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Specific protocols of controlled ovarian stimulation for oocyte cryopreservation in breast cancer patients

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ABSTRACT

Background Fertility preservation is an important concern in breast cancer patients. In the present investigation, we set out to create a specific protocol of controlled ovarian stimulation (cos) for oocyte cryopreservation in breast cancer patients.

Methods From November 2014 to December 2016, 109 patients were studied. The patients were assigned to a specific random-start ovarian stimulation protocol for oocyte cryopreservation. The endpoints were the numbers of oocytes retrieved and of mature oocytes cryopreserved, the total number of days of ovarian stimulation, the total dose of gonadotropin administered, and the estradiol level on the day of the trigger.

Results Mean age in this cohort was 31.27 ± 4.23 years. The average duration of cos was 10.0 ± 1.39 days. The mean number of oocytes collected was 11.62 ± 7.96 and the mean number of vitrified oocytes was 9.60 ± 6.87. The mean estradiol concentration on triggering day was 706.30 ± 450.48 pg/mL, and the mean dose of gonadotropins administered was 2610.00 ± 716.51 IU. When comparing outcomes by phase of the cycle in which cos was commenced, we observed no significant differences in the numbers of oocytes collected and vitrified, the length of ovarian stimulation, and the estradiol level on trigger day. The total dose of follicle-stimulating hormone and human menopausal gonadotropin administered was statistically greater in the group starting cos in the luteal phase than in the group starting in the late follicular phase.

Conclusions Our results suggest that using a specific protocol with random-start ovarian stimulation for oocyte cryopreservation in breast cancer patients is effective and could be offered to young women undergoing oncologic treatment.

Key Words Fertility preservation, breast cancer, ovarian stimulation, oocyte cryopreservation

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INTRODUCTION

Given improved cure rates for cancer in young patients, greater attention has been paid to fertility preservation procedures. Hematologic cancers and other malignancies that affect young people can have 90%–95% 5-year survival rates[1]. Breast cancer is the most common malignancy in adult women, and in the United States, 5%–7% of patients with invasive breast cancer (approximately 11,000 annually) are less than 40 years of age at diagnosis[2]. With the advent of earlier breast cancer diagnosis and effective treatments, survival rates after breast cancer are increasing, with a 5-year survival rate exceeding 80%[3–5]. That survival rate justifies concerns about chemotherapy-related gonadal toxicity in women with reproductive goals.

Chemotherapy treatment can have deleterious effects on the ovarian reserve, affecting the resting pool of primordial follicles or the growing follicle population[4,6]. Moreover, about two thirds of women less than 40 years of age have a hormone receptor–positive cancer and are candidates to receive 10 years of treatment with tamoxifen[7]. To preserve quality of life for those women, fertility preservation

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procedures should be offered. A consideration of early referral to fertility specialists for a discussion of fertility preservation procedures is therefore important[8,9]. Among those procedures, medical ovarian protection, ovarian tissue cryopreservation, and oocyte or embryo cryopreservation are the most common strategies[7,10]. Embryo and oocyte cryopreservation are the most preferred methods of fertility preservation, although ovarian tissue cryopreservation has been demonstrated to be an useful option[11].

For embryo or oocyte cryopreservation, controlled ovarian stimulation (COS) is the first step to be considered. In patients with breast cancer, concerns about COS can emerge, such as delay in starting chemotherapy and exposure to high levels of estradiol consequent to multiple follicle development. Oocyte retrieval requires COS, which might delay oncologic treatment given that conventional COS, initiated at the beginning of the follicular phase, can require up to 6 weeks to conclude.

Random-start ovarian stimulation, which means initiating COS immediately and independently of the menstrual cycle, has become a well-established approach in fertility preservation strategies, allowing oocyte retrieval in no more than 2 weeks in most cases. Moreover, the outcome of random-start ovarian stimulation seems to be similar no matter the phase of the menstrual cycle at the initiation of stimulation[12,13].

Another concern might be the estradiol level consequent to ovarian stimulation. To keep estradiol concentrations low, adjuvant therapy with an aromatase inhibitor, letrozole, is recommended throughout COS. Ovarian stimulation combined with aromatase inhibitors has proved to be an efficient procedure[14–16]. In neoadjuvant chemotherapy for hormone receptor–positive cancers, we propose the administration of tamoxifen in addition to letrozole during ovarian stimulation. To prevent thromboembolic complications, patients are given a prophylactic dose of low molecular weight heparin. With the aim of preventing ovarian hyperstimulation syndrome, which is an important complication of ovarian stimulation, avoidance of human menopausal gonadotropin for triggering final follicular maturation is strongly recommended; a gonadotropin-releasing hormone (GNRH) agonist is therefore used for that purpose[17,18].

The foregoing approaches to ovarian stimulation for breast cancer patients could make the procedure more efficient and safer when the aim is cryopreservation of oocytes. In the present study, we report the outcome of a specific protocol of COS for breast cancer patients, and we assess the outcomes of a random-start approach to initiation of stimulation, no matter the phase of the menstrual cycle.

METHODS

The breast cancer patients reported here were undergoing COS for oocyte vitrification in a tertiary public hospital. From November 2014 to December 2016, we studied 109 patients who underwent random-start COS to retrieve oocytes for fertility preservation. We divided the cycles of COS into 3 groups, according to the phase of the menstrual cycle at COS initiation:

- Initial follicular phase group (IFP, $n = 42$)
  In these patients, COS was initiated at the beginning of the follicular phase, in which no dominant follicle greater than 10 mm was observed.
- Late follicular phase group (LFP, $n = 20$)
  In these patients, COS was initiated in the late follicular phase, in the presence of a dominant follicle greater than 10 mm.
- Luteal phase group (LP, $n = 47$)
  In these patients, COS was initiated in the LP, with either or both of ultrasound evidence of follicular rupture and an endometrium of secretory pattern.

Specifically, COS was performed using either recombinant follicle-stimulating hormone (FSH) or urinary human menopausal gonadotropin, in a daily dose of 150–300 IU. In the follicular phase, ovarian stimulation was performed using urinary human menopausal gonadotropin, and in the LP, the gonadotropin choice was recombinant FSH so as to avoid luteinizing hormone activity. The gonadotropin starting dose was chosen according to the antral follicle count: 150 IU daily with 15 or more antral follicles, 225 IU daily with fewer than 15 but 10 or more antral follicles, and 300 IU daily with fewer than 10 antral follicles. Letrozole was started concomitantly with the gonadotropins, at a dose of 5 mg once daily, independently of the immunohistochemistry of the tumour.

Pituitary suppression to prevent a premature luteinizing hormone surge was performed using 0.25 mg of a GNRH antagonist daily. When COS was initiated in the late follicular phase in the presence of a follicle larger than 10 mm, the GNRH antagonist was introduced concomitantly with the gonadotropin; otherwise, the antagonist was administered in the presence of a follicle 13 mm or larger in size. In cases of neoadjuvant chemotherapy, patients also daily received oral tamoxifen 20 mg and a prophylactic dose of low molecular weight heparin (enoxaparin 40 mg) administered subcutaneously to prevent thromboembolic complications.

During ovarian stimulation, ultrasonography imaging was performed every 48 hours. Final follicular maturation was achieved using 0.2 mg triptorelin in the presence of follicles 19 mm or larger in size, and oocyte retrieval was performed transvaginally under ultrasound guidance, 35–36 hours later.

The endpoints of the study were the number of oocytes retrieved and the number of mature oocytes cryopreserved, total number of days of ovarian stimulation, total dose of gonadotropin administered, and estradiol level on the day of the trigger. Outcomes were also analyzed according to the phase of menstrual cycle in which ovarian stimulation was initiated.

Inclusion Criteria

Patients were included if they had been diagnosed with breast cancer, with an indication for neoadjuvant or adjuvant chemotherapy; if they had plans for reproduction after cancer treatment; and if they were 40 years of age or younger.

Exclusion Criteria

Patients with advanced or metastatic disease and those more than 40 years of age were not included in the fertility preservation program.

Ethics Approval

This research was approved by the Committee of Ethics in Research of the Women's Health Reference Center, Sao Paulo, 29 October 2014, under the number 848.880.

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 compared with LFP, IFP compared with LP, and LFP compared with LP. The level of statistical significance was considered to be a p value less than 0.05.

RESULTS

Of the 109 breast cancer patients included in the study, 42 commenced COS in the LP; 20, in the IFP, and 47, in the LFP. Mean age of the patients was 31.27 ± 4.23 years. The average duration of COS was 10.0 ± 1.39 days. The mean number of collected oocytes was 11.62 ± 7.96, and the mean number of vitrified oocytes was 9.60 ± 6.87. The mean estradiol concentration on triggering day was 706.30 ± 450.48 pg/mL, and the mean dose of FSH administered was 2610.00 ± 716.51 IU (Table I). When comparing outcomes according to the phase of the menstrual cycle in which COS was initiated, we observed no significant differences in the number of oocytes collected and vitrified, in the ovarian stimulation duration, and in the estradiol level on the trigger day. A statistically significant increase in the total dose of FSH administered was observed in the group starting COS in the LP compared with the group starting in the LFP. In Figure 1, the box plots show overall mean values, standard deviations, and outliers of the mean values.

DISCUSSION

Modern treatments for breast cancer, including surgery, chemotherapy, and radiotherapy, have improved cure rates, and the decline in mortality is remarkable in women less than 50 years of age[10]. Nevertheless, gonadal toxicity consequent to cancer therapy can lead to impaired reproductive function in younger patients, such that procedures aiming to preserve reproductive potential are necessary. Quality of life is an important issue to be considered in cancer survivors, and compromised fertility is a concern, particularly for young women.

We can emphasize that reproductive concerns are not meaningless for young women diagnosed with breast cancer, and the demand for fertility preservation techniques by those patients has increased with improved cure rates. The techniques used for fertility preservation include reducing the effect of chemotherapy on the ovaries, cryopreservation of oocytes and embryos, and cryopreservation of ovarian tissue[10]. Given that protection of ovarian function with GnRH analogs is controversial and that cryopreservation of ovarian tissue is still an experimental procedure, cryopreservation of oocytes or embryos is the most important procedure indicated for fertility preservation[19].

Considering the availability of *in vitro* fertilization (IVF) as a technique to treat infertile couples, embryo cryopreservation has become a very efficient procedure, and studies suggest a "freeze all" policy, even in conventional cycles of IVF[20]. Because many young women with breast cancer do not have a male partner at the moment of treatment, because concerns could arise about the destiny of embryos should the disease progress, and because IVF outcomes with vitrified oocytes are comparable to those with fresh oocytes[21], oocyte cryopreservation has become the option of choice.

The method called vitrification, which implies ice-free cryopreservation, represents significant progress in oocyte cryopreservation, being associated with satisfactory rates of pregnancy[22]. Among established methods, oocyte cryopreservation has been postulated to be the preferred option in postpubertal women; in contrast, ovarian tissue cryopreservation is the only possibility for prepubertal girls[23]. Regardless, COS is mandatory for optimization of embryo or oocyte cryopreservation. The procedure normally require 2–6 weeks, depending on the current day of the patient's menstrual cycle, potentially leading to an undesirable delay in initiating chemotherapy.

Given that a receptive endometrium is not necessary in fertility preservation procedures, random-start stimulation is an interesting option. Our specific protocol proposes random-start ovarian stimulation, and our results demonstrate that outcomes are comparable to those

TABLE I Outcomes in 109 breast cancer patients undergoing controlled ovarian stimulation for fertility preservation

Variable	Patient group				*p* Value
	Overall	IFP	LFP	LP	
Patients (*n*)	109	41	21	47	
Mean aspirated oocytes (*n*)	11.62±7.96	10.95±7.23	10.38±8.0	12.77±8.54	NS
Mean vitrified oocytes (*n*)	9.60±6.87	8.927±6.75	7.952±5.38	10.94±7.43	NS
Age (years)	31.27 ± 4.23	31.37±3.48	29.76±4.94	31.85±4.41	NS
Mean FSH or hMG dose (IU)	2610±716.51	2577±670.19	2387±615.31	2738±780.9	0.04457[a]
Mean days of stimulation (*n*)	10±1.39	9.854±1.33	9.714±1.31	10.26±1.45	NS
Mean serum estradiol (pg/mL)	706.3±450.48	761±439.93	677.8±503.39	671.2±440.1	NS

[a] Statistically significant difference between the LFP and LP groups.

IFP = initial follicular phase; LFP = late follicular phase; LP = luteal phase; NS = statistically nonsignificant; FSH = follicle-stimulating hormone; hMG = human menopausal gonadotropin.

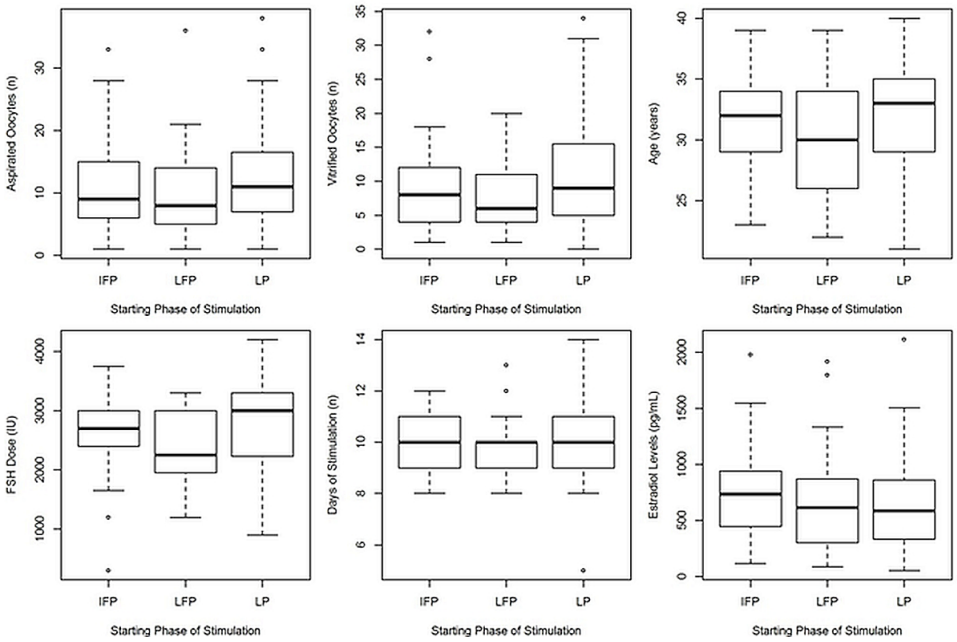

FIGURE 1 Box plots showing the results of random-start ovarian stimulation. The inner black line marks the median; the box delimits the upper and lower quartiles; and small circles mark outliers. IFP = initial follicular phase; LFP = late follicular phase; LP = luteal phase; FSH = follicle-stimulating hormone.

obtained during conventional IVF cycles, which accords with other observations[12,13,24]. Currently, random-start ovarian stimulation is routinely and successfully used for emergency IVF[25–27]. In the present study, results (number of days of stimulation, maturity rate of the oocytes collected) were similar for the initiation of stimulation in women at all three phases of the menstrual cycle, although a statistically significantly higher dose of gonadotropins was administered in the LP group than in the LFP group. Those results accord with the results obtained by von Wolff *et al.*[13], who reported a significant increase in the total dose of gonadotropin when COS was initiated in the LP. However, that finding was not clinically significant. For a few patients, we cryopreserved embryos, and fertilization rates were similar to those obtained with conventional COS (data not shown).

Our data confirm that the concomitant use of an aromatase inhibitor during ovarian stimulation is efficient for preventing the high levels of estradiol commonly observed in conventional COS. The aromatase inhibitor used most often in ovarian stimulation protocols is letrozole, which has proved to be more efficient than anastrozole for this purpose[28]. Ovarian stimulation combined with letrozole at a daily dose of 5 mg has proved to be efficient in this context[14]. On the other hand, some reports suggest that the concomitant use of letrozole with gonadotropins significantly lowers the number of oocytes available for cryopreservation[29]. In our investigation, the mean numbers of oocytes collected and cryopreserved (11.62 ± 7.96 and 9.60 ± 6.87 respectively) were considered adequate, given that recent data suggest that at least 8–10 metaphase II vitrified oocytes are necessary to achieve reasonable success[22]. Recent publications also corroborate that adjuvant therapy with letrozole throughout COS is a safe and efficient approach[15,16,30].

In our patient series, the mean estradiol concentration on triggering day was 760.30 ± 450.48 pg/mL (median: 655 pg/mL). Given that serum estradiol levels during COS are increased by a factor of 10 compared with levels during natural cycles[31], estradiol concentrations on triggering day are expected to reach 2500 pg/mL, which are much higher than the levels observed in our study.

If COS is performed in the presence of cancer, as occurs when the indication is neoadjuvant chemotherapy, we propose using tamoxifen together with letrozole. It is possible that the different mechanisms of action of those agents are complementary, with the aromatase inhibitor lowering the estrogen level, thus allowing tamoxifen to function more effectively as a competitive inhibitor with estradiol. Given that venous thromboembolism is a major concern in cancer patients[32], and considering the risk of administering tamoxifen together with letrozole, our protocol for women undergoing neoadjuvant chemotherapy proposes the addition of daily enoxaparin in a prophylactic dose. Our group recently published a case series in which COS was performed with letrozole, tamoxifen, and enoxaparin for 40 patients who received neoadjuvant chemotherapy (also reported in the present series), obtaining the same outcomes for days of stimulation and number and maturity of oocytes[33].

With respect to triggering of the final oocyte maturation, we believe that there is very little place for a human menopausal gonadotropin trigger in COS for fertility preservation. The use of GnRH agonist to trigger final oocyte maturation is strongly recommended to prevent

the occurrence of hyperstimulation ovarian syndrome[18], which would be an undesirable complication of ovarian stimulation in cancer patients. Moreover, it was recently reported that a GnRH agonist trigger increases the number of mature oocytes available for vitrification in cancer patients[34].

Our data confirm that random-start ovarian stimulation is effective and, compared with conventional stimulation, produces mature oocytes in the same proportion. That important fact reassures patients that an oocyte vitrification procedure will not delay their oncologic treatment. Moreover, the concomitant use of letrozole, an aromatase inhibitor, provides a safe option for women with breast cancer, resulting in estradiol levels that are lower than those in conventional stimulation[15]. The efficacy of cos is not altered by concomitant use of letrozole, and no evidence suggests an increased risk of malformations in newborns conceived while women are taking aromatase inhibitors[35]. Considering the emotional stress of the diagnosis and treatment of cancer at a young age, reassuring patients, family, and oncologists that the fertility preservation procedure is safe and effective is important for reducing the psychological burden to which the patient is exposed.

With respect to the efficacy and safety of cryopreserved oocytes to produce a normal pregnancy, reports in the medical literature confirm that the procedure is safe and efficient in women with cancer. Cancer patients who undergo oocyte cryopreservation before chemotherapy achieve good IVF performance and good perinatal outcomes[36]. However, on a cautionary note, it is important to realize that most of the reports assessing the safety and efficacy of oocyte cryopreservation involve healthy women undergoing conventional IVF or participants in ovodonation programs. Whether the results can be extrapolated to cancer patients remains to be better elucidated, even if the data obtained so far are reassuring[22,29].

CONCLUSIONS

The results observed in our patient series suggest that oocyte or embryo cryopreservation in a specific protocol based on random-start ovarian stimulation for breast cancer patients is effective and safe, and can be offered to young women undergoing oncologic treatment who have concerns related to their reproductive future.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

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