A specific controlled ovarian stimulation (COS) protocol for fertility preservation in women with breast cancer undergoing neoadjuvant chemotherapy

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Aim of the study: The authors present a novel and specific controlled ovarian stimulation protocol for fertility preservation in women with estrogen-positive receptor breast cancer undergoing neoadjuvant chemotherapy. The protocol foresees random start ovarian stimulation and the use of letrozole associated to tamoxifen.

Material and methods: Forty breast cancer patients were included in the study. COS was performed either with recombinant FSH or hMG. Concomitantly with COS, letrozole in a dose of 5 mg and tamoxifen in a dose of 20 mg were given orally on a daily basis. The trigger was performed with 0.2 mg of triptorelin, in the presence of follicles ≥ 19 mm. Oocyte retrieval was scheduled 35–36 hours after triptorelin injection. Our main outcome measures were the number of oocytes collected and number of oocytes vitrified, the length of ovarian stimulation, total dose of gonadotropins administered, and levels of estradiol on the day of the trigger.

Results: The mean age of patients was 30.43 ±4.25 years. Nineteen women commenced COS in the luteal phase, eleven in the early follicular phase and ten in the late follicular phase. The mean number of collected oocytes was 11.78 ±9.12 and the mean number of vitrified oocytes was 9.72 ±7.36. The mean duration of COS was 10.03 ±1.33 days. The mean estradiol concentrations on the triggering day was 623.10 ±441.27, and the mean dose of gonadotropins administered was 2540 ±713.10.

Conclusions: The authors suggest that the protocol is efficient and may be a safe option for oocyte vitrification in these patients.

Key words: breast cancer, fertility preservation, ovarian stimulation, letrozole, tamoxifen.

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Introduction

Breast cancer is the most common malignancy in adult women, and in the United States, 5%–7% of cases of invasive breast cancer (~11,000/year) occur in women who are under age 40 at diagnosis [1]. Given the advent of early breast cancer diagnosis and effective cancer treatments, survival rates following breast cancer are increasing, with a 5-year survival rate over 80% [2, 3]. This fact justifies the concern about chemotherapy-related gonadal toxicity in women with reproductive wishes. Chemotherapy treatment may have deleterious effects on the ovarian reserve, by affecting the resting pool of primordial follicles or the growing follicle population [3] Given that, it is important to consider early referral of breast cancer young patients to fertility specialists, in order to discuss fertility preservation procedures [4–6]. Among these, medical ovarian protection, ovarian tissue cryopreservation, oocytes or embryos cryopreservation are the most common fertility preservation strategies [7–9]. As occurs in conventional *in vitro* fertilization (IVF) techniques, to obtain oocytes or embryos for cryopreservation, controlled ovarian stimulation (COS) is the first step to be considered. Performing COS in breast cancer patients prior to surgery may promote concerns about the risks of delaying chemotherapy treatment and exposing a breast cancer patient to high estradiol levels consequent to multiple follicle development, mainly in hormone-receptor positive tumors [10]. To mitigate the effect of high estradiol levels, the use of aromatase inhibitors has been demonstrated to be safe and efficient [11, 12]. In this series of cases, we performed COS in estrogen-receptor positive breast cancer patients undergoing neoadjuvant chemotherapy, and we proposed the concomitant administration of letrozole and tamoxifen with gonadotropins, which may be a safe approach in such type of cancers. It is possible that their different mechanisms of action would be complementary, with the aromatase inhibitor decreasing the estrogen level and thus allowing tamoxifen to function more effectively as a competitive inhibitor with estradiol. In order to mitigate thromboembolic event, which is a serious complication of cancer [13] and which can be exacerbated by the use of tamoxifen [14], we proposed the prophylactic use of a low molecular weight heparin, enoxaparin, administered on a daily basis, throughout ovarian stimulation.

Material and methods

This is an observational cross sectional study of breast cancer patients undergoing COS for fertility preservation in a tertiary public hospital. Between

November 2014 and December 2016, 40 women with hormone-receptor positive breast cancer and indication of neoadjuvant chemotherapy underwent random start COS for fertility preservation, in a public IVF center in Sao Paulo, Brazil. Given that we propose a specific COS protocol for women who have a breast cancer not removed, so that ovarian stimulation is performed in the presence of an estrogen receptor positive tumor, only patients receiving neoadjuvant chemotherapy were included. The patients were divided in three groups, according to the phase of the menstrual cycle:

Initial Follicular Phase Group (IFP, n = 11): the COS was initiated in the beginning of the follicular phase, in which a dominant follicle (>10 mm) was not detected.

Late Follicular Phase Group (LFP, n = 19): the COS was initiated in the late follicular phase, in the presence of a dominant follicle > 10 mm;

Luteal Phase Group (LP, n = 10): the COS was initiated in the luteal phase, with ecographic evidence of follicular rupture and (or) an endometrium of secretory pattern.

COS was performed either with recombinant FSH or hMG, in a daily dose of 150-300 IU. The gonadotropin starting dose was chosen according to the antral follicle count: 150 IU daily with ≥ 15 antral follicles, 225 IU daily with < 15 ≥ 10 antral follicles and 300 IU daily with < 10 antral follicles. When COS was initiated in the late follicular phase, with the presence of a follicle > 10 mm, a GnRH antagonist was introduced concomitantly with the gonadotropin; otherwise, the antagonist was introduced in the presence of a follicle ≥ 13 mm. Concomitantly with COS, letrozole in a dose of 5 mg and tamoxifen in a dose of 20 mg were given orally on a daily basis. Enoxaparin was given daily in a dose of 40 mg subcutaneously, as a prophylactic measure. The trigger was performed with 0.2 mg of triptorelin, in the presence of follicles ≥ 19 mm. Oocyte retrieval was scheduled 35–36 hours after triptorelin injection. After the oocyte retrieval, the patients discontinued any medication. Our main outcome measures were the number of oocytes collected and number of oocytes vitrified, the length of ovarian stimulation, total dose of gonadotropins administered, and levels of estradiol on the day of the trigger. The secondary outcome measure was to determine whether there are differences in the outcomes according to the phase of the cycle in which COS was initiated.

Criteria of inclusion and exclusion

Patients diagnosed with hormone-positive breast cancer and indication of neoadjuvant chemotherapy were included in this investigation. Patients with advanced and metastatic disease and with age > 40 years were not included in the fertility preservation program.

Ethics

This research was approved by the Committee of Ethics in Research of the Women's Health Reference Center, in Sao Paulo, on 29 October 2014, under the number 848.880. All patients signed an informed consent for undergoing COS with the specific protocol.

Statistical analysis

A hypothesis test was applied to evaluate the statistical differences between the groups. The Kruskal-Wallis test was used to compare the results between groups: IFP versus LFP, IFP vs LF and LFP vs LF. The level of statistical significance was considered to be a p-value of less than 0.05.

Results

This study included 40 patients with hormone receptor positive breast cancer undergoing neoadjuvant chemotherapy. Among the 40 patients, 28 were classified as immunohistochemical subtype luminal B, and twelve as luminal HER2+. No patient's tumor was more advanced than stage IIIA. Neoadjuvant chemotherapy was performed with the aim of downstaging the tumor, allowing for conversion from mastectomy to conservative surgery. The chemotherapy regimen employed in patients undergoing neoadjuvant chemotherapy was cyclophosphamide and doxorubicin (four cycles) and taxol (four cycles). Patients with luminal HER2 tumors received adjuvant trastuzumab therapy. The mean age of patients was 30.43 ±4.25 years (range 21–39). The average duration of COS was 10.03 ±1.33 days (range, 8–13). In all 40 patients it was collected 459 oocytes. Of these, 399 (86.92%) were in metaphase II and were vitrified. The remainders were in metaphase I or germinal vesicle stage, and were discarded. The mean number of collected oocytes was 11.78 ±9.12 (range 1–38) and the mean number of vitrified oocytes was 9.72 ±7.36 (range, 0–34). The mean total dose of FSH administered was 2540.00 ±713.10 IU, and the mean estradiol concentrations on the triggering day were 623.10 ±441.27 pg/ml. Nineteen women commenced COS in the luteal phase (LP), eleven in the initial follicular phase (IFP) and ten in the late follicular phase (LFP). When comparing the outcomes according to the phase of the cycle in which COS was commenced, there were no significant differences in the number of oocytes collected and vitrified, ovarian stimulation length, total dose of gonadotropin administered and estradiol levels on the trigger day phase. The results are shown in Table 1. In Fig. 1, the box plots graphs show the overall mean values, standard deviation and outliers of the mean values.

Discussion

Currently, oocyte vitrification may be considered the gold standard in female onco- fertility preservation, although embryo cryopreservation is still performed for this purpose [15]. Regarding oocyte cryopreservation, it was shown that women treated of breast cancer who have their oocytes vitrified before chemotherapy have good IVF performance and good obstetric outcomes, when compared to age-matched patients [16]. To perform embryos or oocytes cryopreservation, COS is the first step to be considered. Regarding this issue, some concerns may emerge, such as delaying chemotherapy treatment and exposing a breast cancer patient to high estradiol levels, particularly when the tumor is present, as occurs in the cases of neoadjuvant chemotherapy. The stimulation required for oocyte retrieval could delay oncologic treatment, given that

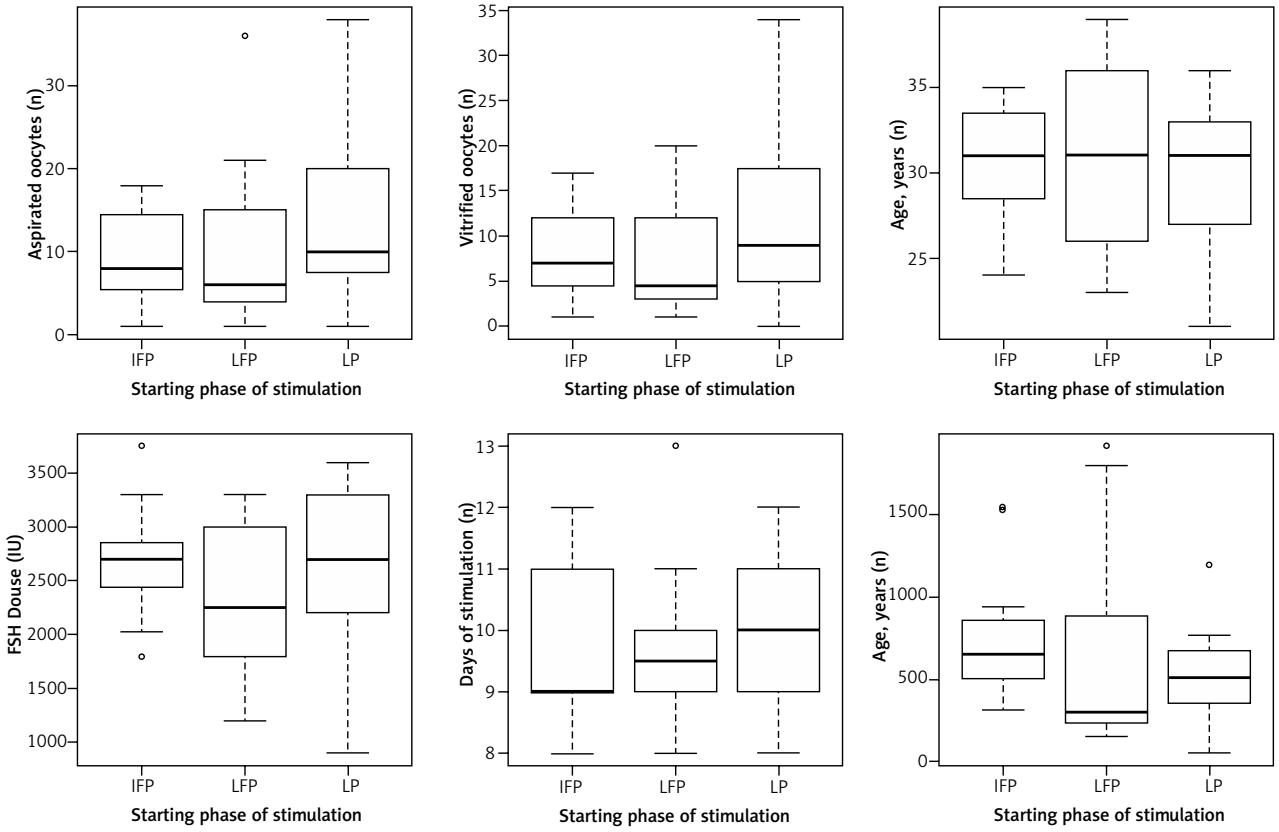
Table 1. Outcomes in 40 breast cancer patients undergoing COS for fertility preservation prior to neoadjuvant chemotherapy

Parameter	All patients (*n* = 40)	IFP (*n* = 11)	LFP (*n* = 10)	LP (*n* = 19)	*P*-value
Age (years)	30.43 ±4.25	30.73 ±3.52	31 ±5.25	29.95 ±4.24	0.697
Collected oocytes	11.78 ±9.12	9.18±6.0	10.90±10.77	13.74±9.67	0.173
Vitrified Oocytes	9.72 ±7.36	8.27 ±5.37	7.90 ±6.44	11.53 ±8.61	0.331
Days of stimulation	10.03 ±0.33	10.00 ±1.41	9.70 ±0.49	10.21 ±1.23	0.653
FSH/hMG total dose (IU)	2,540 ±713.1	2,677 ±544.3	2,260 ±706.6	2608 ±792.2	0.983
Estradiol Levels (pg/ml)	623.1 ±441.2	783.0 ±411.7	661.7 ±666.7	510.2 ±274.8	0.053

Data are expressed as mean ± SD.

IFP – initial follicular phase; LFP – late follicular phase; LP – luteal phase

The limit of significance is a p value ≤ 0.05



IFP – initial follicular phase; LFP – late follicular phase; LP – luteal phase

Fig. 1. Box plot graph showing the results of random-start ovarian stimulation. The graph illustrates the median (inner black line), the upper and lower quartiles (the box) and outliers

the conventional COS, when initiated in the beginning of the follicular phase may require up to 6 weeks to be concluded. Random-start ovarian stimulation, which means initiating COS immediately, regardless of the patient's menstrual-cycle phase, has become a well-established approach in fertility preservation strategies, allowing oocyte retrieval in no more than two weeks, in the majority of the cases [17, 18]. Currently, random-start ovarian stimulation is routinely and successfully employed for emergency IVF [19–21] and the outcome of ovarian stimulation seems to be similar after stimulation initiation during any phase of the menstrual cycles [22]. Recently, it was reported that the formation of euploid blastocyst does not depend on the phase of the cycle in which ovarian stimulation commences [23]. Another concern might be the high estradiol levels consequent to ovarian stimulation, particularly in hormone-positive breast cancer patients undergoing neoadjuvant chemotherapy. In order to reduce estradiol concentrations, ovarian stimulation with aromatase inhibitors has been proved to be an efficient procedure [11]. Recent

publications also confirm that the adjuvant therapy with letrozole, throughout the length of the COS, is a safe and efficient approach [24–26]. The most employed aromatase inhibitor in ovarian stimulation protocols is letrozole, which has been proved to be more efficient than anastrozole for this purpose [27]. Another option to decrease estradiol levels could be the protocol performing profound LH suppression by high and sustained GnRH antagonist dosis, which maintains estradiol levels around the physiological range [28]. This approach, however, has the inconvenient of the high cost due to the elevated daily dose of the GnRH antagonist. When the indication is neodjuvant chemotherapy, and the cancer is hormone-receptor positive, the concerns about estradiol levels augment, because of the presence of the tumor during ovarian stimulation. In this study, we present a specific COS for patients undergoing neoadjuvant chemotherapy. Besides the use of letrozole, we also propose the administration of tamoxifen, which is a selective estrogen receptor modulator with antagonist effect on the breast. The use of letrozole together with tamoxifen may protect the patient by two different mechanisms of action: diminishing estradiol levels with the aromatase inhibitor and by competition in the estradiol receptors by tamoxifen. As well as letrozole, it was already demonstrated that tamoxifen can be safely employed to perform ovarian stimulation in breast cancer patients [29]. Meirow et al. [30] stated that co-administration of tamoxifen for fertility preservation does not interfere with ovarian stimulation outcomes and should be considered safe. The risk of thromboembolic complications can be minimized by the prophylactic use of a low molecular weight heparin, enoxaparin, in a daily basis. Being so, the random start COS prevents the delay to start chemotherapy, and the concomitant use of letrozole and tamoxifen may help to make the procedure safer. It is known that there is an association between tamoxifen therapy and thromboembolic events [31]. Furthermore, considering the possibility of venous thromboembolism as a paraneoplasic syndrome, we propose the prophylactic administration of enoxaparin in order to prevent this complication. The use of GnRH agonist to trigger final oocyte maturation avoids the occurrence of hyperstimulation ovarian syndrome, which is an important complication of ovarian stimulation, making the procedure safer [32]. There is no place for hCG trigger in COS for cryopreservation of oocytes or embryos. Our results showed an adequate number of oocytes retrieved and cryopreserved, with relative low concentrations of estradiol and without occurrence of any complications. The mean estradiol concentrations observed with this protocol (623.10 ±44.27 pg/ml) was considerably lower than in conventional COS for IVF, considering that serum estradiol levels during COS are increased by 10-fold compared with those of natural cycles [33]. Regarding the outcomes evaluated, it was not observed any statistically significant difference among the three groups studied (IFP, LFP and LP). In performing COS with this specific protocol, we observed a high rate of oocyte maturity (86.92%) even considering that in fertility preservation procedures we generally aspirate all follicles, including those very small which would be ignored in a conventional IVF treatment. It was also observed that some patients, although young, have a poor response to ovarian stimulation. In a 34-year-old patient, we retrieved only one immature oocyte, and vitrification was not possible. It was previously registered that the number of oocytes retrieved in breast cancer patients with BRCA1 mutations is significantly lower compared to BRCA negative and untested patients [34]. On the other hand, it was recently described that women with gynecological cancer have less number of retrieved mature oocytes compared with haematological and breast cancer patients [35]. Interestingly, it was also recently reported that both healthy and cancer-affected BRCA mutation women have normal response to COS in IVF cycles [36]. However, the outcomes observed in this investigation were comparable to conventional IVF results, with significant lower estradiol levels. Obviously, women affected with BRCA mutations may have concerns about transmission of the mutation to offspring. In these cases, preimplantation genetic diagnosis to avoid the birth of affected offspring is ethically acceptable [37]. We postulate that the administration of tamoxifen, concomitantly with letrozole, could promote an additional safeness to the procedure, by competing with estrogen receptors. The results of this investigation suggest that a specific protocol of ovarian stimulation for fertility preservation in this group of patients may be an effective procedure, and no complications were observed during the treatment. Pharmacological ovarian stimulation before the initiation of chemotherapy seems to be safe, and one prospective study did not observe a negative impact on patient's survival after this procedure [25]. However, there remains a need for long-term follow up to better determine the safety of COS in patients with hormone-receptor positive breast cancer and indication of neoadjuvant chemotherapy, and we shall stress that our findings should be confirmed with more rigorous reporting and data monitoring in prospective trials of larger populations.

The authors declare no conflict of interest.

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