Serum markers of ovarian reserve and ovarian histology in adult mice treated with cyclophosphamide in pre-pubertal age

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Abstract

Purpose AMH is used to quantify the extent of follicular pool in postpubertal women, but its value after chemotherapy is unclear. We tested AMH as a marker of follicular reserve in adult mice treated with cyclophosphamide (CTX) in prepubertal age.

Methods Mice received placebo or CTX at age 18 days. AMH and FSH were assessed on day 43, 56, and 95 of life. Ovaries were fixed in formalin, embedded in paraffin, and stained with H&E and TUNEL. Follicular apoptosis was graded.

Results All mice exposed to CTX had a decreased number of follicles/mm² and significantly decreased AMH, but only 48 % of pubertal and 81 % of adult mice had increased FSH. Over time, there was an increase in FSH ($p < 0.05$), but not a concurrent decrease in AMH, while in controls, FSH remained stable and AMH decreased. There was no correlation between histological and serological markers.

Capsule AMH is a marker of quantitative, and FSH of qualitative, residual ovarian function after cyclophosphamide administration to pre-pubertal mice.

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Conclusions CTX administration to pre-pubertal mice caused various degrees of residual function, which were reflected by FSH, but not by AMH or by the number of ovarian follicles. AMH served as a marker of quantitative, and FSH of qualitative, residual ovarian function.

Keywords Animal study · Prepubertal · Cyclophosphamide · AMH · FSH · Follicle count

Introduction

Folliculogenesis in the mammalian ovary is characterized by follicular assembly to create primordial follicles, which consist of one oocyte surrounded by a monolayer of flat granulosa cells, and their maturation: primordial follicles will gradually grow into primary, secondary, and tertiary follicles by granulosa cell proliferation and fluid accumulation [20]. Follicular assembly is believed to be complete in utero. The initial stages of follicular assembly and maturation are regulated by still mostly unknown paracrine factors. These factors are determinant in the assembly of primordial follicles and their progression to the stage of secondary follicles. It is only during the last stages of maturation that the follicles are under the control of circulating factors such as FSH (follicle stimulating hormone) and develop into tertiary follicles (or antral follicles), which will progress in their growth until the selection of the dominant follicle. Only the oocyte contained in the dominant follicle will be released through the ovulation process.

Gonadotoxicity is a well-known side effect of cancer therapy in children and adult survivors and ovarian damage seems to be age-dependent [3,12,23]. In pre-pubertal girls younger than 10 years of age, ovarian function may be retained in 50 %, contrasted with girls older than 10 years who uniformly

experience acute ovarian failure after chemo/radiotherapy. In humans, the alkylating agent cyclophosphamide (CTX) was shown to be the agent most often implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner [18,28]. The pathologic mechanism by which CTX damages the ovary has been shown to be the apoptosis of granulosa cells and oocytes [9].

Anti-Müllerian hormone (AMH) is produced by granulosa cells. Its production starts at the stage of primary follicles, soon after the columnar differentiation from the flat granulosa cells of primordial follicles [9]. Production of AMH is maximum in secondary follicles, gradually decreases in early tertiary follicles, and is nil in antral tertiary follicles, when the follicles are primarily under FSH control. AMH is believed to exert an inhibitory role in the recruitment and development of primordial follicles into developing follicles. In fact, in engineered AMH-null mice the ovaries contain almost three-fold greater number of growing secondary follicles and a decreased number of primordial follicles [9]. This holds true for both the pre-pubertal and post-pubertal mice, and indicates that in the absence of AMH, primordial follicles are recruited at a faster pace. Further, it has been seen that in granulosa cell cultures AMH attenuates the FSH-dependent increase in aromatase activity and LH (luteinizing hormone) receptor expression, thus inhibiting FSH-dependent follicular growth [8]. Thus, AMH seems to downregulate two important steps of follicular development: follicle recruitment and cyclic selection for dominance [9,10]. Mouse studies have shown that AMH serum levels reflect the size of the primordial follicle pool and its reduction with aging [13,27]. In addition, human studies have shown that AMH directly correlates with the number of antral follicles assessed by ultrasound [5] and FSH, inhibin-B and estradiol [11]. Despite these results in human studies, serum AMH was found to have no correlation with the extent of germ cell destruction (primordial follicles) in the post-pubertal mouse when measured 10 days after CTX administration [2].

FSH is the most important circulating hormone that stimulates follicular growth and development in post-pubertal mammals. Estradiol and inhibin-B produced by the granulosa cells contribute to the feedback loop of the pituitary-ovarian axis to suppress FSH secretion. The role of FSH is to rescue some of the secondary follicles initially recruited and developed out of the primordial pool. Under FSH stimulation, the increased aromatase activity will promote estrogen production by conversion from androgens produced in the surrounding thecal cells, thus preventing apoptosis. Follicular dependency on FSH is believed to begin at the stage of secondary follicles, but it has been hypothesized that FSH may stimulate primary follicles, [17] thus making the interaction between AMH and FSH in controlling follicular differentiation and growth more complex.

FSH and AMH have been interchangeably used in childhood cancer survivors to assess ovarian reserve after

chemotherapy and radiotherapy, where they correlate with a decreased ovarian volume [1]; however, no study has evaluated the two markers in the same study population. In addition, no human or animal study to date has evaluated the impact of CTX on the ovary when administered to pre-pubertal subjects.

The aim of our study was characterization of a murine model for studying cancer treatment exposure during childhood and its effects on female reproduction, evaluate ovarian histological development through puberty, and correlate ovarian histology with serum AMH and FSH as markers of residual ovarian reserve.

Materials and methods

Pre-pubertal (postnatal day 18) C57BL/6 J female mice (Jackson Laboratory, Bar Harbor, Maine, USA) were randomized to receive placebo, or CTX 120 mg/kg, which was administered with a single intraperitoneal injection (see Fig. 1 for a study design flowchart). The dosage of CTX used was based on previous studies, which demonstrated a significant dose-dependent ovarian toxicity [16,19]. Mice were euthanized when still pubertal (56 days of life) or when adult (95 days of life). The study was approved by the IACUC office at the University of Tennessee Health Science Center at Memphis, TN (protocol number 1980, approved in May 2011).

In the mouse strain C57BL/6 J, puberty is characterized by vaginal opening at approximately 25 days of life, estrus 3–4 days later, and subsequently by regular cycling every 5–6 days, which culminates with mating. Adulthood is considered to be reached at 90 days of life (3 months). Mice were housed in the Animal Center at UTHSC under a 12-h light/dark cycle with food and water ad libitum. The mice euthanized at age 95 days (groups G1₍₉₅₎ and G2₍₉₅₎) underwent blood collection from the retro-orbital plexus venosus at age 43 days (24 days after CTX or placebo administration), and again at the time of euthanasia (76 days after CTX or placebo administration). The mice euthanized at age 56 days (G1₍₅₆₎ and G2₍₅₆₎) underwent blood collection at the time of euthanasia (37 days after CTX or placebo administration), when in late puberty. Serum testing for FSH (follicle stimulating hormone), AMH, and estradiol levels was performed in triplicate with ELISA in a specialized laboratory (DS Biotech, Detroit, MI). Mouse ELISA Kits for AMH (CSB-E13156m) and FSH (M7581) were purchased at Life Sciences Advanced Technologies, Inc. (St Petersburg, Florida, USA) and Biotang (Waltham, MA), respectively. Standards were performed in duplicate, samples in triplicate. Assays were performed according to the manufacturer's protocol and concentrations of AMH and FSH were determined from the standard curve. AMH and FSH measurements were used to characterize ovarian reserve as diminished versus normal. Ovarian function insufficiency

Fig. 1 Flowchart of mouse allocations into the groups

was defined, as previously reported, as decreased ovarian reserve (DOR) by FSH values greater than 15 mIU/ml, and primary ovarian insufficiency (POI) by FSH values greater than 30 mIU/ml [7]. However, in the current study, we unified these two categories in a broader entity called ‘ovarian insufficiency’ and defined it by FSH values greater than 15 mIU/ml.

After dissection, one ovary per mouse was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; ApopTag® Kit S7101, Millipore Corporation, Billerica, MA, USA) to study cellular apoptosis by detecting DNA fragmentation. Five μ m-thick sections were serially cut and every 5th section analyzed for follicular counts. At least three ovaries from different animals were obtained from each group, and at least four sections were analyzed in each ovary. The computerized program used for image analysis, Spectrum (Version 10.2.2.2314; by Aperio, Vista, California, USA), allowed us to examine all the sections at the same time and to accurately count all the follicles in each section without redundancy. One oocyte surrounded by a monolayer of flat or cubic granulosa cells enclosed by a basement membrane would identify a primordial and a primary follicle, respectively. Secondary follicles were identified with one oocyte surrounded by multiple cubic granulosa cells, and tertiary follicles with fluid accumulation amid the granulosa cells, enclosed by a basement membrane. A corpus luteum (CL) was identified with a not-well defined conglomerate of larger and clearer (on H&E staining) granulosa cells blended with capillaries without a distinct basement membrane (with a variable degree of cellular apoptosis on TUNEL immunostaining). The total number of primordial, primary, secondary, tertiary follicles, and corpora lutea in each section was divided by the section area in order to calculate the

number of follicles/mm². Figure 2a demonstrates the different ovarian follicles. The rate of apoptosis (%) was calculated as the number of apoptotic follicles/total number of follicles/mm² × 100. Figure 2b and c show apoptosis in an ovarian section and in an ovary after CTX exposure, respectively.

Because the variables investigated were normally distributed, we used t-tests and two-way ANOVA with post-hoc Bonferroni corrections (SPSS 20.0, Armonk, New York) to compare means in the different groups. We used Pearson correlations to test the relationship between variables and 95 % confidence intervals (95 % CI) to define internal estimates associated with each probability. Data was expressed as mean $\pm$ 95 % CI. Significance was defined as $p < 0.05$.

Results

Data from 41 mice were analyzed in our study. Body weight and length and BMI did not differ between groups at the time of the respective euthanasia dates (mean BMI = 24.1 g/cm², 95 % CI: 22.8–25.4 in groups 1₍₉₅₎ and 2₍₉₅₎, and 22.7 g/cm², 95 % CI: 21.6–23.9 in groups 1₍₅₆₎ and 2₍₅₆₎).

Serologic findings

After CTX exposure, 48 % (14/29 mice) of the mice sampled during the pubertal transition, on day of life 43 and 56, showed ovarian function insufficiency, as defined by FSH values greater than 15 mIU/ml. When sampled as adults on day 95 of life, 81 % (13/16 mice) showed ovarian function insufficiency, all with FSH values greater than 30 mIU/ml (average 94.7, 95 % CI: 76.8–120.0). FSH and AMH mean values in the two groups sampled at age 43, 56, and 95 days of life, are reported in Table 1. AMH levels were not measured in

Fig. 2 **a** Types of ovarian follicles: PDF = primordial follicles, PMF = primary follicles, SEF = secondary follicles, TEF = tertiary follicles. **b** Ovarian section from CTX-treated mouse stained with H&H and TUNEL. **c** Ovary from CTX-treated mouse treated with TUNEL immunostaining

the control group at age 43 days (G1₍₉₅₎) due to low remaining blood for this analysis. As expected, FSH levels were higher in the CTX-exposed groups that developed DOR or POI ($p < 0.05$). Similarly, AMH levels were significantly lower in the CTX-exposed groups ($p < 0.05$).

Figure 3 shows serum FSH (A), AMH (B), and total follicle count (C) trends in the same mice sampled at age 43 and 95 days in controls and CTX-exposed mice (G2₍₉₅₎): from early puberty to adulthood, serum FSH remains stable in controls, but increases in CTX-exposed mice while serum AMH and follicle count decrease in controls (because of missing values, for graphic purposes, we used the average AMH and follicle count values in controls at age 56, instead of age 43), but remain low in CTX-exposed mice. This reflects the fact that the CTX-exposed follicles, while maintaining a constant AMH production, become progressively unable to produce a normal amount of estrogen (which only developing

follicles can produce), thus eliciting soaring FSH production. To support this presumption, we obtained estradiol levels in a subgroup of 13 mice at age 43 days, 6 controls and 7 CTX-exposed, 4 of which developed ovarian insufficiency by FSH levels: estradiol levels were no different regardless of FSH, AMH levels and follicle counts (controls, 9.3 pg/ml, 95 % CI 7.5–11.2; CTX-exposed, 9.9 pg/ml, 95 % CI 7.5–11.4; n.s.).

Pearson's correlation analyses found no correlation between the serum levels of FSH and AMH at any age, in both controls and CTX-exposed mice.

Histological findings

There was no difference in ovarian surface area between CTX-treated and non-treated mice in either age group. However, there was an overall decreased number of follicles/mm² in the CTX-exposed mice, regardless of

Table 1 FSH and AMH mean values in groups G1 and G2 sampled at age 43, 56, and 95 days of life

	GROUP 1 Control (95 % CI)	GROUP 2-Normal-FSH FSH <15 IU/ml (95 % CI)	GROUP 2-Ovarian Insufficiency FSH >15 IU/ml (95 % CI)
FSH (mIU/ml)			
Sampling at age 43 days	7.5 ^{bc} (7.0–7.9), $n=6$	7.5 ^c (7.1–7.9), $n=10$	46.4 ^c (27.3–67.5), $n=6$
Euthanasia at age 56 days	5.8 ^{abc} (3.6–8.0), $n=6$	11.3 ^c (9.5–12.9), $n=5$	28.4 ^c (22.1–35.4), $n=8$
Euthanasia at age 95 days	3.4 ^{abc} (1.7–5.0), $n=6$	1.8 ^c (1.2–2.6), $n=3$	94.7 ^c (86.3–103.1), $n=13$
AMH (ng/ml)			
Sampling at age 43 days	—	4.0 ^c (3.7–4.4), $n=10$	0.5 ^c (0.4–0.7), $n=6$
Euthanasia at age 56 days	6.5 ^{ab} (4.9–8.1), $n=6$	1.5 ^c (0.3–1.8), $n=5$	2.5 ^c (0.8–2.9), $n=8$
Euthanasia at age 95 days	3.5 ^{bc} (2.7–4.4), $n=6$	2.5 ^c (1.5–3.6), $n=3$	0.9 ^c (0.7–1.2), $n=13$

Ovarian insufficiency is defined by FSH >15 mIU/ml [7]

^a $p < 0.05$, G1 vs. G2 Normal FSH; ^b $p < 0.05$, G1 vs. G2 ovarian insufficiency; ^c $p < 0.05$, G2 Normal FSH vs. ovarian insufficiency

Fig. 3 AMH (a), FSH (b) and total follicle count (c) trends in controls, $n=6$, and in the CTX-exposed subgroup of mice, $n=16$, sampled at 43 and 95 days of life (24 and 76 days post-CTX exposure); $*p<0.05$

whether they demonstrated ovarian insufficiency or not. In fact, there was no difference in the total number of follicles/mm² between mice that had normal FSH and those that developed ovarian insufficiency after exposure to CTX. Despite an overall decreased follicle number at age 56 and 95, the difference was significant only in the mice sacrificed in late puberty ($G2_{(56)}$), which received the higher CTX dose (Fig. 4a). These results were due to the higher number of follicles in pubertal compared to adult, and not to the higher CTX dose used in this group, since the number of residual follicles was similar in the two treated groups, $G2_{(56)}$ and

$G2_{(95)}$. Primordial and primary follicles suffered the greatest decrease in number, -80% and -68% , respectively, while secondary follicles and tertiary follicles decreased by about 50% (Table 2).

No apoptosis was noted in primordial and primary follicles, irrespective of CTX exposure. The rate of apoptosis in secondary follicles was nil in controls and, among the CTX-treated mice, was detectable only in those sacrificed in late puberty ($G1_{(56)}$ and $G2_{(56)}$). Instead, in the mice sacrificed as adults, secondary follicles showed some degree of apoptosis, in both treated and untreated mice (average 41% , 95% CI 34% –

Fig. 4 Follicular counts in the mice euthanized at age 56 (**a**) and 95 days (**b**); PDF = primordial follicles, PMF = primary follicles, SEF = secondary follicles, TEF = tertiary follicles, * $p < 0.05$

47 %). The rate of apoptosis in tertiary follicles was similar across all groups, in both controls and CTX-exposed, whether they developed ovarian insufficiency or not (average 53 %, 95 % CI 46–60 %). Corpora lutea were present in all ovaries, irrespective of CTX exposure and development of DOR or POI, and the number was no different in all groups (2.3/mm², 95 % CI 0.9–3.7, in G1₍₉₅₎ and 1.2/mm², 95 % CI 0.4–1.5, in G1₍₅₆₎ versus 2.2/mm², 95 % CI 1.7–2.6, in G2₍₉₅₎, and 2.0/mm², 95 % CI 0.9–3.1, in G2₍₅₆₎).

There was no relationship between serum levels of FSH and AMH and follicular count in the controls at both time points after CTX administration (56 and 95 days of life).

Similarly, there was no correlation between AMH and the follicular count in the CTX-exposed ovaries, even when broken down in the FSH-level subgroups in both age groups.

Discussion

CTX treatment in pre-pubertal mice causes follicular destruction and ovarian hormonal dysfunction, but does not preclude somatic development. Our results show that in mice treated at a pre-pubertal age and sacrificed in late pubertal or adult age,

Table 2 Follicle numbers in controls and CTX-treated mice (at least three ovaries from different animals from each group, and at least four sections analyzed in each ovary)

Group	PDF/mm ² (95 % CI)	PRF/mm ² (95 % CI)	SEF/mm ² (95 % CI)	TEF/mm ² (95 % CI)	Total Follicles/mm ² (95 % CI)
G1 (Control, 95 days)	0.7 (0.2–1.2)	0.9 (0.5–1.2)	1.8 (0.9–2.6)	0.8 (0.9–1.4)	4.2 (2.6–5.7)
G2 (120 CTX, 95 days)	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.8 (0.3–1.2)	0.3 (0.1–0.4)	1.8 (1.2–2.4)
<i>p</i> -value	ns	ns	ns	ns	ns
G1 (Control, 56 days)	1.6 (1.4–1.8)	1.5 (1.2–1.9)	1.8 (1.0–2.6)	1.3 (1.1–1.5)	6.2 (5.1–7.3)
G2 (200 CTX, 56 days)	0.3 (0.1–0.5)	0.5 (0.2–0.4)	0.9 (0.8–1.1)	0.6 (0.3–0.8)	1.9 (1.2–2.5)
<i>p</i> -value	<0.002	<0.02	<0.05	<0.05	<0.002

PDF primordial follicles, PMF primary follicles, SEF secondary follicles, TEF tertiary follicles

there is a decreased number of all follicle types, but no change in ovarian surface area and in the rate of apoptosis in all follicles when compared to untreated age-matched mice. These histological changes are concurrent with an increase in serum FSH and a decreased serum AMH. In addition, FSH, but not AMH, changes become more severe with time in CTX-exposed mice.

The novelty of our study resides in multiple aspects: 1) we treated pre-pubertal mice, which still preserve their lifetime maximal ovarian reserve, 2) we analyzed their residual ovarian function as assessed by the two most important serum markers of ovarian function, FSH and AMH, during puberty and as young adults; and, 3) we compared those with histological indices at the two different time points after CTX administration.

Forty-eight percent of the pre-pubertal mice exposed to CTX, and 81 % during adulthood, had an increased FSH reflective of an impaired ovarian hormonal production, which was independent from the CTX doses used and worsened with increasing age after the initial insult. This confirms our previous findings [7]. These figures are higher than what has been reported in human studies [15,22,23] and it is probably due to the different assessment methods of ovarian function in those studies (presence or absence of puberty or menses) compared to ours (serologic concentrations of AMH and FSH). Backing our assessment methods was the belief that even a mildly decreased ovarian reserve can progress to severe enough (ovarian insufficiency) to have a major impact on the subject's health and fertility and, therefore, it deserves to be assessed and diagnosed.

In pre-pubertal mice exposed to CTX there was no 'all-or-none' effect on ovarian function, but rather several degrees of damage similar to what we find in the adult population (as commonly assessed by serum FSH). We previously classified the degrees of damage into three main categories (normal FSH, DOR, and POI) by serum FSH values [7]. However, in the current study we unified DOR and POI under the category 'ovarian insufficiency' and found that the histological damage to the ovary was identified by equally low AMH serum levels. When we assessed FSH and AMH levels longitudinally in CTX-exposed mice (puberty and adulthood), we found that FSH levels significantly increased despite stable AMH levels and number of ovarian follicles, while estrogen production was similar to controls. The observed decreased AMH levels after CTX confirms the findings of human studies [6]. In controls, instead, AMH levels and follicle number significantly decreased, while FSH and estradiol serum levels remained stable, similar to what happens in humans. This discrepancy supports the belief that the rate of follicular development with every menstrual cycle *depends* upon the number of primordial follicles [22], but it suggests that this rate also depends upon the ability to produce estradiol (and other for the most part unknown factors), which is accomplished only by *intact*

developing follicles. This is particularly true for CTX-treated ovaries.

Our findings confirm AMH as a marker of *quantity* of the follicular pool, as previously reported [21]. They also suggest that serum FSH would represent not only a marker of *quantity* of residual mature follicles (secondary and tertiary follicles), but also of *quality* of the maturing follicles. For *quality* of follicular function we mean the ability of granulosa cells to produce the necessary amount of 'factors' capable to feedback pituitary FSH production, the most important of which is estrogen. Within the follicle, the influence of granulosa cells on oocyte function, i.e., the ability to be fertilized and to develop into a healthy embryo and pregnancy, remain to be understood. However, a link between FSH levels and pregnancy outcomes has been found in infertility patients in whom an increased FSH serum level was linked to an increased risk of aneuploidy in the offspring [14,26]. From this perspective, serum FSH would be a better indicator of residual follicular function and quality after CTX exposure, while AMH would be a better indicator of the extent of follicular damage. Since AMH is produced in the granulosa cells and represents a direct rather than an indirect marker of granulosa cell function, we would foresee that serum AMH became a marker of residual *qualitative* ovarian function, as well. Possibly in the future we will be able to define categories of residual ovarian function based on serum AMH levels alone.

Our study showed no difference in the rate of follicular differentiation into secondary follicles and tertiary follicles in controls and CTX-treated mice, which is consistent with previous observations that a decreased AMH production causes follicular recruitment and differentiation [9,10]. However, we could not find a significant correlation between serum AMH and the extent of follicular destruction. We hypothesize this is due to dyshomogeneous granulosa cell apoptosis after CTX, which would lead to varied AMH production in the affected follicles.

The rate of apoptosis detected as DNA fragmentation by TUNEL immunostaining was nil in primordial and primary follicles of all mice, whether they were exposed to CTX or not. As well, there was no apoptosis in secondary follicles of younger mice (G1₍₅₆₎ and G2₍₅₆₎). There was, instead, apoptosis in secondary follicles of adult mice (G1₍₉₅₎ and G2₍₉₅₎) and tertiary follicles in both age groups, which was no different in controls and in the CTX-treated mice. These findings confirm the study by Tingen et al. [24], where a negligible amount of follicular apoptosis was found in primordial, primary and secondary follicles of normal pre-pubertal mice. In addition, we confirmed other studies on adult mice where apoptosis was noted to involve large secondary and tertiary follicles, predominantly starting in the granulosa cells [4,25]. We also witnessed a physiologic follicular development into secondary and tertiary follicles also in cases of previous damage from chemotherapy: after the initial insult from CTX, follicular

development progressed in a similar fashion in controls and CTX-exposed mice. In fact, corpora lutea were present in all ovaries, irrespective of CTX exposure and of FSH levels, and the number was no different in all groups. Corpora lutea were present also in mice that developed ovarian insufficiency by serum FSH levels, indicating an ongoing follicular development up to the time of euthanasia.

In conclusion, we confirmed a deleterious role for CTX on ovarian development when administered in pre-pubertal age, which was more extensive than previously recognized in human studies. We found various degrees of residual function, as opposed to an all-or-none effect, but the degrees were reflected by FSH serum levels (which reflect granulosa cells function), and not by AMH (which reflect granulosa cells number) or the number of residual follicles. We identified AMH mostly as a marker of residual quantitative, and FSH mostly as a marker of qualitative, ovarian function: FSH would thus discriminate *functioning* from *exhausted* ovaries in the case of a low AMH measurement. Based on these results, in women treated with chemotherapy, both markers should be monitored for assessment of ovarian function. In this case, we suggest that AMH be measured as early as one *ovarian cycle* (about 2 weeks in mice and 8 weeks in humans) after chemotherapy is completed to appreciate the extent of the damage, and be repeated every few months to possibly predict the progression into definitive ovarian insufficiency. FSH, being produced as a response to ovarian hormonal production, should be measured in conjunction with AMH to assess follicular quality in order to counsel about fertility potential.

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