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ORIGINAL RESEARCH

Long-acting gonadotropin-releasing hormone agonist trigger in fertility preservation cycles before chemotherapy

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Available online xxx

Background: Oocytes/embryo cryopreservation and ovarian function suppression with gonadotropin-releasing hormone (GnRH) agonists (GnRHas) are two established strategies for preserving fertility in patients with cancer, frequently both being offered to the same woman. As the first injection of GnRHa should be administered before chemotherapy, it is usually performed in the luteal phase of the urgent controlled ovarian stimulation (COS) cycle. The GnRHa flare-up effect on recently stimulated ovaries may cause ovarian hyperstimulation syndrome (OHSS) and this risk may discourage some oncologists to offer an ovarian function preservation method with proven efficacy. We suggest the long-acting GnRHa as an option to trigger ovulation for egg retrieval in oncological patients, whenever ovarian suppression during chemotherapy is planned.

Patients and methods: We retrospectively analyzed prospectively collected data from all consecutive ovarian stimulation cases in oncological patients for oocyte cryopreservation from 2016 to 2021 in a single academic referral center. The COS was performed according to good clinical practice standards. Since 2020 long-acting GnRHa trigger was offered to all patients for whom ovarian suppression after cryopreservation was planned. All other patients served as controls, stratified for the triggering method used: highly purified chorionic gonadotrophin 10 000 UI or short-acting GnRHa 0.2 mg.

Results: Mature oocytes were collected, with the expected maturation rate, in all the 22 cycles triggered with GnRHa. The mean number of cryopreserved oocytes was 11.1 $\pm$ 4, with a maturation rate of 80% (57%-100%), versus 8.8 $\pm$ 5.8, 74% (33%-100%) with highly purified chorionic gonadotrophin and 14 $\pm$ 8.4, 80% (44%-100%) with short-acting GnRHa. No case of OHSS was observed after long-acting GnRHa triggering and by 5 days after egg retrieval most patients had reached luteinizing hormone levels showing suppression.

Conclusions: Our preliminary data show that long-acting GnRHa is efficacious in inducing the final oocytes' maturation, reducing OHSS risk and suppressing ovarian function by the start of chemotherapy.

Key words: oncofertility, fertility preservation, GnRH analog, oocyte cryopreservation, OHSS

INTRODUCTION

The counseling about the possible gonadotoxic effects of cancer therapies is considered standard of care in all pre-menopausal patients, as endorsed universally by scientific societies.[1] Oocytes and embryo cryopreservation are safe and efficacious standard options to preserve fertility,[2,3] with chances of future pregnancy dependent on the woman's age and ovarian reserve,[4] but have no impact on the long-term endocrine function of the ovaries. On the contrary, ovarian suppression with long-acting gonadotropin-releasing hormone agonists (GnRHas) during chemotherapy is not recommended as a stand-alone fertility preservation technique, but rather as a strategy to preserve ovarian function to be used in combination with other interventions.[1] After an initial flare-up, it causes a profound ovarian suppression inhibiting the hypothalamus—hypophysis—ovaries axis that needs pulsatile secretion of GnRH as its *primum movens*.[5] Its efficacy in preserving the ovarian function was proved by a meta-analysis of five randomized controlled trials reporting a significant 16.8% absolute

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reduction of post-treatment permanent amenorrhea in patients with breast cancer.[6]

Being the only medical option to preserve ovarian function, the use of long-acting GnRH during chemotherapy for breast cancer is currently endorsed by all fertility preservation guidelines.[1] The limitations of this option are that the evidence is not equally strong for neoplasms other than breast cancer,[7] and that the biological rationale behind its beneficial effect is not yet completely understood.[8]

The first injection of long-acting GnRHa should be done before chemotherapy, aiming for ovarian suppression by the start of the therapies. In patients that underwent controlled ovarian stimulation (COS) for oocyte/embryo cryopreservation, it is frequently offered few days after eggs retrieval, with the ultimate aim of avoiding further delays in cancer therapies. Cases of ovarian hyperstimulation syndrome (OHSS) have been described in this setting, as a consequence of the luteotropic effect of its initial gonadotrophins flare-up on recently stimulated ovaries. In particular, Christ et al.[9] recently described three cases of severe OHSS after oocyte cryopreservation cycles with short-acting GnRHa trigger and long-acting GnRHa administration in the luteal phase. The authors therefore advised caution when administering long-acting GnRHa in the luteal phase after an ovarian stimulation with high ovarian response.

OHSS is indeed 'the great enemy' in assisted reproduction treatments and, in the oncofertility setting, may cause complications such as an increased thromboembolic risk and a possible delay in the start of chemotherapy.[9] Such a threat to safety may discourage gynecologists and oncologists to propose a technique of proven efficacy.

We suggest long-acting GnRHa as an option to trigger ovulation after COS in oncological patients. Conceptually, the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) rise obtained with long-acting GnRHa triggering is sustained enough to meet the requirements for final oocytes maturation and ovulation induction, as it is a consequence of the release of FSH and LH stored in the pituitary, with the same mechanism as short-acting GnRH. Furthermore, the long-acting formulation has the advantage of initiating ovarian suppression for chemotherapy, guaranteeing complete suppression in $\sim$10 days.[5] In literature there is only one case report of an oncologic patient who used triptorelin 3.75 mg as ovulation trigger, with results as good as expected in terms of mature oocytes yielded and vitrified.[10] Here we report on our experience with long-acting GnRHa triggering.

PATIENTS AND METHODS

We retrospectively analyzed prospectively collected data on consecutive ovarian stimulation for oocyte cryopreservation in oncological patients from 2016 to 2021 in a single academic referral center.

The COS was carried out according to good clinical practice standards. The protocol used was the random start antagonist protocol for all cycles. The type and dosage of gonadotropins were chosen based on women's age and ovarian reserve. GnRH antagonist was added when the leading follicle had a diameter of at least 12-13 mm and/or estrogens levels were $>$200 pg/ml. In women with hormone-sensitive breast cancer letrozole 5 mg/day was coadministered during the ovarian stimulation.

In our center, GnRH agonist for ovarian function protection is offered to all young patients facing chemotherapy. During counseling, the current evidence about its efficacy is illustrated (including the high-quality data we have for breast cancer and more conflicting results for other neoplasms) as well as the possible adverse effects of temporary iatrogenic menopause.

Since 2020 long-acting GnRHa trigger (triptorelin 3.75 mg, 36 h before egg retrieval) was offered to all patients in whom ovarian suppression after cryopreservation was planned, based on the biological rationale of the triggering effect of the GnRHa flare-up on stimulated ovaries, and it was used in 22 patients. All other patients served as controls, stratified for the triggering method used: highly purified chorionic gonadotrophin 10 000 UI or short-acting GnRHa 0.2 mg. All patients signed an informed consent form for the use of their anonymized clinical data for scientific research, and we obtained the institutional review board approval (CERLiguria, n. 428/21) for analysis and publication of results.

Continuous data were reported as mean and standard deviation and compared with Student's *t*-test or reported as median and range and compared with the Mann–Whitney *U* test, depending on whether they were normally distributed or not (assessed with the Kolmogorov–Smirnov test). Categorical data, reported as number and percentage, were compared with chi-square test. A P value <0.05 was considered statistically significant.

The main outcome of the study was to assess whether the long-acting GnRHa triggering would lead to the retrieval of mature oocytes with a maturation rate, expressed as mature metaphase II (MII) oocytes/total oocytes retrieved, comparable with the other triggering methods.

RESULTS

Our cohort included 82 consecutive ovarian stimulation cases (oncological patients) for oocyte cryopreservation from 2016 to 2021. Of these, 22 women received the long-acting GnRHa trigger (triptorelin 3.75 mg; group A). All other patients received the final ovulation triggering with highly purified chorionic gonadotrophin 10 000 UI (34 women; group B) or short-acting GnRHa 0.2 mg (26 women; group C).

Table 1 reports on the demographic and clinical parameters of the three groups. Patients in group C had a slightly higher ovarian reserve (antral follicular count: 15.6 $\pm$ 12.7 versus 10.7 $\pm$ 5.5 in group B and 12.9 $\pm$ 7.2 in group A) and therefore retrieved more oocytes (18 $\pm$ 10.3 versus 11.5 $\pm$ 6.7 in group B and 13.9 $\pm$ 5.2 in group A), although these differences were not statistically significant.

Table 1. Demographic and clinical parameters of women undergoing ovarian stimulation for oocytes cryopreservation, triggered with long-acting GnRHa, triptorelin 3.75 mg (group A), highly purified chorionic gonadotropin 10 000 UI (group B), or short-acting GnRHa, triptorelin 0.2 mg (group C)

	Group A (n = 22)	Group B (n = 34)	Group C (n = 26)
Age (years)	33.7 ± 4.8	31.9 ± 5.0	31.3 ± 5.9
Neoplasm, *n* (%)			
Breast cancer	16 (73)	20 (59)	16 (62)
Hematological cancer	4 (18)	10 (29)	5 (19)
Other	2 (9)	4 (12)	5 (19)
Letrozole, *n* (%)	14 (64)	17 (50)	14 (54)
Antral follicular count, *n*	12.9 ± 7.2	10.7 ± 5.5	15.6 ± 12.7
Stimulation length (days)	12 ± 1.7	12 ± 2.9	13.4 ± 7.1
Follicles >14 at trigger, *n*	11 ± 5.1	7.3 ± 3.2	13.3 ± 5.9
Estradiol levels at trigger (pg/ml)	657 (108-5000)	643 (52-3598)	618 (69-3835)
Oocytes retrieved, *n*	13.9 ± 5.2	11.5 ± 6.7	18 ± 10.3
MII oocytes vitrified, *n*	11.1 ± 4	8.8 ± 5.8	14 ± 8.4
Maturation rate (%)	80 (57-100)	74 (33-100)	80 (44-100)
OHSS, *n* (%)	0 (0)	1 (2.9)	1 (3.8)

Continuous data are reported as mean ± standard deviation or median (range), depending on normality (tested with the Kolmogorov—Smirnov test of normality); categorical data are reported as absolute number (percentage).
GnRHa, gonadotropin-releasing hormone agonist; MII, metaphase II; OHSS, ovarian hyperstimulation syndrome.

The higher percentage of women in group A were patients with breast cancer (16 women; 73%), the most common neoplasm in women of that age. Moreovre, breast cancer patients were probably more likely to accept ovarian suppression during chemotherapy due to the published evidence of efficacy.

The mean number of mature cryopreserved oocytes in group A was 11.1 ± 4, with a maturation rate of 80% (57%-100%), versus 8.8 ± 5.8 with a maturation rate of 74% (33%-100%) in group B, and 14 ± 8.4, 80% (44%-100%) in group C (no statistically significant difference).

In patients triggered with long-acting GnRHa, 5 days after oocyte retrieval (7 days after trigger), serum FSH median level was 1.29 mUI/ml (0.48-2.50 mUI/ml), LH median level was 1.04 mUI/ml (0.26-2.46 mUI/ml), estradiol 165.5 pg/ml (min 20-max 1676 pg/ml), and progesterone 32.1 ng/ml (0.79-124 ng/ml).

tConsidering the risk of OHSS among patients from whom >15 oocytes were collected, 9 were at risk in group A (40.9%), 8 in group B (23.5%), and 12 in group C (46.2%). There was no case of OHSS in group A. In group B, 1/8 developed moderate OHSS after administration of long-acting GnRHa in the luteal phase three after oocytes retrieval, and, among the other 7, 2 declined GnRH agonist before chemotherapy. The five patients that received the first long-acting injection after egg retrieval (between 1 and 5 days) reported mild abdominal discomfort for which they had at least one follow-up visit to the fertility center, but not clinical OHSS. In group C, 1/12 patients at a high risk of OHSS developed it after administration of long-acting GnRHa (5 days after oocytes retrieval), 1 declined the GnRH agonist, and the other 10 accepted it with mild abdominal discomfort (6 patients) or without clinical symptoms (4 patients).

In both cases OHSS was moderate with abdominal distension and discomfort, ascites, mild hemoconcentration, and increased ovarian size. No thrombotic events were registered and antithrombotic prophylaxis was performed. Hospitalization was not necessary and symptoms were resolved within 2 weeks. However, the patients faced significant discomfort and chemotherapy initiation was postponed by few days.

DISCUSSION

In humans, 14-18 h of LH surge are needed to restart oocyte meiosis, while a 28-h exposure is needed to reach MII.[11,12] The midcycle LH surge, responsible for spontaneous ovulation, consists of three phases: a 14-h ascending phase, a 14-h plateau, and a 20-h descending phase.[13] Estrogen levels increase with the LH surge and then decline rapidly. Progesterone levels start to rise 12 h before the LH surge, rise for other 12 h, reach a plateau until ovulation, and then increase again reaching luteal phase levels.[14]

In patients treated for infertility, ovulation is traditionally triggered with human chorionic gonadotropin (hCG), either recombinant or purified. LH and hCG are molecularly and structurally similar (they bind to the same receptor), but hCG is pharmacologically easily available.[15] The main difference between endogenous LH and hCG is the half-live, 60 min versus >24 h,[16] with hCG having an increased luteotropic effect and an increased OHSS risk.

Numerous trials report on the efficacy of GnRHa in triggering the final oocyte maturation and inducing ovulation,[17] with increased safety and reduced OHSS risks.[18] The administration of GnRHa on an estrogen-primed woman triggers the pituitary release of FSH and LH, with a resulting LH surge able to induce ovulation. The amplitude of this surge is similar to the one seen in spontaneous ovulation; however, it has a different pattern, consisting of a shorter ascending phase of at least 4 h and a long descending phase of >20 h.[19] Estrogen levels peak during the first 12 h after GnRHa administration and gradually decline before the retrieval of oocytes. Progesterone levels similarly rise right after GnRHa administration and then fall, returning to baseline by day 5 after trigger in most cases.[20] The GnRHa triggering is also associated with lower levels of follicular and circulating vascular endothelial growth factor (VEGF)

compared with traditional hCG triggering, probably due to an increased expression of pigment epithelium-derived factor, an antiangiogenic factor that lowers secretion of VEGF and may contribute to luteolysis by reducing the blood vessel infiltration of the corpus luteum and by lowering luteal phase estrogen levels.[21] However, for the same reason, GnRHa trigger alone is insufficient for supporting the luteal phase for implantation without the addition of minimal amounts of hCG or recombinant LH.[22]

Of course, because the majority of trials are performed in patients with infertility, when an embryo transfer is planned, they focus on short-acting GnRHa and then on luteal phase support. On the contrary, in a fertility preservation cycle a swift luteolysis is welcomed, because the patient is bound to start chemotherapy as soon as possible.

Conceptually, the LH rise obtained with long-acting GnRHa triggering is sustained enough to meet the requirements for final oocytes maturation and ovulation induction, as it is consequence of the release of FSH and LH stored in the pituitary, with the exact same mechanism as short-acting GnRH. The reach and amplitude of the LH surge do not change because it is induced by the said gonadotropins stored in the pituitary. In our sample, not only mature oocytes were retrieved, but also the percentage of mature MII oocytes on the total number of oocytes collected was comparable among groups. Of all the patients in group A, up to 40.9% were at a risk of OHSS, based on the number of oocytes retrieved, but no case of clinical OHSS was reported. We have other strategies to reduce OHSS risk, such as the use of short-acting GnRHa trigger, but it does not eliminate it, especially when a subsequent long-acting GnRHa causes a flare-up on recently stimulated ovaries.[9] Even when there is no clinical evidence of OHSS, the patients experience discomfort and need for additional visits, which add stress in an already demanding time. In groups B and C some patients at a high risk of OHSS opted out of ovarian suppression with long-acting GnRHa. While the decisional process is complex and we cannot say that fear of OHSS was the only factor weighing down the option, we cannot exclude that it may have had an impact. Another possible consequence is a few days delay in the administration of GnRHa injection for the OHSS threat, with the risk of not having a complete suppression by the start of chemotherapy. The trigger with the long-acting formulation has the advantage of initiating ovarian suppression in time for chemotherapy, guaranteeing complete suppression in ~10 days.[5] In our sample, by 5 days most patients had already reached LH levels showing suppression.

Conclusion

Long-acting GnRHa triggering is efficacious in fertility preservation patients and should be proposed when ovarian suppression during chemotherapy is planned. Other than reducing OHSS risk, it simplifies the procedures in a difficult phase, reducing medicalization and promoting the prompt initiation of chemotherapy. Although the rationale for long-acting GnRHa triggering is clear, this option is rarely discussed in the fertility preservation community and even less frequently proposed to patients. The next research step for an evidence-based use should focus on obtaining multicentric data, possibly through a randomized controlled trial, to demonstrate the noninferiority of this trigger option compared with the traditional ones and if it is beneficial, compared with long-acting GnRHa after eggs retrieval, in avoiding OHSS.

ACKNOWLEDGEMENTS

Paola Anserini acknowledges the support by the Italian Ministry of Health - Ricerca Corrente (no grant number). Matteo Lambertini acknowledges the support by the Associazione Italiana per la Ricerca sul Cancro (AIRC; grant number MFAG 2020 ID 24698) and the Italian Ministry of Health—5 × 1000 funds 2017 (no grant number).

FUNDING

None declared.

DISCLOSURE

ML has acted as a consultant for Roche, Novartis, Lilly, AstraZeneca, Exact Sciences, MSD, Pfizer, and Seagen and received speaker honoraria from Roche, Novartis, Lilly, Pfizer, Takeda, Ipsen, and Sandoz outside the submitted work. All other authors have declare no conflicts of interest.

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