versus standard ovarian stimulation. There were also no differences in the number of oocytes or embryos banked. There was one serious adverse event after standard ovarian stimulation: one woman was admitted to the hospital because of ovarian hyperstimulation syndrome.

LIMITATIONS, REASONS FOR CAUTION: The available literature on which we based our hypothesis, power analysis and sample size calculation was scarce and studies were of low quality. Our study did not have sufficient power to perform subgroup analysis on follicular, luteal or random start of ovarian stimulation.

WIDER IMPLICATIONS OF THE FINDINGS: Our study showed that adding tamoxifen or letrozole to a standard ovarian stimulation protocol in women with breast cancer does not impact the effectiveness of fertility preservation and paves the way for high-quality long-term follow-up on breast cancer treatment outcomes and women's future pregnancy outcomes. Our study also highlights the need for high-quality studies for all women opting for fertility preservation, as alternative ovarian stimulation protocols have been introduced to clinical practice without proper evidence.

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TRIAL REGISTRATION DATE: 6 August 2013.

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Key words: breast cancer / fertility preservation / ovarian stimulation / tamoxifen / letrozole / oocyte banking / embryo banking / cumulus-oocyte complexes

Introduction

Young women with cancer are at risk for future infertility as cancer treatment can be lifesaving but negatively impacts ovarian reserve (Bines *et al.*, 1996; Wallace *et al.*, 2005; Sonmezer and Oktay, 2006; Hulvat and Jeruss, 2009; Rodriguez-Wallberg and Oktay, 2010; Mulder *et al.*, 2021). Breast cancer is the most common malignancy in women of reproductive age (Bray *et al.*, 2018). Women with breast cancer have the option to bank oocytes or embryos prior to their treatment, which requires ovarian stimulation involving short-term exposure to high levels of estrogens (Barbieri, 2019; Strauss and Lessey, 2009; Practice Committee of the American Society for Reproductive Medicine, 2019). This increased level of estrogen has led to concerns about the safety of standard ovarian stimulation in terms of cancer recurrence, despite reassuring data on the safety of estrogen exposure during pregnancy and after ART in breast cancer survivors (Goldrat *et al.*, 2015; Hartman and Eslick, 2016; Iqbal *et al.*, 2017; Nye *et al.*, 2017; Lambertini *et al.*, 2018; Rosenberg *et al.*, 2019). Nevertheless, these concerns paved the way for the introduction of additional medication in ovarian stimulation regimens to counterbalance estrogen exposure in women with breast cancer undergoing ovarian stimulation for fertility preservation (Oktay *et al.*, 2005; Revelli *et al.*, 2013). Since then, multiple prospective and retrospective cohort studies have been published comparing various ovarian stimulation protocols in women with breast cancer (Rodgers *et al.*, 2017; Bonardi *et al.*, 2020). These alternative stimulation protocols consist of addition of the selective estrogen receptor (ER) modulator tamoxifen or the aromatase-inhibitor letrozole, but their effectiveness has never been compared to standard ovarian stimulation in any randomized controlled trial (RCT; Dahhan *et al.*, 2013).

The aim of the current study was to evaluate the effectiveness of ovarian stimulation with the addition of tamoxifen or letrozole compared to standard ovarian stimulation in terms of the number of cumulus-oocyte complexes (COCs) retrieved in women with breast cancer undergoing ovarian stimulation to bank oocytes or embryos.

Materials and methods

Study design

This study was designed as an international, multicentre, open-label randomized controlled two-sided superiority trial carried out in seven

university hospitals and three non-university hospitals in the Netherlands participating in the Dutch Consortium for Women's Health Research and one university hospital in Belgium.

The trial protocol and all subsequent amendments were approved by the Medical Ethical Committee of the Amsterdam University Medical Center, location AMC (MEC 2013_070) and by the board of directors of all participating centres. Serious adverse events were reported to the Medical Ethical Committee. A serious adverse event was defined as any unwanted medical occurrence or effect at any dose that requires hospitalization, results in disability, is life threatening or results in death. This study was designed and conducted in line with the guidelines for good clinical practice as well as the Declaration of Helsinki. The study protocol has been published previously (Dahhan et al., 2017).

Participants

Women aged between 18 and 43 years were eligible for the study if they had a diagnosis of breast cancer, regardless of ER status and opted for banking of oocytes or embryos. Women were excluded if they used medication that opposed the effect of study medication, such as antidepressants paroxetine or fluoxetine, which are strong inhibitors of the enzyme cytochrome p450 2D6 (CYP2D6). Randomization followed if women fulfilled the inclusion criteria, and written informed consent was obtained.

Randomization and masking

Women were randomly assigned to one of three treatment arms: ovarian stimulation plus 60 mg of tamoxifen daily, ovarian stimulation plus 5 mg of letrozole daily or standard ovarian stimulation. Women were randomized by the research nurse or local investigator. Allocation concealment was ensured by the use of a web-based randomization program, as the persons who registered participants for randomization could not see how many participants had already been randomized or what their allocation was. Randomization was performed in a 1:1:1 ratio with permuted block randomization. Women were stratified for oral contraception usage at the start of ovarian stimulation, positive ER status and positive lymph nodes. Oral contraception usage, positive ER status and positive lymph nodes were chosen because these may influence breast cancer prognosis and a follow-up study of the women is intended. The allocated treatment appeared directly online and an automatic e-mail with allocation code was sent to the research nurse and the data manager.

Procedures

In the ovarian stimulation plus tamoxifen arm, women received 60 mg tamoxifen orally per day, starting on cycle Day 2 in addition to the standard ovarian stimulation with 225 IU recombinant FSH (rFSH; Puregon®; Organon, Oss, the Netherlands or Gonal-F®; Merck Serono, Switzerland or Ovaleap®; Theramex, Dublin Ireland). Tamoxifen was discontinued on the day of the GnRH agonist trigger (Decapeptyl®, 0.2 mg; Ferring BV, Hoofddorp, Triptofem®, 0.2 mg; Goodlife BV Lelystad). In the ovarian stimulation plus letrozole arm, women received 5 mg letrozole per day orally, starting on cycle Day 2 in addition to the standard ovarian stimulation regimen. Ovarian stimulation was started with 225 IU recombinant rFSH on cycle Day 4. Letrozole was discontinued on the day of the GnRH agonist trigger. Women restarted letrozole on the day of follicle aspiration to prevent a rebound increase in estradiol levels, for 3 days. In the standard ovarian stimulation arm, women started with 225 IU rFSH on cycle Day 2, which was continued until one or more follicles reached 18–20 mm, followed by GnRH agonist trigger. On Day 5 of rFSH stimulation, a GnRH antagonist (Orgalutran® 0.25 mg; Organon, Oss, the Netherlands or Cetrotide® 0.25 mg, Merck Serono, Switzerland) was administered to prevent a premature LH surge and discontinued on the day of the GnRH agonist trigger. In case of extreme time limitations related to the start of breast cancer treatment and her cycle, luteal or random start of ovarian stimulation was allowed in all treatment arms.

In all treatment arms, a transvaginal ultrasound-guided follicle aspiration was performed 34–36 h after the GnRH agonist trigger. Follicle aspiration was performed in outpatient clinics under local anaesthesia or light sedation according to local protocol. In the IVF laboratory, COCs were collected. After denudation, metaphase II oocytes were vitrified with the use of cryoprotectants and the ultra-rapid freezing technique, according to the Kitazato protocol (Kuwayama et al., 2005; Kuwayama, 2007). In case of embryo-cryopreservation, oocytes were fertilized by ICSI with subsequent embryo banking on day 3, 4 or 5 of development, according to the local slow-freezing protocol.

When designing the trial in 2012, we primarily focused on the safety of controlled ovarian stimulation, with peak estradiol as a proxy for safety. Following new insights, after approval of all investigators and the Medical Ethical Committee, we changed our focus to effectiveness in March 2015, with number of cumulus oocytes retrieved as the effectiveness outcome.

Outcomes

The primary outcome measure was the mean number of COCs retrieved at follicle aspiration. Secondary outcomes were the number of metaphase II oocytes, number of oocytes or embryos banked, peak estradiol levels defined as serum estradiol level measured on the day of ovulation trigger, and number of women with cancelled cycles. We only used data of the first cycle of ovarian stimulation.

Statistical analysis

Based on the literature available when designing the trial, we estimated the mean number of COCs in women with standard ovarian stimulation to be 10, while tamoxifen could lead to 4 COCs more and letrozole to 4 COCs less with an SD of 6 (Bodri et al., 2011; Revelli et al., 2013; Meirow et al., 2014). Our null hypothesis was that no difference would exist for number of COCs. To show a two-sided mean difference of at least 4.0 COCs with a common standard deviation of 6.0 between the ovarian stimulation plus tamoxifen and ovarian stimulation plus letrozole arms with the standard ovarian stimulation arm, we needed to include a total of 144 women. Compensating for 7% lost to follow-up, we intended to enrol 159 women in total. The sample size calculation was originally calculated with STATA 14.2 (StataCorp LLC; TX, USA) and confirmed with PASS 2020 (NCSS; UT, USA), V20.0.2.

We followed the intention-to-treat principle (ITT). Baseline characteristics, breast cancer data and ovarian stimulation data are presented as the mean with SD or as proportion (%), depending on the variable. Residual analysis was used to test normality of continuous outcomes. For all continuous outcomes, we compared the ovarian stimulation

plus tamoxifen and ovarian stimulation plus letrozole treatment arms with the standard ovarian stimulation treatment arm. Mean differences with 95% CI were calculated for all continuous outcomes. For the primary outcome, the number of COCs, we calculated mean differences both with and without covariate adjustment for the stratification variables using univariate regression analysis (UNIANOVA) with Dunnett's testing. Additionally, we calculated mean differences following imputation of zero COCs in case of cancelled cycles and drop-outs. The number of cancelled cycles was expressed as a relative risk with 95% CI.

A pre-planned independent interim analysis was performed by the Data Safety Monitoring board of the Dutch Consortium for Women's Health Research when 25% of the sample size was reached to exclude large differences in COCs and to assess whether any adverse events occurred related to the ovarian stimulation. The Data Safety Monitoring board advised to proceed with the trial as planned.

The STIM trial was registered within the Netherlands Trial Register (NTR4108).

Results

Between 1 January 2014 and 31 December 2018, 162 women were randomized. Fifty-four women were assigned to the ovarian stimulation plus tamoxifen arm, 53 women to the ovarian stimulation plus letrozole arm and 55 women to the standard ovarian stimulation arm. Of these, 154 had a follicle aspiration. All 162 women were included in the ITT analysis (Fig. 1). Baseline characteristics are presented in Table I and showed a similar distribution among groups. The mean age of the women was 32 years.

Data on breast cancer status are shown in Table II. Three out of 53 women (6%) in the ovarian stimulation plus letrozole arm had a bilateral tumour, while all other women had a unilateral tumour. The tumour, node, metastasis (TNM) staging for breast cancer was used to determine disease stage (Amin *et al.*, 2017). The tumour stage was I–II in 45 women (83%) in the ovarian stimulation plus tamoxifen arm, 45 women (85%) in ovarian stimulation plus letrozole arm and 46 women (84%) in the standard ovarian stimulation arm. Invasive breast cancer of no special type, previously named ductal invasive breast cancer, was present in 44 women (82%) in the ovarian stimulation plus tamoxifen arm, 44 women (83%) in ovarian stimulation plus letrozole arm and 48 women (87%) in standard ovarian stimulation arm. Additional characteristics regarding breast cancer treatment are shown in Supplementary Table SI.

Details on ovarian stimulation are shown in Table III. Nineteen women had two cycles of ovarian stimulation and one woman three cycles; only data from the first cycles were included in the analyses. The mean length of ovarian stimulation was 10.6 days. Seventy-seven out of 162 women (48%) started ovarian stimulation in the follicular phase of the cycle, 25 (15%) in the luteal phase and 56 (35%) random in their menstrual cycle.

Primary and secondary outcomes are shown in Table IV. The mean (±SD) number of COCs retrieved at follicle aspiration did not differ between treatment arms and was 12.5 (10.4) after ovarian stimulation plus tamoxifen, 14.2 (9.4) after ovarian stimulation plus letrozole and 13.6 (11.6) after standard ovarian stimulation. After adjusting for oral

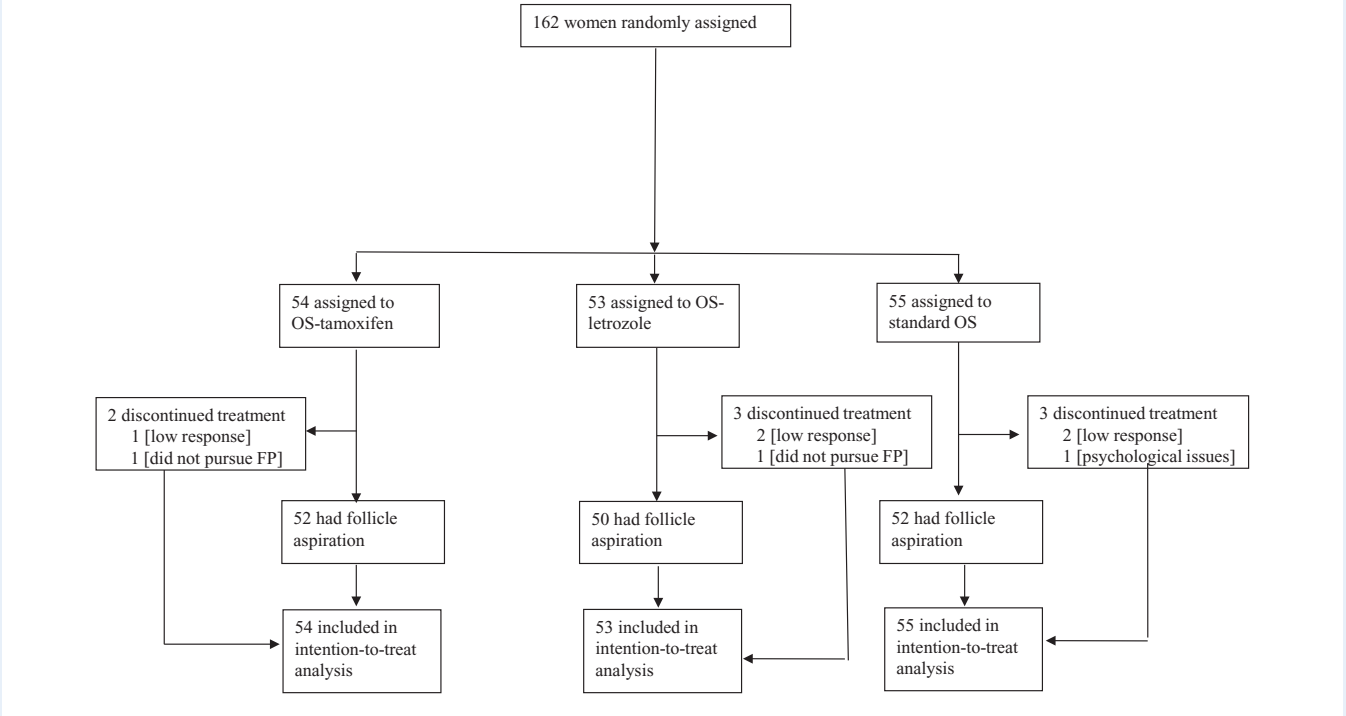

Figure 1. Flow diagram of randomization of women with breast cancer in a randomized controlled trial of various ovarian stimulation protocols for fertility preservation. OS, ovarian stimulation; FP, fertility preservation.

Table I Baseline characteristics of women with breast cancer in a randomized controlled trial of various ovarian stimulation protocols for fertility preservation.

Characteristic	OS-tamoxifen n = 54	OS-letrozole n = 53	Standard OS n = 55
Mean age, years (SD)	31.8 (4.4)	32.3 (3.8)	31.4 (4.0)
Cycle pattern			
Regular menstrual cycle, n (%)	28 (53)	33 (64)	25 (46)
Oligomenorrhoea/amenorrhoea	3 (5.6)	1(1.9)	4 (7.3)
Contraception use, n (%)			
Oral contraceptives	14 (26)	16 (30)	17 (31)
Hormonal- IUD	3 (5.6)	8 (15)	3 (5.5)
Other	5 (9.3)	4 (7.5)	7 (13)
No contraceptive use	28 (52)	23 (43)	27 (50)
Nulliparous, n (%)	42 (78)	33 (62)	32 (58)
Relationship, n (%)	36 (67)	39 (74)	34 (62)
Mean BMI (kg/m^2) (SD)	23.6 (4.3)	23.2 (2.8)	24.1 (6.1)
AFC (mean, SD)	14.0 (7.8)	15.0 (8.8)	18.9 (10.2)
AMH µg/l (mean, SD)	2.6 (2.3)	2.7 (2.1)	3.1 (3.0)

OS, ovarian stimulation; AMH, anti-Müllerian hormone; AFC, antral follicle count; IUD, intrauterine device.
There was no data available on menstrual cycle information in 13 women, contraception use in two women, parity status in 12 women, relationship in five women, BMI in 29 women, AFC in 28 women and AMH in 21 women.

Table II Breast cancer characteristics of the women in the trial.

Characteristic	OS-tamoxifen n = 54	OS-letrozole n = 53	Standard OS n = 55
TNM tumour stage, n (%)			
I	18 (33)	17 (32)	13 (24)
II	27 (50)	28 (53)	33 (60)
III	5 (9.3)	4 (7.5)	2 (3.6)
IV	2 (3.7)	2 (3.8)	–
Unknown	2 (3.7)	2 (3.8)	7 (13)
Histologic tumour grade, n (%)			
I	5 (9.3)	1 (1.9)	2 (3.6)
II	15 (28)	13 (25)	15 (27)
III	16 (30)	24 (45)	23 (42)
Positive estrogen receptor, n (%)	34 (63)	35 (66)	34 (62)
Positive progesterone receptor, n (%)	30 (56)	29 (55)	27 (49)
Positive HER-2-NEU receptor, n (%)	17 (32)	11 (21)	20 (36)
Genetic mutation carrier, n (%)			
BRCA-1	7 (13)	9 (17)	4 (7.3)
BRCA-2	3 (5.6)	2 (3.8)	–
CHEK 2	4 (7.4)	1 (1.9)	4 (7.3)
Other	–	1 (1.9)[A]	–
None	36 (67)	36 (68)	35 (64)

TNM, tumour stage version 7. According to the Dutch guidelines hormone sensitivity was defined as: Estrogen receptor ≥10% positive tumour cells and/or Progesterone receptor ≥10% positive tumour cells. HER2-positivity was defined according to ASCO/CAP guidelines (Deyarmin *et al.*, 2013).
OS, ovarian stimulation; HER-2-NEU, Human Epidermal growth factor Receptor 2; BRCA, breast cancer gene; CHEK, checkpoint kinase gene; TNM, tumour, node, metastasis. There were no data available on histologic tumour grade in 48 women, estrogen, progesterone and HER-2-Neu receptor status in 4 women and genetic carrier status in 18 women. [A]One woman had a PALB-2 mutation (PALB-2, partner and localizer of BRCA2).

contraception usage at start of ovarian stimulation, positive ER status and positive lymph nodes, the mean differences (95% CI) in number of COCs were -1.11 (-5.58 to 3.35) after ovarian stimulation plus tamoxifen versus standard ovarian stimulation and 0.30 (-4.19 to 4.78) after ovarian stimulation plus letrozole versus standard ovarian stimulation. There were also no differences in the number of oocytes or embryos banked. Of the six cancelled cycles, five were cancelled because of low ovarian response and one for psychological reasons. Peak estradiol was significantly lower in ovarian stimulation plus letrozole compared to standard ovarian stimulation, but there was no difference between ovarian stimulation plus tamoxifen and standard ovarian stimulation.

One woman (standard ovarian stimulation) was admitted to the hospital for one night because of ovarian hyperstimulation syndrome (OHSS). Outside of the study period, one woman (who received ovarian stimulation with letrozole) died of secondary acute myeloid leukaemia 15 months after participation in the trial and one woman (who received ovarian stimulation with tamoxifen) died of breast cancer 22 months after participation in the trial. There were some protocol violations; seven women were prescribed a lower dosage (150/175/200 IU) and two a higher dosage (250/300 IU) of rFSH. Three women were prescribed urinary FSH instead of rFSH and another three women were prescribed a different ovarian stimulation protocol (flare-up or agonist protocol). In one woman, ovulation was triggered by hCG instead of by GnRH agonist. One woman was treated in a large non-university hospital that was not registered as participating centre.

Discussion

In this multicentre, open-label, RCT in women with breast cancer who opted for fertility preservation, alternative ovarian stimulation protocols that included tamoxifen or letrozole did not affect the number of COCs retrieved at follicle aspiration. There was also no evidence of a difference in number of oocytes or embryos banked and no difference in number of cancelled cycles. Peak estradiol was significantly lower in the ovarian stimulation plus letrozole compared to standard ovarian stimulation group.

The strength of the study is that we chose a pragmatic study design that reflects daily clinical practice in which acute interventions are frequently necessary (Flink *et al.*, 2017). As such, we included women who underwent 'emergency' IVF starting ovarian stimulation in the follicular phase, luteal phase or random in their menstrual cycle, irrespective of breast cancer characteristics (including ER-receptor status), which are often not yet available at the start of ovarian stimulation. Also, we provided information about baseline breast cancer characteristics that are important for future safety follow-up on breast cancer outcomes. In addition, we used a standard ovarian stimulation protocol

with a GnRH antagonist for pituitary down-regulation and GnRH agonist as a trigger, which is an established protocol to minimize the risk of OHSS (Cakmak *et al.*, 2013; Rodgers *et al.*, 2017). In women with breast cancer, this is especially relevant since they need to undergo cancer treatment shortly after ovarian stimulation. From this perspective, the low percentage of cancelled cycles (3.8%) is encouraging. We chose the number of COCs as primary outcome, because this variable is available for both women who bank oocytes or embryos.

Our study also has limitations. The available literature on which we based our hypothesis to perform our power analysis and calculated our sample size was scarce and studies were of low quality (Bodri *et al.*, 2011; Revelli *et al.*, 2013; Meirow *et al.*, 2014). The SD of the oocytes retrieved was higher than estimated; this is a clear limitation of our design as this leads to lower ability to detect group differences. Our study did not have sufficient power to perform subgroup analysis on female age, BRCA (BReast CAncer 1 or 2 gene mutations) status or follicular, luteal or random start of ovarian stimulation. We cannot report on a pick-up rate of banked oocytes and embryos at this moment in time. It will take many years before all women finish their breast cancer treatment and obtain permission from their oncologist to become pregnant. Oocyte retrieval rates should be part of large and long-term follow-up studies.

Recently, the TALES trial was published, a single-centre RCT that compared ovarian stimulation with letrozole versus ovarian stimulation with tamoxifen in women with ER-positive breast cancer (Letourneau *et al.*, 2021). In the TALES study, 96 women were included, 45 received ovarian stimulation with tamoxifen 20 mg and 51 received ovarian stimulation with letrozole with a starting dose of 5 mg and titrated up to as much as 10 mg per day with the goal of maintaining estradiol levels (<500 pg/ml). As a secondary comparison of the primary outcome, a prospectively collected non-randomized comparison arm was built in with 38 women with ER-negative breast cancer who received standard ovarian stimulation. In contrast to the TALES trial, we also randomized for standard ovarian stimulation, which is quintessential to answer the question of whether there is a place for alternative protocols at all. Studies on long-term safety of the various protocols will determine which protocol to use, and such studies are ongoing. If these studies conclude that safety is improved by estrogen modulation, one should implement a protocol with estrogen modulations since oocyte yield will not be diminished. If, however, safety is not affected by estrogen modulation, there is no rationale for these alternative protocols. We also included women with ER positive, ER negative and unknown ER status, which is clinically relevant since ER status is not

Table III Ovarian stimulation details for the three different protocols in the trial.

Characteristic	OS-tamoxifen n = 54	OS-letrozole n = 53	Standard OS n = 55
Ovarian stimulation, n (%)			
Follicular start	25 (46)	26 (49)	26 (47)
Luteal start	10 (19)	7 (13)	8 (15)
Random/OCP	18 (33)	19 (36)	19 (35)
Not started/unknown	1 (2)	1 (2)	2 (4)
Follicles ≥15 mm on last US before follicle aspiration, mean (SD)	9.0 (5.1)	8.9 (7.0)	9.1 (6.7)
Total amount of FSH IU, mean (SD)	2371 (537)	2225 (716)	2389 (546)
Length of stimulation in days, mean (SD)	10.6 (2.4)	10.2 (3.2)	10.8 (2.3)

OS, ovarian stimulation; OCP, oral contraceptive pill; US, ultrasound.
Two women did not start ovarian stimulation. There were no data available on number of follicles in three women and total amount of FSH in one woman.

Table IV Primary and secondary outcomes of ovarian stimulation in women with breast cancer.

Characteristic	OS-tamoxifen n = 54	OS-letrozole n = 53	Standard OS n = 55	Tamoxifen versus standard	Letrozole versus standard
				Mean difference (95% CI)	
Number of COCs,[a] mean (SD)	12.5 (10.4)	14.2 (9.4)	13.6 (11.6)	−1.13 (−5.70 to 3.43)	0.58 (−4.03 to 5.20)
Adjusted difference[b]				−1.11 (−5.58 to 3.35)	0.30 (−4.19 to 4.78)
Zero imputed for cancels	12.0 (10.5)	13.4 (9.7)	12.9 (11.4)	−0.85 (−5.38 to 3.67)	0.52 (−4.03 to 5.07)
Metaphase II	11.2 (10.6)	11.2 (8.2)	10.5 (9.3)	0.77 (−4.23 to 5.77)	0.74 (−4.09 to 5.57)
Oocytes banked, mean (SD) n = 123	10.2 (7.9)	10.2 (8.1)	9.8 (9.8)	0.33 (−3.78 to 4.44)	0.35 (−3.71 to 4.40)
Embryos banked, mean (SD) n = 46	5.6 (4.5)	5.2 (3.9)	4.6 (4.0)	1.01 (−2.34 to −4.36)	−0.63 (−2.79 to 4.04)
Peak estradiol (at day of trigger) pmol/l[c]	6101 (6525)	1798 (1285)	5675 (5208)	425 (−1891 to 2641)	−3877 (−6193 to −1561)
				Relative risk (95% CI)	
Cycles cancelled, n (%)	1 (1.9)	2 (3.8)	3 (5.5)	0.36 (0.04 to 3.3)	0.71 (0.12 to 4.1)

OS, ovarian stimulation; COCs, cumulus-oocyte complexes; ER, estrogen receptor.
[a]Two women did not start ovarian stimulation. Not all women had oocyte retrieval due to cancelled cycles; one cancel in OS-tamoxifen, two cancels in OS-letrozole and three cancels in standard stimulation.
[b]Adjusted for oral contraception usage at start of ovarian stimulation, positive ER status and positive lymph nodes. [c]Peak estradiol was unknown in 28 women. Metaphase II was only available if women banked oocytes and was missing in two women.

always known at time of fertility preservation counselling. We chose to also include women with known ER-negative breast cancer, as these tumours can still contain a small fraction of estrogen positive stained cells (Yi et al., 2014; Marklund et al., 2020). Similar to the findings of the TALES trial, we found no evidence of a difference in number of mature oocytes between letrozole, tamoxifen and standard ovarian stimulation. In our study, peak estradiol levels were lower in the ovarian stimulation plus letrozole arm compared to the other two arms, which might be explained by the mechanism of action of letrozole (the prevention of conversion of androgens into estrogens). Similar results were observed in the TALES trial and in a recent meta-analysis of 11 non-randomized cohort studies that included women receiving either standard controlled ovarian stimulation alone or with additional letrozole (Bonardi et al., 2020). Also, a recent Swedish observational multicentre study in which 224 women underwent ovarian stimulation with letrozole and 156 women without letrozole showed no differences in overall survival after a mean follow-up time of 6.3 years (Marklund et al., 2020); however, this trial included few ER+ women such that no solid inferences on safety can be made. It is the actual follow-up of women with breast cancer after ovarian stimulation that will provide the ultimate answer about the safety of ovarian stimulation for fertility preservation. The recent guideline 'Female Fertility Preservation' from ESHRE emphasizes that further studies are needed on the long-term effects of ovarian stimulation with tamoxifen or letrozole co-administration (Anderson et al., 2020).

In conclusion, the number of COCs retrieved and number of oocytes or embryos banked were not affected by the alternative protocols in women with breast cancer. Since the main purpose of estrogen modulation is long-term safety, long-term follow-up in terms of breast cancer recurrence rates will determine which type of ovarian stimulation to be used.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data will be shared on reasonable request to the corresponding author. Requests will be processed by involving the trial bureau and methodologist and will include protocol, the used informed consent form, de-identified participant data and will follow national laws for data sharing.

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Authors' roles

T.D., C.C.M.B., K.F., D.S., A.M.E.B., C.B.L., R.S., S.C.L., F.v.d.V., M.v.W. and M.G. designed the trial protocol and applied for the research grant. E.M.E.B. and M.G. coordinated the trial. All authors are responsible for inclusion of eligible women. E.M.E.B., C.C.M.B., K.F., D.S., A.M.E.B., C.B.L., R.S., J.M.J.S., L.A.L., A.E.P.C., J.P.d.B. and M.G. collected data. E.M.E.B. and M.v.W. performed the statistical analysis. E.M.E., T.D., M.v.W., F.v.d.V., S.C.L. and M.G. interpreted the data and wrote the manuscript. All authors revised the manuscript and approved the final submitted version.

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Conflict of interest

M.G., C.B.L. and R.S. declared that the Center for Reproductive Medicine, Amsterdam UMC (location VUMC) has received unconditional research and educational grants from Guerbet, Merck and Ferring, not related to the presented work. C.B.L. declared a speakers fee for Inmed and Yingming. S.C.L. reports grants and non-financial support from Agendia, grants, non-financial support and other from AstraZeneca, grants from Eurocept-pharmaceuticals, grants and non-financial support from Genentech/Roche, Novartis, grants from Pfizer, Tesaro, Immunomedics and other from Cergentis, IBM, Bayer, and Daiichi-Sankyo, outside the submitted work; In addition, SCL has a patent UN23A01/P-EP pending that is unrelated to the present work. J.M.J.S. reported payments and travel grants from Merck and Ferring. C.C.M.B. reports her role as unpaid president of the National guideline committee on Fertility Preservation in women with cancer. K.F. received unrestricted grants from Merck Serono, Good Life and Ferring not related to present work. K.F. declared paid lectures for Ferring. D.S. declared former employment from Merck Sharp & Dohme (MSD). KF declared paid lectures for Ferring. DS reports grants from MSD, Gedeon Richter and Ferring paid to his institution; consulting fee payments from MSD and Merck Serono paid to his institution; speaker honoraria from MSD, Gedeon Richter, Ferring Pharmaceuticals and Merck Serono paid to his institution. D.S. has also received travel and meeting support from MSD, Gedeon Richter, Ferring Pharmaceuticals and Merck Serono. No payments are related to present work.

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