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High progesterone levels during the luteal phase related to the use of an aromatase inhibitor in breast cancer patients

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Abstract. – OBJECTIVE: To evaluate the hormonal profile in three breast cancer patients who underwent controlled ovarian stimulation in the presence of the aromatase inhibitor letrozole.

PATIENTS AND METHODS: In IVF University referral center, a case series of three breast cancer patients who underwent controlled ovarian stimulation (COS) with recombinant FSH and letrozole were investigated. Ovulation was induced with hCG (case No. 1) or with GnRH agonist (case No. 2-3). The primary outcome of our study was the detection of progesterone levels in the luteal phase.

RESULTS: Very high progesterone values (mean 186.6 ± 43.6 ng/mL) during the luteal phase were recorded in all three cases.

CONCLUSIONS: High progesterone levels can be related to the use of letrozole independently of the most commonly used trigger regimen. Although progesterone has long been considered a protective factor against breast cancer, several studies have demonstrated that progesterone could expand a transformation-sensitive stem cell population in the mammary glands. The estrogen negative feedback effect on the hypothalamus-pituitary axis and the disruption of steroid biosynthesis and could represent an intriguing reason behind this phenomenon. Our results highlight the need to evaluate further the increase in progesterone levels in the luteal phase in women with breast cancer undergoing COS with letrozole.

Key Words:
Breast cancer, Controlled ovarian stimulation, Aromatase inhibitor, Letrozole, Fertility preservation.

Introduction

Breast cancer accounts for one-third of all neoplasms in women of reproductive age[1,2].

Adjuvant chemotherapy regimens commonly used to treat breast cancer may impair fertility and lead to premature ovarian insufficiency (POI)[3]. Thanks to recent advances in the field of fertility preservation, many women who experience chemotherapy-induced POI are able to bear children[4]. For its feasibility and quickness, controlled ovarian stimulation and immediate oocyte cryopreservation are the most frequently used fertility preservation measures for women affected by breast cancer[4,5].

Breast cancer cells are often characterized by the expression of estrogen receptors[6] and respond to estrogen exposure with increased growth. Therefore, protocols for controlled ovarian stimulation (COS) and multiple follicle developments have been established based on the use of an aromatase inhibitor (i.e., letrozole) in combination with conventional gonadotropin preparations to avoid high levels of estrogen, which may stimulate the growth of malignant cells. Specifically, 5 mg letrozole per day is given on the second day of the menstrual cycle until ovarian triggering, together with daily injections of recombinant FSH (r-FSH) depending on ovarian response and the patient's characteristics. Gonadotropin-releasing hormone antagonists are used to prevent premature oocyte ovulation[7]. After oocyte retrieval, letrozole is usually reinitiated and continued until estradiol levels drop to below 50 pg/mL.

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Given the growing body of evidence linking prolonged estrogen exposure to the etiology of breast cancer, many oncologists, and breast cancer patients are reticent to use assisted reproductive technologies[8]. Consequently, letrozole is used to mitigate the prolonged exposure of estrogen during COS. However, there is evidence that also exposure to progesterone negatively impacts on the patient's prognosis[9,10] Little is known about the levels of progesterone induced by letrozole, probably because oncological patients undergoing oocyte/embryo preservation are not candidates for embryo transfer immediately after ovarian stimulation[11].

Here, we report a case series of three breast cancer patients stimulated by r-FSH and letrozole in whom high progesterone levels were detected during the luteal phase.

Case Series

Case 1

A 31-year-old woman was referred with a history left quadrantectomy for breast cancer. Post-surgery histology showed an infiltrating ductal carcinoma (G_3, ER: 70%, PgR: 75%, Ki67: 50%, $HerbB_2$: 3+, pT1 Ns M0 – stage I). After discussion with oncologists and embryologists, we opted for COS with an aromatase inhibitor. Ultrasound transvaginal examination performed on the first day of the cycle revealed an enlarged retroverted uterus characterized by the presence of three myomas (< 15 mm mean diameter). Ovaries were normal with no evidence of lesions. Eleven antral follicles were observed. Before ovarian stimulation, anti-müllerian hormone (AMH) was 2.7 ng/mL.

Five mg per day of letrozole (Femara, Novartis Farma S.p.A., Milan, Italy) was started on cycle day 2. Daily injections of r-FSH (150 IU per day) (Gonal F, Merck Serono S.p.A., Rome, Italy) were prescribed two days later (day 4). A GnRH antagonist (0.25 mg) (Cetrorelix, Merck Serono S.p.A, Rome, Italy) was administered when the mean diameter of the leading follicle measured 14 mm. All medications were discontinued on the day of human chorionic gonadotropin (hCG) administration, in the presence of at least one follicle > 17 mm in diameter. Letrozole was reinitiated after oocyte retrieval and continued until E_2 levels fell to < 50 pg/mL.

During ovarian stimulation, blood levels of estrogen and progesterone were respectively, 160 pg/mL and 1 ng/mL on the third stimulation day; 242 pg/mL and 0.6 ng/mL on the fifth stimulation day. Estrogen levels were (474 pg/mL) on the seventh stimulation day. On day 9 of the cycle, 10,000 IU of hCG (Gonasi HP, IBSA Farmaceutici, Lodi, Italy) were administered, and oocyte picks up (OPU) performed 36 hours later. Nine oocytes were retrieved and all were MII. Two days after oocyte retrieval, a depot formulation of triptorelin (3.75 mg) was administered (Decapeptyl, Ipsen S.p.A., Milan, Italy) to prevent ovarian damage during chemotherapy.

Sex hormones were measured to determine when letrozole treatment could be suspended. Progesterone and estradiol levels were 140 ng/mL and 7 pg/mL, respectively four days after OPU. Sex hormone values were confirmed by another laboratory. Based on these results, letrozole administration was suspended.

Case 2

The second patient was a 38-year-old woman with a history of breast cancer subjected to quadrantectomy. Histology showed an infiltrating ductal carcinoma (G_3, ER: 0%, PgR: 2%, Ki67: 70%, $HerbB_2$: 1+, pT1 N0 M0 – stage I). After discussion with oncologists, also in this case we carried out ovarian stimulation with aromatase inhibitors. Ultrasound transvaginal examination, performed on the second day of the cycle, showed a normal uterus and ovaries with 10 antral follicles measuring between 2 and 9 mm in diameter.

Letrozole was started on cycle day 3. Daily injections of r-FSH (150 IU per day) were started two days later (day 5). On cycle day 7, the dose of r-FSH was increased to 187.5 IU daily. A GnRH antagonist (0.25 mg) was administered when the mean diameter of the leading follicle measured 14 mm. All medications were discontinued on the day of triggering which was performed by a bolus injection of 0.2 mg of triptorelin (Decapeptyl, Ipsen S.p.A., Milan, Italy). Letrozole was reinitiated after oocyte retrieval and continued until E_2 levels fell to < 50 pg/mL.

During COS, blood levels of estrogen and progesterone were respectively, 55 pg/mL and 0.8 ng/mL on the second stimulation day, and 154 pg/mL and 0.9 ng/mL on the fifth stimulation day. Estrogen level was 464 pg/mL on the seventh stimulation day. On the same day (which corresponded to cycle day 11), final maturation of oocytes was induced with an injection of a GnRH agonist (GnRH-a). Oocytes were retrieved 36 hours later. Twenty oocytes were retrieved, 10 of which were in MII stage.

We measured progesterone and estradiol levels 5 days after OPU to determine when to suspend letrozole administration. Progesterone was 191 ng/mL and estradiol 57.3 pg/mL. The patient started chemotherapy 7 days after OPU.

Case 3

The third case was a 34-year-old woman with a history of right quadrantectomy for infiltrating ductal carcinoma (G2, ER: 80%, PgR: 32%, Ki67: 10%, HerbB2: 2+, pT1 N0 M0 – stage I). The level of AMH before the procedure was 5.24 ng/mL. Ultrasound transvaginal examination was carried out in the follicular phase and revealed a normal uterus and ovaries with nine antral follicles measuring between 2 and 9 mm. COS was started on day 19 of the menstrual period soon after ovulation due to the patient's late decision to undergo COS.

Letrozole was started at the dose of 5 mg per day together with a starting dose of r-FSH consisting of 150 IU per day. A GnRH antagonist (0.25 mg) was prescribed when a 14 mm leading follicle was detected on ultrasound. Estrogen and progesterone values were 64.5 pg/mL and 8.7 ng/mL respectively on the third stimulation day, and 219.6 pg/mL and 0.8 ng/mL on the seventh stimulation day.

Final maturation of follicles was induced by a bolus injection of 0.2 mg triptorelin. OPU was performed 36 hours after ovulation induction and 20 oocytes were retrieved, 15 of which were in MII stage. Twenty-four hours after OPU, letrozole (2.5 mg per day) was administered until estradiol levels decreased to below 50 pg/mL, and triptorelin (0.1 mg per day) was administered for gonadal protection. Chemotherapy was started three days after OPU. Progesterone values reached 206.0 ng/mL and 227.6 ng/mL five and seven days after OPU, respectively.

Discussion

To our knowledge, this is the first report to describe very high levels of progesterone in the mid-luteal phase in connection with the use of letrozole adopting GnRH antagonist short protocol and GnRH-a triggering during the luteal phase.

Letrozole is a third-generation aromatase inhibitor that very effectively reduces circulating levels of estradiol shortly after initiating administration in the early follicular phase[12].

All three patients reported herein continued letrozole treatment after oocyte collection, and had extraordinarily high progesterone levels during the luteal phase (> 140 ng/mL). In a recent observational trial, Goldrat et al[11] found that luteal phase progesterone levels did not differ significantly (p = 0.092) between 21 breast cancer patients who underwent COS with aromatase inhibitors and a control group of 21 infertile patients treated with standard GnRH-a COS. All women underwent triggering with hCG. The authors also reported a slight increase (≤ 60 ng/ml) in progesterone levels in women treated with aromatase. Conversely, in our small series, all three women had progesterone levels >140 ng/ml. This discrepancy may be related to different triggering modalities. Our experience suggests that progesterone levels might be higher when GnRH-a is used for ovarian triggering (Table I). Notably, although Case No. 1 of our series received hCG triggering, a GnRH-a depot formulation was ad-

Table I. Characteristics and controlled ovarian stimulation results.

Characteristics	Case 1	Case 2	Case 3
Age (years)	31	38	34
Stage (TNM)	I	I	I
Histology	Infiltrating ductal carcinoma	Infiltrating ductal carcinoma	Infiltrating ductal carcinoma
COS starting day	2	3	19
Endometrial thickness (mm)	5.0	6.2	11.0
AMH (ng/mL)	2.7	n.a.	5.24
Antral follicle count (number)	11	10	9
Triggering	hCG 10,000 IU	Triptorelin 0.2 mg/mL	Triptorelin 0.2 mg/mL
GnRH antagonist starting day	Cycle day 5	Cycle day 7	Cycle day 24
Peak E_2 (pg/mL)	474.0	464.0	219.6
Progesterone levels during luteal phase (ng/mL)	140.7	191.0	227.6

AMH, anti müllerian hormone; COS, controlled ovarian stimulation; E2, 17β estradiol; GnRH, gonadotropin releasing hormone; hCG human chorionic gonadotropin; n.a., not available.

ministered two days after OPU to prevent ovarian damage during chemotherapy. Another possible explanation of the differences between the two studies is the use of GnRH-antagonist during the luteal phase in 10 of the 21 women treated with letrozole[11].

Progesterone levels are frequently increased during COS where multiple follicles are involved in steroidogenesis[13,14]. During standard IVF procedures, i.e., without letrozole, progesterone level should exceed 80-100 nmol/L (approximately 25 ng/mL) during the luteal phase to obtain a high reproductive outcome[14]. hCG triggering of final oocyte maturation results in higher progesterone levels than does triptorelin triggering[15]. Nonetheless, on the day of transfer and one week later, progesterone levels do not usually exceed 60 ng/mL and 50 ng/mL, respectively[14]. Even when progesterone plus hCG supplementation is prescribed in cycles with hCG triggering, the mean progesterone level was found to be below 95 ng/mL 6 days after oocyte retrieval[16].

There are two possible explanations for the discrepancy between standard and letrozole-associated COS. One is related to the fact that letrozole reduces estradiol production during the luteal phase thereby inhibiting the estrogen-negative feedback on the hypothalamus-pituitary axis. Consequently, LH levels may rise above physiological values, which are around 4-10 IU/L, and so stimulate progesterone synthesis. Alternatively, disruption of the steroid biosynthesis pathway associated with letrozole-induced inhibition of aromatase could provoke upstream accumulation of steroid precursors including progesterone[11]. However, this possibility is less likely because estradiol is measured in pg/mL concentrations and progesterone in ng/mL concentrations, and thus the difference in concentration is usually more than a thousand times.

The role of progesterone in the pathogenesis of breast cancer is still unclear[17]. Although progesterone has long been considered a protective factor against breast cancer[10], several studies have demonstrated that progesterone expands a transformation-sensitive stem cell population in the mammary glands[18-20]. Moreover, progesterone may activate the stem-cell pool and accelerate the formation of tumors including breast tumors[21]. It is conceivable that this effect could be mediated by a paracrine mechanism through the receptor activator of nuclear factor kappa-B ligand (RANKL) paracrine signaling[21].

Epidemiological data seem to confirm progesterone's involvement in breast cancer development. For instance, the Women's Health Initiative trial showed an increased risk of breast cancer among patients who underwent combined estrogen/synthetic progestin therapy versus estrogen monotherapy[22]. However, breast cancer risk seems to differ according to the type of progestin used, with a lower no significant relative risk among patients who used natural progesterone 1.00 (CI 0.83-1.22, $p > 0.05$)[23]. Our three patients were affected by breast cancer, which is associated with a high risk of recurrence, and even the effect of native progesterone should not be underestimated in such subjects. However, larger series of patients are required to substantiate these preliminary findings.

Conclusions

Our results highlight the need to evaluate further the increase in progesterone levels in the luteal phase in women with breast cancer undergoing COS with letrozole.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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