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Efficacy and safety of controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: a multicenter retrospective study

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Abstract:	introduction Fertility preservation (FP) is recommended in young breast cancer (BC) patients before (neo)adjuvant treatment. Letrozole-associated controlled ovarian hyperstimulation (LetCOH) is used worldwide to collect mature oocytes for FP, but its efficacy and safety compared to conventional protocols (cCOH) is still debated. Aims To compare efficacy and safety of FP procedure using LetCOH or cCOH in BC patients in terms of oocyte maturation rate and disease-free survival rates after at least 2 years of follow-up. Methods This multicenter retrospective study compared outcomes of 107 cycles in 97 non-metastatic BC patients aged ≤40 years who underwent cCOH (n=56) or LetCOH (n=41) for FP in CHU-Lille and Erasme Hospital, respectively between December 2012 and January 2017. Results Patients and oncological characteristics were similar except for tumor size and HER2 status which were less favorable in the LetCOH group. Patients underwent adjuvant chemotherapy in 96.4% and 48.8% of the cases in cCOH and LetCOH groups, respectively. Hence, 51.2% of LetCOH patients underwent neoadjuvant chemotherapy ($p<0.001$). Estradiol peak at ovulation trigger was lower in LetCOH compared to cCOH group while oocyte maturation rates were significantly higher ($p<0.001$), without impacting final number of mature oocytes collected. Seven and 4 patients relapsed in LetCOH and cCOH groups respectively, and one patient died in each group after a median follow-up of 4 years. Conclusion

	LetCOH is as effective as cCOH for FP. At this time-point, there were no safety concerns regarding cCOH in adjuvant setting but longer follow-up is warranted.

Title

Efficacy and safety of controlled ovarian hyperstimulation with or without letrozole for fertility preservation in breast cancer patients: a multicenter retrospective study

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ABSTRACT

Introduction

Fertility preservation (FP) is recommended in young breast cancer (BC) patients before (neo)adjuvant treatment. Letrozole-associated controlled ovarian hyperstimulation (LetCOH) is used worldwide to collect mature oocytes for FP, but its efficacy and safety compared to conventional protocols (cCOH) is still debated.

Aims

To compare efficacy and safety of FP procedure using LetCOH or cCOH in BC patients in terms of oocyte maturation rate and disease-free survival rates after at least 2 years of follow-up.

Methods

This multicenter retrospective study compared outcomes of 107 cycles in 97 non-metastatic BC patients aged ≤40 years who underwent cCOH (n=56) or LetCOH (n=41) for FP in CHU-Lille and Erasme Hospital, respectively between December 2012 and January 2017.

Results

Patients and oncological characteristics were similar except for tumor size and HER2 status which were less favorable in the LetCOH group. Patients underwent adjuvant chemotherapy in 96.4% and 48.8% of the cases in cCOH and LetCOH groups, respectively. Hence, 51.2% of LetCOH patients underwent neoadjuvant chemotherapy ($p<0.001$).

Estradiol peak at ovulation trigger was lower in LetCOH compared to cCOH group while oocyte maturation rates were significantly higher ($p<0.001$), without impacting final number of mature oocytes collected. Seven and 4 patients relapsed in LetCOH and cCOH groups respectively, and one patient died in each group after a median follow-up of 4 years.

Conclusion

LetCOH is as effective as cCOH for FP. At this time-point, there were no safety concerns regarding cCOH in adjuvant setting but longer follow-up is warranted.

KEYWORDS

Fertility preservation, early breast cancer, letrozole, controlled ovarian stimulation, oocyte maturation rate, disease-free-survival

1. Introduction

Although the majority of breast cancer (BC) diagnoses occur after menopause, 5%-10% of the cases occur in young women during their reproductive years (≤40 years) [1, 2], when they have often not yet completed their family planning. Multimodal gonadotoxic therapy [3, 4] has radically improved long-term survival over the last few decades, but can potentially lead to infertility and/or premature ovarian failure, by reducing ovarian reserve and inducing stromal fibrosis and/or vascular injury [5, 6].

Quality of life in young BC patients, particularly with regard to motherhood, has become a major concern in cancer care over the last few years [7]. Oocyte and/or embryo freezing following controlled ovarian hyper-stimulation (COH) is the established standard option for fertility preservation (FP) [8, 9]. COH is however accompanied by a substantial increase in estradiol (E2) levels, raising some safety concerns especially in hormone-responsive (ER+) tumors [10, 11]. Consequently, an alternative COH protocol associated with letrozole (Let-COH)[12, 13], has been implemented in order to recover several mature oocytes while keeping low estradiol levels. Although LetCOH is now used almost worldwide, controversy still remains regarding its efficacy in terms of oocyte maturation rate[13-15], and only few reports have been published on long term disease-free survival (DFS) [16-18]. In this retrospective multicenter study, we have compared ovarian response and relapse rate between BC patients who underwent LetCOH and conventional COH (cCOH).

2. Patients and methods

This multicenter retrospective observational study was conducted in two reference onco-fertility centers in Belgium and France, the CUB-Erasme Hospital in Brussels, and the Lille University Hospital (CHU-Lille), and the breast cancer unit of the Oscar Lambret Center in Lille. The Ethics Committees of the participating centers approved this study.

2.1. Patient population

Eligible cases included non-metastatic BC patients of ≤40 years who underwent fertility preservation (oocyte or embryo freezing) before oncological treatment between December 2012 and January 2017. We excluded patients with metastatic disease, ovarian insufficiency (follicle stimulating hormone (FSH) >20 mIU/mL) and with anti-Müllerian hormone (AMH) levels <0.5 ng/mL (poor ovarian reserve) or >8 ng/mL (severe polycystic ovary syndrome (PCOS)). AMH measures were performed using the Roche Elecsys® AMH Plus assay in both centers with a limit of detection (LOD) of 0.01 ng/ml.

The study group included patients who were previously referred to CUB-Erasme Hospital by several oncological centers in Belgium to participate in a long-term prospective trial on fertility preservation in BC patients (BROVALE trial, Clinicaltrials.gov NCT02661932). All patients in this group underwent Let-COH before adjuvant or neoadjuvant chemotherapy (LetCOH). The control group included patients who were referred for FP to CHU-Lille, Jeanne de Flandres Hospital by several oncological centers in France and who participated in an observational prospective study on chemotherapy impact on fertility markers (Clinicaltrials.gov "Cancer-et-fertilité-1104" NCT 01614704). All patients in this group underwent conventional COH without letrozole, before administration of chemotherapy in an adjuvant setting (cCOH) [19]. Patients in both groups had signed informed consent before undergoing fertility preservation procedures. In Cancer-et-fertilité-1104, patients were enrolled in the study before starting chemotherapy after receiving full information from the investigators regarding the research. Written consent was not required from patients according to French laws governing non-interventional studies.

2.2. Data collection

Medical records were retrieved for all eligible patients in both groups. Collected data were age and body mass index, anti-Müllerian hormone (AMH) level for ovarian reserve, oncological characteristics at diagnosis, BC treatment and follow-up of at least two years or until disease recurrence. Characteristics of stimulation cycles were also collected in each center. The primary end-point was number of obtained mature oocytes.

2.3. COH protocols

All patients in both groups underwent gonadotropin releasing hormone (GnRH) antagonist cycles with recombinant FSH or urinary human menopausal gonadotropin. Daily doses were based on patient age, BMI, antral follicle count (AFC), and serum AMH levels, and varied between 150 and 300 IU/d in LetCOH and between 150 and 450 IU/d in cCOH groups respectively. GnRH antagonist was initiated when the lead follicle reached 12-14 mm in diameter and/or when E_2 levels reached 250 pg/ml in the Let-COH, or at the 6th day of stimulation in the cCOH group. In patients who received LetCOH, ovulation trigger was achieved with human chorionic gonadotropin (hCG) (10,000 IU) or GnRHa (0.2 mg) when at least two follicles reached >19-20 mm in diameter, based on previously published data suggesting a lower oocyte maturation rate when trigger is performed at follicular size of 17mm,

as recommended for standard COH protocol [13]. In the cCOH group, ovulation triggering was performed with GnRHa (0.2 mg), hCG (6,500 IU) or dual trigger (GnRHa 0.2mg and hCG 1,500 IU) as soon as ≥ 3 leading follicles reached ≥17 mm and a majority of intermediary follicles reached ≥14 mm, based on standard practice [19].

Depending on the woman's menstrual cycle phase at the time of referral, each BC patient underwent standard or random-start COH protocol in both groups in order to avoid any delay before starting chemotherapy. In the LetCOH group, 5 mg of letrozole was administered during the entire stimulation until the day of ovulation triggering. Pelvic ultrasound and blood tests were performed during COH in both groups. Transvaginal oocyte retrieval was performed 36 hours after ovulation trigger. After stripping the cumulus cells, mature oocytes were cryopreserved or fertilized for embryo storage, according to patient choice.

2.4. Oncological follow-up

Follow-up information was collected during return visits to the fertility clinic or by contacting the patient's referring oncological clinic. For the current analysis, women were followed-up for during at least 2 years. Recurrence was defined as detection of loco-regional or distant disease after the initial oncological treatment, and time to recurrence was calculated from time of diagnosis.

2.5. Statistical analysis

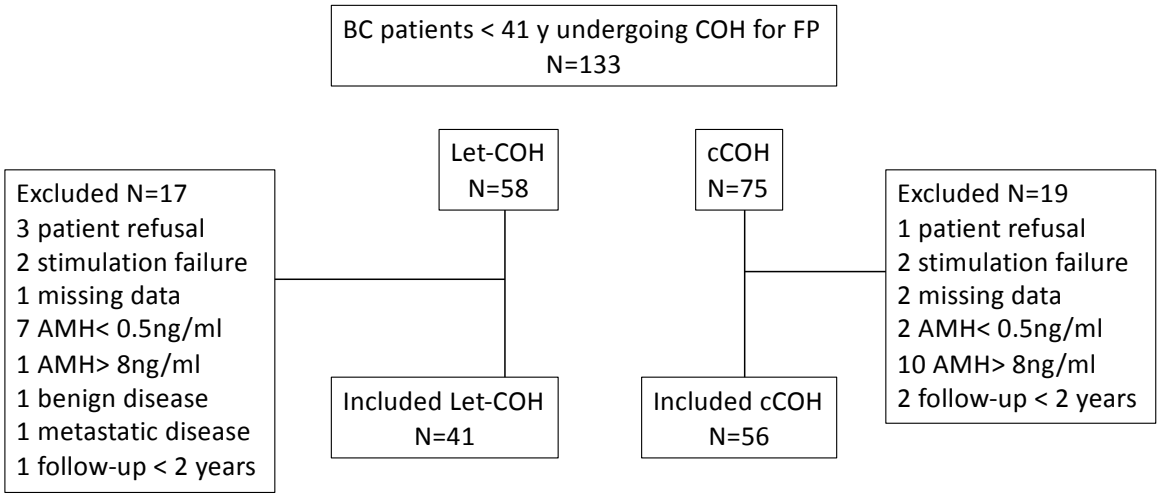
Statistical analysis was performed with Prism GraphPad 9.1.2. Mann-Whitney test was used for comparison of quantitative data and χ^2-test and Fisher exact test for comparison of proportions. A *p*-value <0.05 was considered statistically significant.

3. Results

3.1. Patient selection

A total of 133 BC patients who underwent fertility preservation between December 2012 and January 2017 were screened for potential inclusion (Flowchart fig. 1). Eight patients were not eligible because they had refused to pursue their FP procedure or because of COH failure due to mismanagement of injections or premature LH surge. Another 23 patients were excluded for important missing data (n=3) and AMH <0.5 ng/mL and >8 ng/mL (n=20). An additional 5 patients were excluded for non-invasive breast carcinoma (n=1), metastatic disease during Let-COH cycle (n=1), and available follow-up data under 2 years.

Figure 1: flowchart



AMH: anti- mullerian hormone, BC: breast cancer, LetCOH: letrozole associated controlled ovarian hyperstimulation, cCOH: conventional controlled ovarian hyperstimulation

3.2. Patient and oncological characteristics

Patient, BC characteristics and treatments are presented in **Table 1**. Although most of patients and tumors characteristics were similar between groups, including triple negative tumor rate, patients in LetCOH group had taken more hormonal contraceptives, presented with larger tumors at diagnosis (p=0.014), and higher positive HER-2/neu status (p=0.005) compared to the cCOH group. Adjuvant chemotherapy was administered to almost all patients (54/56) in the cCOH group while patients in the LetCOH group received adjuvant or neo-adjuvant treatment in similar proportions. All HER2+ patients received trastuzumab therapy and hormonal receptor positive patients in both groups received adjuvant endocrine therapy (Table 1).

Table 1. Patient and oncological characteristics

	LetCOH n= 41	**cCOH n=56**	***P*-values**
Age, years	32 (29; 34)	31 (28.25; 33)	0.123
BMI, kg/m^2	22.90 (20.25; 26.30)	22.15 (20; 24.75)	0.324
Prior use of contraception			
Yes, n (%)	29 (70.7)	13 (23.2)	0.006
No, n (%)	11 (26.8)	28 (50)	
Unknown, n (%)	1 (2.4)	15 (26.8)	
Histologic type			
Ductal carcinoma, n (%)	40 (97.6)	51 (91.1)	0.878
Lobular carcinoma, n (%)	-	1 (1.8)	
Other types (mucoid, medullary, etc.), n (%)	1 (2.4)	3 (5.4)	
Unknown, n (%)	-	1 (1.8)	
Tumor size*			
T1, n (%)	13 (31.7)	30 (53.6)	
T2, n (%)	22 (53.7)	24 (42.9)	0.014
T3, n (%)	6 (14.6)	1 (1.8)	
Unknown, n (%)	-	1 (1.8)	
Nodal status			
Negative, n (%)	24 (58.5)	35 (62.5)	0.812
Positive, n (%)	17 (41.5)	20 (35.7)	
Unknown, n (%)	-	1 (1.8)	
Grade			
I, n (%)	2 (4.9)	3 (5.4)	0.722
II, n (%)	16 (39)	24 (42.9)	
III, n (%)	23 (56.1)	27 (48.2)	
Unknown, n (%)	-	2 (3.6)	
Hormone receptor status			
Negative (ER and PR negative), n (%)	13 (31.7)	26 (46.4)	0.144
Positive (ER and/or PR positive), n (%)	28 (68.3)	30 (53.6)	
HER2 status			
Negative, n (%)	23 (56.1)	46 (82.1)	
Positive, n (%)	18 (43.9)	9 (16.1)	0.005
Unknown, n (%)	-	1 (1.8)	
Germline mutations			
No mutation, n (%)	26 (63.4)	40 (71.4)	0.055
BRCA1 and 2 mutations, n (%)	14 (34.2)	9 (16.1)	
Other (P53, PALB2), n (%)	1 (2.4)	2 (3.6)	
Unknown, n (%)	-	5 (8.9)	
Type of surgery			
No surgery, n (%)	-	1 (1.8)	0.815
Conservative, n (%)	21 (51.2)	25 (44.6)	
Mastectomy, n (%)	20 (48.8)	30 (53.6)	

Chemotherapy			
No chemotherapy, n (%)	-	2 (3.6)	< 0.001
Neo-adjuvant, n (%)	21 (51.2)	-	
Adjuvant, n (%)	20 (48.8)	54 (96.4)	
Other adjuvant therapies**			
Trastuzumab, n (%)	18 (43.9)	9 (16.1)	
Endocrine therapy, n (%)	28 (68.3)	29 (51.7)	
Unknown, n (%)	-	1 (1.8)	
Follow-up, median months (quartiles)	55.6 (43;71)	50.7 (37;72)	0.325
Relapse			
No relapse, n (%)	34 (82.9)	51 (91)	0.190
Local, n (%)	3 (7.3)	3 (5.4)	
Distant, n (%)	4 (9.8)	1 (1.8)	
Unknown, n (%)	0	1 (1.8)	
Death, n	1	1	

*Clinical and pathological stagings were performed for neo adjuvant and adjuvant settings respectively.

** adjuvant therapies include trastuzumab alone or in combination with endocrine therapy. All continuous variable results are presented in median and 25th and 75th percentiles. BM: body mass index; LetCOH: letrozole-associated controlled ovarian hyperstimulation; cCOH: conventional COH

3.3. Controlled ovarian hyperstimulation outcomes

One hundred and eight cycles were performed in the 97 included BC patients (table 2). Forty-six Let-COH cycles were performed in the LetCOH group (n=41), and 61cycles in the cCOH group (n=56). Although AMH levels and the number of stimulation days were comparable between groups, the total dose of gonadotropin used was significantly lower in the LetCOH group compared to the cCOH group. As expected, the peak median level of E_2 was significantly lower in the Let-COH group compared to the cCOH group (343 pg/mL (178; 472) versus 1009 pg/mL (668; 2072), respectively; p<0.001). Ovulation trigger was achieved using mainly GnRHa (0.2 mg) in the Let-COH group, and dual trigger (0.2 mg GnRHa+ 1,500 IU hCG) in the cCOH group. Numbers of total and mature oocytes collected were comparable between groups. However, oocyte maturation rates were significantly higher in the LetCOH group compared to the cCOH group (83 % vs 65% respectively, p<0.001).

Table 2. Controlled ovarian hyperstimulation cycle characteristics

	LetCOH n=47	**cCOH n=61**	***P*-values**
Baseline AMH, ng/mL	1.93 (1.00; 4.15)	2.46 (1.28; 3.95)	0.369
Timing stimulation			
Standard, n (%)	22 (46.8)	39 (63.9)	0.113
Random-start, n (%)	25 (53.2)	22 (36.1)	
Stimulation days, n	10 (8; 12)	10 (9; 12)	0.062
Total gonadotropins dosage, IU	2250 (1700; 2975)	2700 (2150; 3650)	0.008
E2 at trigger, pg/mL	343 (178; 472)	1009 (668; 2072)	< 0.001
Ovulation trigger			
hCG+/- GnRHa, n (%)	17 (36.2)	41 (67.2)	0.002
GnRHa, n (%)	30 (63.8)	20 (32.8)	
Total number of oocytes	8.00 (5.00; 13.00)	9 (4.5; 15)	0.688
Number of mature oocytes	6 (4; 10)	6 (2.50; 9.50)	0.281
Oocyte maturation rate, %	0.83 (0.67; 1)	0.65 (0.50; 0.80)	< 0.001

AMH, anti-Müllerian hormone; E2, estradiol; LetCOH, letrozole-associated controlled ovarian hyperstimulation; cCOH, conventional COH; hCG, human chorionic gonadotropin; GnRHa, gonadotropin releasing hormone antagonist. All continuous variable results are presented in median and 25th and 75th percentiles.

3.4. Follow-up and relapse rate

Follow-up was at least 48 months after diagnosis (median 55.6 and 50.7 months in the LetCOH and cCOH groups, respectively), with no significant difference between groups. During this period, disease recurrence occurred in 7 (17%; 3 local/contralateral and 4 metastatic) and 4 (7.2%; 3 local/contralateral and one metastatic) patients in the LetCOH and cCOH groups respectively. One patient died in each group.

4. DISCUSSION

FP counseling is a priority for young women diagnosed with cancer[20] , as it offers information about treatment gonadotoxicity and possible options to achieve pregnancy at remission. Oocyte and/or embryo cryopreservation remains the standard procedure for FP but COH in breast cancer patients is still controversial. Although data regarding the efficacy of LetCOH are reassuring, a recent meta-analysis showed that studies comparing COH protocols in breast cancer populations are scarce and safety data remain largely incomplete [21]. In this meta-analysis including 11 studies, only 3 compared the efficacy of LetCOH versus the standard protocol in BC cohorts. In our study, we investigated efficiency and safety parameters in two large breast cancer cohorts recruited at two reference oncofertility centers. We showed that the number of total and mature oocytes was similar in both cohorts despite significantly lower gonadotropin dose used in the LetCOH group compared to the cCOH group. However, oocyte maturation rates were significantly higher in the LetCOH compared to cCOH group. These results suggest that LetCOH was as effective as the standard COH protocol and did not require higher gonadotropin dose to obtain adequate ovarian response. In contrast with other studies [15, 22], maturation rates were higher in the LetCOH compared to cCOH group. In a recent study including BC patients, the authors observed a significantly lower oocyte maturation rates in patients who received LetCOH (64.9+/- 22.8%) compared to cCOH (77.4+/- 19.3%) group after using GnRHa for triggering in both groups [15]. However, ovulation trigger was carried out in follicles > 15 mm, although less favorable outcomes were previously reported using the LetCOH protocol when performing triggering before follicular size has reached 19-20mm [13]. Another recent and large retrospective study in BC patients showed slightly but significantly lower maturation rate (71% vs 79%) following LetCOH (n=224) and cCOH (n=156) cycles, respectively. Follicular size at trigger in each group was not described in this study but the total dose of gonadotrophins was higher for a lower number of stimulation days in the LetCOH compared to cCOH group [18]. Nevertheless, a similar number of mature oocytes was collected in both groups. Follicle size at trigger has been shown to impact maturation rate, with higher maturation rates and fertilization in larger follicles [23, 24]. In our study, follicular size of leading follicles was at least 19-20 mm in the Let-COH and 17mm in the cCOH group, consistent with previous recommendations [13], but additional stimulation days or gonadotropins doses were not required in the LetCOH group to achieve this size. In contrast with the Swedish policy that recommended addition of letrozole in case of hormonal receptor presence, all patients with BC received the LetCOH protocol in our Belgian center, irrespective of oncological characteristics, while letrozole was not allowed in this indication in

the French centers. Therefore, cCOH was offered to patients receiving chemotherapy, almost exclusively in an adjuvant setting. Our study and the recent Swedish study both highlight the difference in attitudes between countries regarding COH in breast cancer patients and reveal the urgent need for more data to provide strong recommendations on letrozole use during ovarian stimulation in this population.

Recurrence risk due to hormonal environment represents the main argument for letrozole administration during ovarian stimulation. As previously demonstrated [10, 13, 15, 18, 25], we confirmed that letrozole allows maintenance of E2 levels within physiological range in the large majority of patients. In a recent study published by our group [26], we observed reassuring evidence with no increase in circulating tumoral DNA in 14 out of 15 BC patients following LetCOH for FP. Nevertheless, strong evidence regarding LetCOH safety as well as its usefulness are still lacking. We previously observed very high progesterone levels during luteal phase following LetCOH triggered with hCG [25]. Consequently, we suggested using GnRH agonist for ovulation trigger in all patients, regardless of ovarian hyperstimulation syndrome risk, and avoiding administration of letrozole during luteal phase. Indeed, progesterone may act through paracrine and/or autocrine signaling pathways, and activate mammary stem cell proliferation, factors which are involved in breast tumorigenesis [27, 28].

Half of the LetCOH cohort in our study was scheduled for neoadjuvant chemotherapy (within 2-3 weeks after diagnosis) while adjuvant treatment was planned in all cCOH patients (within 6 weeks in both groups after diagnosis). After a similar follow-up period, a higher number of patients relapsed in the LetCOH group compared to the cCOH group. However, LetCOH patients also had larger tumor size and higher rates of HER-2/ positive disease that are known to be associated with poorer prognosis. In a study published by Marcklund et al., survival was comparable between 45 LetCOH patients and 132 cCOH patients at 5-year follow-up. Relapse rate was however not reported in the study, nor were the oncological characteristics, limiting the interpretation of their safety analysis.

Although this is one of the only studies to analyze the safety of different COH protocols in BC patients, our study has several limitations. It is an observational retrospective study from two different centers (Belgium and France) with different attitudes regarding COH in BC, as it was proposed only in the adjuvant setting in France. Moreover, patients in the study group presented with larger tumors and higher numbers had HER2+ positive status, which could have contributed to the difference in relapse rates, but grade and nodal involvement were comparable between groups. Additionally, patient cohorts were limited, even if larger compared to many others [21]. Finally, although patients had a follow-up of more than 4 years, a follow-up of 5-

10 years would be of high importance to evaluate disease-free-survival, especially considering relapse in hormone dependent tumors may occur after a longer follow-up period [29].

Our study results confirmed that letrozole does not impact efficacy of the procedure compared to standard protocol, and even showed a benefit in terms of maturation rates in our cohort, likely related to follicular size at trigger. More importantly, follow-up was reassuring in the cCOH group, irrespective of tumor's hormonal sensitivity. Although this should be confirmed by large and long-term prospective trials, our results suggest that COH without letrozole may be a viable option in selected breast cancer patients according to their oncological characteristics and chemotherapy setting.

5. CONCLUSION

Our results suggest triggering ovulation at an adapted follicular size leads to higher oocyte maturation rate in the LetCOH protocol compared with conventional protocol for fertility preservation purposes. Consequently, letrozole seems to be beneficial regarding the number of mature oocytes retrieved while minimizing the risk of supraphysiologic estrogen exposure. Nevertheless, more patients in the LetCOH group, who had poorer prognosis, relapsed. A prospective trial is warranted to include BC characteristics at diagnosis, primary therapy setting, and long-term follow-up of patients undergoing COH with and without letrozole to assess its usefulness as well as safety of cCOH.

Authors' contribution

Oranite Goldrat methodology, validation, formal analysis, data curation; writing-review & editing **; Manon De Cooman** formal analysis, investigation, data curation, writing original draft **; Audrey Mailliez** conceptualization, methodology, validation, resources ; **Anne Delbaere** resources, writing review and editing ; **Emmanuelle D'Orazio** investigation, data curation; **Isabelle Demeestere** conceptualization, methodology, resources, writing-review & editing, supervision ; **Christine Decanter** conceptualization, methodology, resources

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Declaration of interest statement

Isabelle Demeestere acted as consultant/advisor for Roche, has received speaker's honoraria from Novartis and support for congress participation from Ferring and Theramex, outside of the present study. All remaining authors have declared no conflicts of interest.

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Authors' contribution

Oranite Goldrat methodology, validation, formal analysis, data curation; writing-review & editing **; Manon De Cooman** formal analysis, investigation, data curation, writing original draft **; Audrey Mailliez** conceptualization, methodology, validation, resources ; **Anne Delbaere** resources, writing review and editing ; **Emmanuelle D'Orazio** investigation, data curation; **Isabelle Demeestere** conceptualization, methodology, resources, writing-review & editing, supervision ; **Christine Decanter** conceptualization, methodology, resources