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EDITORIAL

Appraising the Biological Evidence for and Against the Utility of GnRHa for Preservation of Fertility in Patients With Cancer

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Tremendous advances have been made within the past 15 years in the field of fertility preservation.[1] Although embryo cryopreservation was an already available standard technique, new ovarian stimulation techniques with aromatase inhibitors[2,3] to reduce estrogen exposure in patients with breast cancer and random-start strategies[4] to shorten the delay to chemotherapy have increased the acceptability of this approach in patients with cancer. Oocyte cryopreservation emerged as an established technique for single women who did not wish to use donor sperm, and success rates justified its removal from the experimental category.[5] Ovarian cryopreservation has also shown a giant leap since its first successful use to restore ovarian endocrine function in 1999,[6] as live birth rates have exceeded 30% in those who undergo ovarian transplantation,[7,8] though it is still considered experimental by the American Society of Reproductive Medicine. Given that the recent advances in cryopreservation and transplantation techniques[7] may improve the longevity of ovarian transplants with cryopreserved tissue, and given that the number of live births is increasing exponentially, it may not be too long before ovarian cryopreservation is also added to the list of established fertility preservation procedures.

In the area of medical preservation of ovarian function, however, we have seemingly continued to spin our wheels without apparent progress. The initial idea of gonadotropin-releasing hormone analogs (GnRHa) to preserve fertility during cancer treatments came from misinterpreted observations that children are less likely to develop ovarian failure after chemotherapy. On the basis of that thought, simulation of a prepubertal hormonal milieu by pituitary suppression was proposed, to possibly guard the ovary against chemotherapy agents. This led to a number of retrospective and inadequately controlled studies, which suggested some benefit of ovarian suppression in the preservation of menstrual function during chemotherapy. In fact, recent studies with long-term follow-up showed that children are also equally vulnerable to chemotherapy-induced ovarian reserve loss; however, because of their larger ovarian reserves at the time of chemotherapy, this vulnerability is not immediately apparent in short-term follow-up.[9]

Although a number of randomized controlled studies have been performed in the past decade, none were blinded or placebo controlled. To the dismay of many, these studies also gave contradicting results, which divided physicians into camps of those who believe and do not believe in the benefit of GnRHa in preservation of fertility. In the article that accompanies this editorial, the study by Demeestere et al[10] may get us as close to the biologic truth as possible.

A small, overlooked, yet well-designed study by Waxman et al[11] was the first randomized study to investigate the benefit of ovarian suppression in patients of both genders (13 men and 18 women) with lymphoma who received gonadotoxic chemotherapy. The study, ahead of its time, used follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and gonadotropin-releasing hormone (GnRH) stimulation tests to confirm ovarian suppression. The end point for men was sperm counts, and the incidence of amenorrhea up to 3 years after chemotherapy was the end point for women. At the end of the study, all men were oligospermic, and similar proportions of women experienced persistent amenorrhea. This study and similar observations led to the abandonment of GnRHa for fertility preservation in men. Yet, for women, the argument continues 30 years later. This is, however, easy to interpret, because a simple semen analysis offers us the ability to quantify male germ cells (sperm) and left no room for ambiguity in randomized studies. In contrast, reliance on highly subjective surrogates in women, such as menstruation, for estimation of the remaining ovarian reserve and fertility left studies liable to many confounders.

Gonadotoxic chemotherapy agents cause severe DNA double-strand breaks and trigger apoptotic cell death in resting primordial follicle oocytes that make up the ovarian reserve.[12] We found some evidence, however, that oocytes are capable of mounting a DNA damage response,[13] and this may lead to the survival of some oocytes, even in the face of genotoxic stress. The varying ability of human oocytes may explain why chemotherapy does not result in the death of all follicles in all patients. Primordial follicles do not contain gonadotropin receptors,[14] and their growth is not affected by hormonal manipulations.[15] It therefore becomes clear that GnRHa cannot have any influence on primordial follicle survival in the face of DNA damaging, non–cell-cycle-dependent chemotherapy agents (Fig 1). A recent translational study has already proven this point. That study showed in ex vivo and in vitro models of human ovary and granulosa cells that the coadministration of GnRHa does not confer protection against DNA damage and

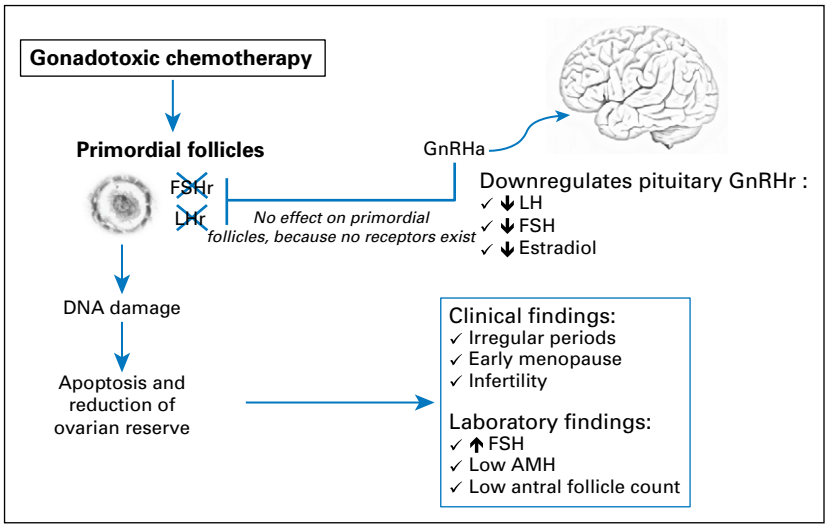

Fig 1. Impact of gonadotoxic chemotherapy and gonadotropin-releasing hormone analog (GnRHa) on ovarian reserve and function. Gonadotoxic chemotherapy reduces ovarian reserve, which is made up of resting and hormone-insensitive primordial follicles, by induction of DNA damage and apoptotic death. GnRHa reduces pituitary gonadotropin-releasing hormone (GnRH) production and, as a result, blocks the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary, which in turn results in the cessation of late-stage follicle development. Because primordial follicles do not have FSH, LH, or GnRH receptors, GnRHa cannot have a direct influence on ovarian reserve. AMH, anti-Müllerian hormone; FSHr, FSH receptor; LHr, LH receptor; GnRHr, GnRH receptor.

apoptotic follicle death caused by numerous gonadotoxic chemotherapy agents.[16] These molecular findings also rule out any theoretical indirect protective effects on ovarian follicles, as speculated by some.

Why then do some of the highly publicized studies[17] conclude a benefit of GnRHa for ovarian protection? We have recently performed a quality-based analysis of the literature and discussed the reasons in detail elsewhere,[18] so we will be brief here. In general, studies that showed benefit used amenorrhea or menstrual resumption rates as primary outcome measures in assessment of the success of GnRHa suppression. This is troublesome, because, in these young women with relatively short follow-up times, irregular menstruation, rather than amenorrhea, is the most common symptom of premature ovarian failure (POF). The most established definition of POF is irregular periods or amenorrhea with FSH levels greater than 40 mIU/mL in women younger than age 40 years. None of the studies, except the current, original study by Demeestere et al,[10] has used an appropriate definition of POF. Because the previous studies in general relied on patient self-descriptions of a normal period and did not compare the actual frequency of periods between the treatment and control groups, there is a significant potential for reporting bias in these unblinded, non–placebo-controlled studies.

However, other studies used a number of quantitative ovarian reserve markers, such as FSH, anti-Müllerian hormone, and antral follicle counts, as secondary outcome measures. None of those studies showed any reproductive benefit from GnRHa suppression in women with hematologic malignancies or breast cancer. This assessment is carefully summarized in a recent meta-analysis[19] and additionally discussed in a letter.[20]

What about fertility as the outcome measure? It is intuitive to assume that the real gauge of a fertility preservation technique is the pregnancy rate. However, this is not as easy to gauge as it sounds. To be able to assess the fertility preservation potential of a hormonal method, one has to look at the reproductive-lifetime fertility potential in those who are actively attempting pregnancy. As can be seen from the current report by Demeestere et al[10], spontaneous pregnancy and live birth can occur even in patients who meet the biochemical criteria for POF. This is not biologically surprising, because, despite the low reserve in a young post-chemotherapy patient, the remaining oocytes still possess age-appropriate high quality, which permits conception in the short run. However, if these patients were observed for their entire reproductive lives, a difference would emerge in the total number of pregnancies in these patients compared with in a healthy population of women who also were attempting pregnancy, because the former group would exhaust their ovarian reserves sooner. Furthermore, the studies of GnRHa suppression studies were not blinded, so those patients who received GnRHa might have been more motivated to attempt pregnancy; in fact, this seemed to be the case in the POEMS (Prevention of Early Menopause Study) trial. There was a trend for a higher proportion of women who attempted pregnancy in the GnRHa group, and, when we recalculated the pregnancy rates on the basis of those who expressed intent for conception, there was not statistical difference between the groups.[21] The study by Demeestere et al[10] also showed us that some of the study participants may be receiving infertility and assisted reproductive technology treatments—a detail that was hardly reported in past randomized studies, despite the fact that it could significantly skew the outcomes.

It has been shown that a publication bias does exist,[22] and cancer trials with positive outcomes are more likely to be published in high-impact journals[23]; *Journal of Clinical Oncology* must be commended for going against this trend. The study by Demeestere et al[10] has numerous strengths, and it eludes the majority of weaknesses cited above. It used an established biologic description of POF with long-term follow-up; it was the only randomized study with that healthy combination. It validated its findings by secondary outcome measures, such as the lack of diminished ovarian reserve reflected by serum FSH levels less than 15 mIU/mL, longitudinal serum anti-Müllerian hormone measurements, and fertility rates, all of which were in agreement with the primary outcome measure. Moreover, the study proved its internal consistency by showing the positive correlation of POF with age and cyclophosphamide dose.

Gonadotoxic chemotherapy agents cause ovarian damage via the common mechanism of DNA damage, regardless of the underlying cancer type. From a biologic point of view, we cannot

expect GnRHa to be ovarian protective in one cancer type (eg, breast cancer) and not in another (eg, lymphomas). The differential results originate not from the differences in biology but from the variations in methodology. Patients with lymphoma are often young and receive highly gonadotoxic chemotherapy. In trials of breast cancer, the patients are often older, many within the range of normal menopause, and are subjected to a variety of regimens with or without tamoxifen treatment. Tamoxifen, an ovarian stimulant, can also cause menstrual abnormalities. The patient populations in trials of lymphoma may yield more uniform study groups with fewer confounders, which can explain the better agreement about the lack of benefit of GnRHa for ovarian preservation in that population.

A medical, noninvasive method of fertility preservation would be highly desirable for patients. However, when carefully scrutinized from a scientific point of view and considered together with ovarian biology, ovarian suppression by GnRHa is highly unlikely to be the answer, especially given the current strong study by Demeestere et al.[10] We must not despair, though; potential agents, such as the sphingosine-1-phosphate,[24] are in preclinical development. When confirmed in phase I trials, these agents may give us the medical fertility preservation options for which we have been searching for the past 30 years. In the meantime, let's not sway our patients away from established fertility preservation techniques.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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