Progesterone levels in letrozole associated controlled ovarian stimulation for fertility preservation in breast cancer patients

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STUDY QUESTION: Are progesterone levels after letrozole-associated controlled ovarian stimulation (COS) for fertility preservation in breast cancer patients, lower than after standard in vitro fertilization (IVF) cycles?

SUMMARY ANSWER: During the luteal phase of letrozole-associated COS cycles (triggered with human chorionic gonadotrophin (hCG)) progesterone levels are similarly elevated to those obtained after standard COS without letrozole.

WHAT IS KNOWN ALREADY: Current fertility preservation strategies for breast cancer patients include association of COS with the aromatase inhibitor letrozole to harvest several mature oocytes while maintaining low estradiol levels. Data on progesterone levels are however lacking despite growing evidence of the role of progesterone in breast tumorigenesis.

STUDY DESIGN, SIZE, DURATION: This is a prospective observational study comparing estradiol and progesterone levels of 21 breast cancer patients undergoing letrozole-associated COS with 21 infertile patients undergoing standard COS for IVF and/or intra cytoplasmic sperm injection (ICSI).

PARTICIPANTS/MATERIALS, SETTING, METHODS: All patients underwent COS with a GnRH antagonist protocol. In the fertility preservation group, ovulation induction was started in the follicular or luteal phase depending on the chemotherapy schedule and in 10 cases a GnRH antagonist was administered during luteal phase to induce luteolysis. Final oocyte maturation was induced by hCG in all patients. Estradiol and progesterone levels were measured on the day of hCG, at oocyte retrieval, and on days 3 and 8 after oocyte retrieval. Hormone levels in fertility preservation patients were compared with those observed in infertility patients.

MAIN RESULTS AND THE ROLE OF CHANCE: While estradiol levels were significantly lower in the fertility preservation group compared with the control group ($P < 0.001$), progesterone levels were similar at all times, including patients receiving a GnRH antagonist during the luteal phase.

LIMITATIONS, REASONS FOR CAUTION: The studied populations (breast cancer and infertile patients) are different, which may induce selection bias. The small sample size limits the study’s statistical power and the possibility to perform multivariate analysis. Recruitment of the study and control patients was completed at the same time; however, enrollment of controls started at a later time.

WIDER IMPLICATIONS OF THE FINDINGS: While the use of letrozole in fertility preservation patients has a favorable effect on estrogen levels, no benefit is seen for progesterone levels which are high and comparable with progesterone levels after standard COS in IVF patients. As progesterone has been associated with tumor cell proliferation, caution is mandatory. Modified protocols including GnRH agonist triggering should be investigated.

Key words: breast cancer / fertility preservation / letrozole / luteal phase / progesterone
Introduction

Breast cancer is the most frequent malignancy diagnosed in women with 5–15% of cases occurring during the reproductive years (Lee et al., 2010). Early diagnosis and greatly improved treatments have evolved over the last two decades, leading to an increased survival rate, especially among young women (Azim et al., 2008). Many breast cancer survivors aim to start or complete their families when they achieve remission. Unfortunately, chemotherapeutic agents used for breast cancer treatments are frequently gonadotoxic and may have the potential to induce infertility and premature ovarian failure (Diedrich et al., 2011).

Several options for fertility preservation have been developed, such as ovarian tissue cryopreservation and oocyte or embryo freezing (Demeestere et al., 2009, 2012; Donnez et al., 2013; Lamberti et al., 2013; Imbert et al., 2014). Ovarian tissue cryopreservation is still considered experimental, but it offers encouraging results. However, the risk of reintroduction of the disease by later-grafted tissue containing micrometastases is a major concern, especially in advanced breast cancer (Bockstaele et al., 2012). Moreover, breast cancer occurs more often in patients in their 30s. At this age, ovarian reserve is known to decrease, potentially reducing the pregnancy success rate after ovarian tissue grafting.

Oocyte and embryo cryopreservation on the other hand require controlled ovarian stimulation (COS), leading to a supra-physiological rise in estrogen levels. However, high estrogen levels should be avoided in cases of hormone-dependent malignancies, such as breast cancer, in order to prevent tumor cell growth (Oktay et al., 2005; Turan et al., 2013). Almost 10 years ago, Oktay et al. investigated a new protocol combining standard COS with letrozole, an aromatase inhibitor, for fertility preservation in breast cancer patients (Oktay et al., 2006). Letrozole was shown to be effective in maintaining infra-physiological estrogen levels, while harvesting several mature oocytes for subsequent oocyte or embryo vitrification (Oktay et al., 2006; Reddy and Oktay, 2012).

Control of the hormonal environment during and after COS with letrozole is crucial for avoiding any potential effects of the treatment on tumor cell progression. Low estradiol levels have largely been reported as maintained using this protocol; however, no study has so far investigated its effect on progesterone levels. Nevertheless, there is growing evidence for a role of progesterone in breast tumorigenesis, suggesting that high progesterone levels may detrimentally affect the oncological outcome.

The objective of this study was to assess progesterone levels in breast cancer patients undergoing letrozole-associated COS for fertility preservation. Progesterone levels were measured on the day of ovulation trigger and of oocyte retrieval, as well as during the early and mid-luteal phase and were compared with levels observed in fertile women undergoing COS without letrozole for IVF/ICSI.

Materials and Methods

Population

Young breast cancer patients less than 41 years of age wanting to preserve their fertility before gonadotoxic treatment were referred to Erasme Hospital by several oncological centers in Belgium for participation in a long-term prospective trial (BROVALE trial, EudraCT/CCB: B406201214697). When a delay of at least 14 days before starting chemotherapy was possible, letrozole-COS was offered for vitrification of oocytes or embryos. Patients with metastatic disease, known ovarian insufficiency, or basal follicle-stimulating hormone (FSH) > 20 UI/l were excluded. Steroid levels measured at different time points were compared with results obtained in a prospective control group of infertile patients undergoing standard GnRH antagonist COS for IVF or ICSI. Inclusion criteria for controls were tubal and/or male infertility and idiopathic infertility. Patients with severe polycystic ovary syndrome (PCOS), endometriosis or ovarian insufficiency were excluded.

Ethical approval

The Ethical Committee of Erasme Hospital approved the study, and patients from both groups signed a specific informed consent.

Ovarian stimulation protocol

Each breast cancer patient underwent classic or ‘random start’ letrozole-COS, depending on whether the patient was in the early (n = 10), or late follicular phase or in the luteal phase (n = 11). The cycle phase was defined based on the last menstruation period and on the hormonal profile before COS.

In the classic protocol, letrozole 5 mg/day (Femara®, Novartis, Switzerland) was started on day 2 of the menses. Administration of FSH (150–300 IU/day, Gonal-f®, Serono, Germany or Menopur®, Ferring, Switzerland) was started the following day, after an antral follicular count (AFC) and evaluation of hormonal levels. Serum estradiol and progesterone levels as well as follicular size evaluation by ultrasound were measured throughout the ovarian stimulation according to local protocol. GnRH antagonist (0.25 mg/day Cetrotide®, Serono, Germany) was administered as soon as at least one follicle reached 14 mm or when estradiol levels reached 250 pg/ml.

If the time before starting chemotherapy was very limited and the patient was not in the early follicular phase, the ‘random start’ COS procedure was applied. Letrozole, gonadotrophins and GnRH antagonists were administered together throughout the stimulation until ovulation triggering occurred.

Ovulation trigger was achieved by 10 000 IU human chorionic gonadotrophin (hCG) (Pregnyl®, MSD, Switzerland) when at least two follicles reached 18 mm. Administration of GnRH agonist (tripotrenol, Decapeptyl 0.2 mg, IPSEN, France) for ovulation trigger was reserved for patients with high risk of ovarian hyperstimulation syndrome (OHSS). Letrozole administration was discontinued on the day of triggering final oocyte maturation. For the first 11 breast cancer patients enrolled in the study, letrozole was administered the evening following oocyte pick-up (OPU), when estradiol levels were > 250 pg/ml at trigger (n = 4) (initial protocol). The protocol was modified after we observed high progesterone levels during the luteal phase. All patients (n = 10) received GnRH antagonist 0.25 mg/day after OPU for 1 week, or until chemotherapy started (modified protocol) (Fig. 1).

The control group underwent standard COS according to local protocol. Briefly, gonadotrophins were administered at day 3 of the follicular phase, after the hormonal and AFC evaluation. GnRH antagonist 0.25 mg/day was started after 5 days of ovarian stimulation. Ovulation was triggered by hCG 10 000 IU if at least three follicles reached 17 mm. After OPU, all patients received luteal phase supplementation with progesterone (3 × 200 mg per day Utrogestan®, Besins, France) until the pregnancy test.

Hormonal measurements

Estradiol and progesterone levels were measured and compared at four time points: ovulation trigger, OPU, and on days 3 and 8 after OPU (luteal phase days 3 and 8). They were assayed by electrochemiluminescence immunoassay using a competitive immunoassay (Modular E170—Roche diagnostics, Germany).

The inter-assay coefficient of variation was less than 5% for both assays.
Statistical analysis

Statistical analysis was performed using SPSS 22 (IBM, Brussels, Belgium) on Mac OS X. Estradiol and progesterone levels were compared for each time with the Mann–Whitney U-test. The ANOVA tests for repeated measures were performed to investigate the evolution of the estradiol and progesterone with time and compare them between groups. Proportions were analyzed with the χ²-test, and the Spearman rank-correlation test (calculation of r-values) was used for correlations. P-values of <0.05 were considered to indicate statistical significance.

Results

Between November 2012 and December 2014, 23 breast cancer patients underwent 28 letrozole-COS cycles for fertility preservation in our department. None of these patients had a previous history of infertility. We excluded two patients who stopped the protocol after 6 and 13 days, respectively, due to non-response to the stimulation. Five patients performed a second cycle that was also excluded from analysis to avoid bias in estradiol and progesterone levels. A total of 21 patients undergoing 21 letrozole-associated COS cycles were analyzed. During luteal phase, 11 patients were subjected to the initial protocol and 10 received GnRH antagonist according to the modified protocol (Fig. 1). Progesterone levels were obtained at trigger, OPU, and at least at one of the two time points of the luteal phase (L3 and/or L8) for 18 patients in the study group and for all patients in the control groups.

A total of 21 patients undergoing standard antagonist COS for IVF/ICSI were recruited for the control group (Fig. 1) between August and November 2014. The characteristics of the patients and the cycles are presented in Table 1. No difference in the ethnic origin was observed between groups (19 were Caucasian and 2 were African in each group). A total of 144 and 167 oocytes were obtained in the study and control groups, respectively.

As expected, estradiol levels were significantly lower (P < 0.001) in the letrozole-COS group compared with the control group at all times (Fig. 2a). In contrast, progesterone levels were as high in the study group as in the control group receiving progesterone supplementation during the luteal phase (Fig. 2b). No difference was found between the groups concerning their progesterone levels evolution pattern with time (P = 0.092). In patients with low estradiol levels at trigger (<250 pg/ml), progesterone levels were similar regardless of whether GnRH antagonist treatment was administered during the luteal phase (n = 7) or not (n = 6) (Fig. 3a). Similarly, high progesterone levels were observed in patients with high estradiol levels at trigger (>250 pg/ml) regardless of the treatment received during the luteal phase (Fig. 3b). Furthermore, progesterone levels at ovulation triggering as well as during the luteal phase were similar between patients who underwent ‘standard’ versus ‘random start’ letrozole-COS (data not shown).

A significant bilateral correlation was observed between progesterone levels on day 3 of the luteal phase and the number of oocytes retrieved in the study group (P < 0.001), but not in the control group (P = 0.421).

Discussion

Letrozole is an aromatase inhibitor that prevents androgen aromatization into estrogens (Bhatnagar, 2007). During COS for fertility preservation,
Letrozole was shown to be effective in maintaining infra-physiological estrogen levels while harvesting several mature oocytes for oocyte or embryo vitrification (Casper and Mitwally, 2011; Lee et al., 2011; Roy et al., 2012). No significant neonatal abnormalities or malformations were described in 26 pregnancies and 11 live births obtained using this protocol, but its safety must be further evaluated (Lee and Oktay, 2012). Only one controlled study has so far evaluated the disease-free survival rate of 79 breast cancer patients who elected to undergo letrozole-COS, with a median follow-up of 2 years (Azim et al., 2008). Results were reassuring, but data on long-term follow-up are lacking.

Although both estradiol and progesterone may enhance tumor cell progression, only data on estradiol levels during letrozole-COS are available. Our study aimed to investigate progesterone levels in breast cancer patients undergoing ovarian stimulation in the presence of letrozole and to compare them with those of infertile couples undergoing standard ovarian stimulation for IVF/ICSI.

Although sample size was small, we observed high progesterone levels during early luteal phase, regardless of the estradiol levels at hCG trigger in the fertility preservation group. Progesterone levels were similar to those observed in control IVF/ICSI patients supplemented with...
progesterone. Furthermore, the administration of GnRH antagonist during the luteal phase in fertility preservation group was not sufficient to reduce progesterone levels.

Progesterone levels were correlated with the number of oocytes retrieved in the study group, but not in the control group (which was supplemented with progesterone during the luteal phase). These results suggest that the presence of letrozole during follicular development, combined with hCG administration for final maturation, induces high progesterone production during luteinization.

Two sequential enzymatic reactions convert progesterone into androgens (by 17α hydroxylase and C17,20 lyase), which are subsequently transformed into estrogens by the aromatase enzyme. If the aromatase enzyme is blocked, accumulation of precursors such as progesterone, testosterone and 17α-progesterone can occur (Stanczyk, 1997). However, this study does not provide evidence for a direct effect of letrozole on progesterone levels during the luteal phase.

The role of progesterone in breast development and tumorigenesis is not clear. Several studies indicate that its action might be different in normal mammary cells compared with that in cancer cells. In 1962, Huggins et al. showed an accelerated progesterone-induced proliferation of in vivo rat breast cancer cells (Huggins et al., 1962). One of the investigated mechanisms suggests that the progesterone receptor (PR) induces activation of cytoplasmic kinase-cascades in addition to the usual nuclear effects on transcription factors. These kinase-cascades enhance cell cycle progression toward proliferation (Skildum et al., 2005). Another mechanism probably involves a paracrine action of progesterone. In the adult female mouse, 7–10% of mammary epithelial cells are PR positive and non-proliferative. Adjacent cells, which are PR negative, showed proliferation after progesterone administration, which suggests paracrine action via mediators (i.e. RANK) between cells (Kim et al., 2013). Altogether, these studies suggest a pro-tumorigenic effect of progesterone that is not fully understood and thus needs further evaluation.

Our study has some limitations that should be taken into account when interpreting its results. Breast cancer patients wishing to preserve their fertility were included in a prospective clinical trial in our department (BROVALE trial, EudraCT/CCB: 8406201214697). However, the control patients for this study were recruited later, while both enrollment processes ended at the same time. The studied populations (breast cancer and infertile patients) differed, which might have induced selection bias. The inclusion and exclusion criteria were however strictly defined, to limit confounding factors that could potentially impact hormonal profile. Luteal phase treatments were different between the two groups as all control patients received progesterone supplementation. Moreover, half of the patients in the fertility preservation group started COS with letrozole during the luteal phase (‘random start’). However, we did not observe any impact of the protocol (random start versus standard) on progesterone levels. Finally, sample sizes were small, which limited the study’s statistical power as well as the opportunity to perform multivariate analysis. Despite these shortcomings, to our knowledge this study is the first to describe progesterone levels following letrozole-COS for fertility preservation in breast cancer patients. While progesterone’s role in breast cancer is not fully understood, its effect on tumor cell proliferation may actually be as relevant as of estradiol. Our results suggest that future evaluation of letrozole-COS protocols’ safety is needed. In particular, administration of progesterone in the luteal phase should probably be avoided. Other strategies such as the use of GnRH agonist instead of hCG to trigger final oocyte maturation can be implemented to limit the increase of progesterone levels (Reddy et al., 2014). Further studies should be carried out to evaluate the impact of these modified protocols on progesterone during the luteal phase.

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Authors’ roles

O.G and I.D designed the study, performed statistical analysis and wrote the manuscript. The results were interpreted by O.G, I.D and C.G. Y.E. and A.D. participated in the study design and revised the manuscript. All authors approved the final version of this article.

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Conflict of interest

None declared.

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