The Impact of Depot GnRH Agonist on AMH Levels in Healthy Reproductive-Aged Women

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Context: GnRH agonists (GnRHa) are being used experimentally in an attempt to preserve fertility in young female cancer patients undergoing chemotherapy. Anti-Müllerian hormone (AMH) produced by ovarian granulosa cells may serve as a marker of ovarian reserve, but it is not clear whether this marker is useful during GnRHa treatment.

Objective: The purpose of this study was to determine the effect of a depot GnRHa on AMH levels.

Design: Depot leuprolide (3.75 mg) was administered in the midluteal phase (MLP) in healthy women. Assessments of AMH, FSH, LH, estradiol, and progesterone were performed in the early follicular phase (EFP) and MLP before GnRHa treatment and approximately 7, 14, and 30 days after GnRHa administration.

Setting: The study was conducted in a university research center.

Patients: Participants were 33 healthy, premenopausal women aged 18 to 45 years old with regular menses.

Results: EFP and MLP AMH levels were similar before GnRHa administration. Relative to MLP AMH levels, AMH decreased 7 days after GnRHa administration by a median of 24% (P < 0.001) and then increased above pretreatment levels 14 and 30 days after GnRHa by 13% and 32%, respectively (P < 0.001). Changes in AMH levels did not correlate with changes in gonadotropins, estradiol, or progesterone.

Conclusions: Significant changes in AMH levels occur in the first 4 weeks after depot leuprolide administration, suggesting that AMH may not be a reliable marker of ovarian reserve during this interval. Changes in AMH occurred independent of gonadotropin levels, supporting a direct effect of GnRHa on granulosa cell expression of AMH or an indirect effect of GnRHa on the development and/or dynamics of the follicle pool. (J Clin Endocrinol Metab 98: E1961–E1966, 2013)

Anti-Müllerian hormone (AMH) is a glycoprotein expressed by granulosa cells in preantral and small antral ovarian follicles. AMH levels provide an indication of the size of the growing follicle pool, with expression disappearing as antral follicles develop (1). Because of the correlation of AMH levels with outcomes of assisted reproduction, fecundability, and menopause, AMH is a commonly used biomarker of ovarian reserve in reproductive-aged women (2–4).

In young female patients with cancer, GnRH agonists (GnRHa) are being used experimentally to preserve fertility (5). With administration of GnRHa, FSH levels cannot be used to measure ovarian function because of gonadotropin down-regulation. AMH levels appear rela-
tively stable across the menstrual cycle (6) and with administration of birth control pills (7, 8), suggesting gonadotropin independence. AMH might therefore be a useful biomarker of ovarian reserve with GnRHa treatment.

The use of GnRHa for fertility preservation during chemotherapy is controversial because of inconclusive outcome data on fertility (9) and because the mechanism by which GnRHa may act to preserve fertility is unknown. The major known function of GnRHa is to suppress gonadotropins, acting indirectly on ovarian follicles. However, recent in vitro studies demonstrating that GnRH modulates expression of GnRH and GnRH receptors in human luteinized granulosa cells and induces granulosa cell apoptosis (10, 11) raise the possibility that GnRHa may also act directly at the ovary. Determining the effect of GnRHa on AMH levels is an essential prelude to use of AMH as a marker of ovarian reserve in cancer patients receiving GnRHa.

The study objective was to examine the response of AMH in normal women over a 4-week exposure to depot leuprolide, a GnRHa used widely in patients with cancer. Because small, growing ovarian follicles that express AMH are not gonadotropin dependent, we hypothesized that AMH levels would not be altered by the initial flare or subsequent down-regulation of pituitary gonadotropins by GnRHa.

Subjects and Methods

The study was approved by institutional review boards at Massachusetts General Hospital and University of California, San Diego. Participants were healthy, premenopausal women aged 18 to 45 years with monthly menstrual cycles, were ovulatory with older age (P < .01) but was not associated with BMI or race (P > .05). The median (IQR) days from GnRHa administration were 7 (1) days for GnRHa + 7, 14 (1) days for GnRHa + 14, and 30 (7) days for GnRHa + 30 time points.

FSH, LH, E2, and P4 levels from all time points are depicted in Figure 1. Before GnRHa administration, FSH and LH levels were lower in the MLP than in the EFP (P < .001). Compared with MLP levels, FSH was reduced further after GnRHa administration at GnRHa + 7 (P < .001) and GnRHa + 14 (P < .001). At 30 days, there was partial recovery of pituitary function, because FSH levels approached MLP levels (P = .69). After GnRHa administration, LH levels at 14 and 30 days were significantly

Results

There were 33 participants with a mean (SD) age of 30.3 (8.5) years. Mean body mass index (BMI) (SD, range) was 25.7 (4.7, 18.4–36.7) kg/m². The cohort included 20 (61%) whites, 6 (18%) blacks, and 7 (21%) other/not reported; 6 (18%) were Hispanic. None had infertility. Average cycle length (SD) was 28 (2) days. Median (IQR) cycle days of EFP and MLP blood samples were 6 (3.5) and 22 (2), respectively. MLP AMH was significantly lower with older age (P = .02), non-Hispanic ethnicity (P = .01), and shorter cycle length (P = .01) but was not associated with BMI or race (P > .05). The median (IQR) days from GnRHa administration were 7 (1) days for GnRHa + 7, 14 (1) days for GnRHa + 14, and 30 (7) days for GnRHa + 30 time points.

FSH, LH, E2, and P4 were measured by chemiluminescent immunoassays (Architect; Abbott Diagnostics) with assay sensitivities of 0.3 IU/L, 0.7 IU/L, and 0.1 ng/mL and interassay CVs of <6%, <7%, and <5% for LH, FSH, and P4, respectively (6, 13). Estradiol (E2) was measured by liquid chromatography/mass spectrometry with an 8.6% interassay CV (Mayo Medical) (14).

Analyses were conducted using Stata (StataCorp). Because only AMH levels met normality assumptions, nonparametric testing was used for analysis. AMH levels were compared among all time points by the Friedman nonparametric repeated-measures test, followed by Wilcoxon signed rank tests for pairwise comparisons between MLP levels and levels from (1) pre-GnRHa EFP, (2) GnRHa + 7, (3) GnRHa + 14, and (4) GnRHa + 30. MLP levels were used as baseline values for post-GnRHa changes because of temporal proximity to GnRHa exposure. Correlations between percent changes in AMH from midluteal levels to the 3 post-GnRHa intervals (GnRHa + 7, GnRHa + 14, and GnRHa + 30) and percent changes in FSH, LH, E2, P4, and baseline covariates were assessed by Spearman ρ for continuous variables and linear regression for discrete variables. Statistical tests were 2-tailed. Values of P < .05 were considered significant. Data are presented as medians (interquartile range [IQR]) unless otherwise stated.

An a priori sample size calculation was based on prior studies of AMH in premenopausal women, where log-transformed mean AMH (SD) was 0.69 (0.75) ng/mL (4). With 33 participants, 80% power, and an α error of .05, the detectable difference would be 0.43 SD.
decreased compared with midluteal levels \((P < .001)\). In response to GnRHa, \(E_2\) and \(P_4\) levels declined sharply from MLP levels \((P < .001)\) with the lowest values apparent at 14 and 30 days after GnRHa.

AMH levels were not significantly different between the follicular phase and MLP (median [IQR], 2.4 [3.2] ng/mL vs 2.4 [2.9] ng/mL, respectively; \(P = .67\)). After GnRHa administration, AMH levels declined significantly from the MLP (median [IQR], 2.4 [3.2] ng/mL) to GnRHa+7 (1.7 [2.3] ng/mL, \(P < .001\)), representing a median decrease of 24\% (Figure 2). AMH levels subsequently rose to a median (IQR) of 2.5 (2.5) ng/mL at GnRHa+14 (\(P = .09\)) and 3.1 (2.6) ng/mL at GnRHa+30 (\(P < .001\)), 13\% and 32\% higher than midluteal values. The pattern of change with GnRHa exposure was similar within each group when participants were dichotomized according to the median MLP AMH (2.4 ng/mL) (data not shown). There were no associations of change in AMH in response to GnRHa with age, race, ethnicity, BMI, or cycle length (all \(P > .05\)).

The decline in AMH from the MLP to GnRHa+7 was associated with concurrent decreases in \(E_2\) \((r = 0.46, P = .01)\) and \(P_4\) \((r = 0.57, P = .002)\) but not with concomitant changes in FSH \((r = -0.15, P = .43)\) or LH \((r = -0.28, P = .13)\). Increases in AMH from 7 to 14 days and from 14 to 30 days after GnRHa administration were unrelated

![Figure 1.](https://example.com/figure1.png)

**Figure 1.** FSH (A), LH (B), estradiol (C), and progesterone (D) expressed as a percentage of MLP levels obtained in the follicular phase, MLP, and approximately 7 days (GnRHa+7), 14 days (GnRHa+14), and 30 days (GnRHa+30) after administration of the depot GnRHa leuprolide (3.75 mg im), indicated by the dashed vertical line \((n = 33)\). REF, reference. Data are depicted as medians with 25th to 75th percentile values, and absolute levels are reported immediately below the graph. *, \(P < .0001\) vs MLP level.

![Figure 2.](https://example.com/figure2.png)

**Figure 2.** AMH expressed as a percentage of MLP levels in the follicular phase, MLP, and approximately 7 days (GnRHa+7), 14 days (GnRHa+14), and 30 days (GnRHa+30) after administration of the depot GnRHa leuprolide (3.75 mg im), indicated by the dashed vertical line \((n = 33)\). REF, reference. Data are depicted as medians with 25th to 75th percentile values, and absolute levels are reported immediately below the graph. *, \(P < .0001\) vs MLP level.
to concurrent changes in FSH, LH, E₂, or P₄ (all \( r < 0.26 \), all \( P > .18 \)).

**Discussion**

In reproductive-aged women undergoing depot leuprolide treatment, AMH levels changed dynamically across the initial 4 weeks after midluteal administration. AMH levels reached the observed nadir after 7 days of GnRHa and then increased progressively to levels exceeding baseline midluteal levels by 14 and 30 days of GnRHa. Whereas lower AMH levels were observed in the MLP in older and in non-Hispanic women, this biphasic response of AMH response to GnRHa was observed regardless of age, pre-treatment AMH levels, or ethnicity. These data indicate that AMH is not likely to be a reliable marker of ovarian reserve in patients with cancer over the first month of GnRHa treatment. Our data also show that changes in AMH levels did not correlate with concurrently measured LH and FSH levels, raising the possibility of a direct, gonadotropin-independent effect of GnRHa on granulosa cells.

The role of gonadotropins and GnRHa in modulation of AMH secretion is controversial. GnRHa administration causes an initial increase in LH and FSH, which can stimulate follicle development and E₂ secretion (flare), followed by gonadotropin desensitization and sustained suppression of gonadotropins and ovarian steroids. The initial agonist-induced flare is minimized with luteal phase administration, as used in this protocol, because of suppression of the FSH flare by the negative feedback of E₂ and inhibin A on FSH. AMH measured weekly in the first month after GnRHa administration demonstrated a biphasic response, helping to resolve apparent conflicting results of previous studies in which AMH was measured at different time points during GnRHa administration (15). In this study, AMH levels were significantly lower 3 and 6 months after institution of GnRHa AMH levels suggest some degree of gonadotropin independence. Consistent with prior reports (6), AMH levels were stable between follicular and luteal phases, whereas FSH and LH levels were lower in the MLP. In addition, neither the initial decline nor subsequent rise in AMH levels after GnRHa administration correlated with changes in gonadotropins. GnRH receptors are expressed by human granulosa cells and up-regulated, in part, by GnRH (10). The AMH decrease after 7 days of treatment may have resulted from up-regulation of GnRH receptors combined with the antiproliferative and apoptotic effects of short-term GnRHa exposure on granulosa cells (10, 11). Rodent studies showing that granulosa cell GnRH receptor expression declines with longer GnRHa administration may explain the subsequent increase we observed in AMH levels starting at 14 days of GnRHa. The observed decrease in AMH at 7 days may have allowed an expansion of AMH-secreting preantral and small antral follicular pools, resulting in subsequent increases in AMH levels at days 14 and 30 (20). Whether GnRHa directly affects only granulosa cells or modulates follicle dynamics is key to discerning whether GnRHa administration is likely to protect fertility in patients with cancer undergoing chemotherapy.

In the current study, we did not detect a gonadotropin flare but did not sample until 7 days after GnRHa. Therefore, an alternative explanation for the observed AMH changes is that the initial decrease in AMH resulted from an undetected gonadotropin flare, which advanced follicular pools, resulting in subsequent increases in AMH levels.
ular development in gonadotropin-responsive larger antral follicles, thereby reducing AMH (15). After the flare, suppression of FSH and LH precluded additional advancement of FSH-dependent follicles, thereby increasing the total number of gonadotropin-responsive follicles secreting AMH. More frequent blood sampling immediately after GnRHa administration to demonstrate a flare with a concomitant decrease in AMH levels would test this hypothesis.

An important strength of this study is that subjects served as their own controls, providing AMH levels from the EFP and MLP before GnRHa administration as evidence of stability of AMH across the menstrual cycle and in sharp contrast to the changes in AMH observed after GnRHa administration. Several limitations must be considered. Longer follow-up is needed to determine whether AMH levels become partially suppressed again with prolonged exposure, as suggested previously (18, 21). Follow-up data are important for interpreting ovarian reserve measurements in young female patients with cancer who are receiving prolonged GnRHa therapy. This study did not include antral follicle counts, so changes in follicular populations can only be surmised using AMH levels. However, others have not detected an increase in the number of smaller follicles concurrent with elevated levels of AMH after 14 days of GnRHa (15), suggesting that changes in the follicle population may occur below ultrasound resolution.

This study provides evidence that dynamic changes in AMH levels occur in the first 4 weeks after depot leuprolide administration, suggesting that AMH will not be a reliable marker of ovarian reserve in patients with cancer receiving GnRHa during this time period. The clinical question of how to measure ovarian reserve in women receiving prolonged GnRHa treatment remains unanswered. However, the finding that fluctuations in AMH levels occurred independent of gonadotropins, whether the result of a direct effect of GnRHa on granulosa cell AMH expression and/or an indirect effect of GnRHa on follicle pool development and/or dynamics, supports continued efforts to elucidate the role of GnRHa in fertility preservation.

Acknowledgments

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References


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