Observational study

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A prospective cohort study with serum anti-mullerian hormone levels change in patients undergoing uterine preservation after gestational trophoblastic neoplasia treatment with a methotrexate regimen

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Abstract

Objectives. To monitor changes in serum anti-Mullerian hormone (AMH) levels of the patients with gestational trophoblastic neoplasia (GTN) who have undergone uterine preservation during treatment with a Methotrexate (MTX) regimen and associations with AMH variations.

Methods. This observational prospective cohort study included 35 patients with low-risk GTN with uterine preservation during single–agent MTX chemotherapy at Hanoi Obstetrics and Gynecology Hospital from August 2021 to August 2022. Serum AMH levels were measured before initiation of chemotherapy and after the 1st, 2nd, and 3rd chemotherapy cycles. AMH evolution and its associations with some factors were analyzed.

Results. The median basal AMH level before chemotherapy was 2.87 ng/mL (0.96 – 7.9 ng/mL) and negatively correlated with age. The serum AMH levels decreased significantly after each chemotherapy cycle (2.87 vs. 1.16, 0.91, 0.41 ng/mL). The median magnitude of the AMH levels decline after 1st, 2nd, and 3rd chemotherapy cycles were 51.2%, 69.4%, and 84.6% ($p<0.001$), respectively. AMH variation was associated with the basal AMH level, but not with age, βhCG at diagnosis and menstrual status.

Conclusion. Our study has shown that the serum AMH levels declined rapidly and steadily in all patients during chemotherapy for GTN. Although AMH cannot be used to monitor fertility potential lonely, these new studies improve our knowledge of ovarian toxicity and ovarian reserve during chemotherapy and strongly support the use of fertility preservation strategies. ***Clin Ter 2024; 175 (2):128-134 doi: 10.7417/CT.2024.5045***

Keywords: *gestational trophoblastic neoplasia, AMH (Anti-Mullerian Hormone), MTX (methotrexate)*

Introduction

Gestational trophoblastic neoplasia (GTN) refers to a group of malignant neoplasms or disorders which have the potential to turn malignant. These tumors arise from abnormal proliferation of trophoblastic tissue after abnormal fertilization.[1] Follow-up with human chorionic gonadotropin (hCG) is essential for early diagnosis of GTN. GTN must be distinguished from other causes of an elevated hCG level (such as a normal pregnancy, an aborting pregnancy, an ectopic pregnancy, hCG-releasing nontrophoblastic tumors or pituitary source of hCG).[2]

GTNs are highly sensitive to chemotherapy even with distant metastasis. In recent years, given the improved management and prognosis of patients with GTN, the overall cute rate has increased to over 98% and fertility preservation has become a pressing clinical issue.[3] Reproductive potential has an important role on individual women. This is also associated with ovarian reserve and reflected by the number and quality of oocytes. Chemotherapy agents are known to have impact on ovarian reserve via mechanism leading to depletion of growing and primordial follicles.[4]

Anti – Mullerian hormone (AMH) is produced by granulosa cells of growing follicles and is expressed throughout folliculogenesis. AMH is widely used as a surrogate for ovarian reverse and has potential as a diagnostic and predictive biomarker for reproductive lifespan in women undergoing anticancer treatments.[5–7] However, the degree of decrease in ovarian reverse is related to the type of chemotherapy agents, dosage, time of treatment as well as individual.[8] Methotrexate (MTX) is a commonly chemotherapeutic agent that kills cancer cells by binding dihydrofolate reductase, which is associated with DNA synbiothesis.[9] Due to its non-selectivity,

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MTX also impairs normal cell and causes damage to healthy tissue, such as ovarian cell.[10] This has been demonstrated in breast cancer, ovarian germ cell tumors, and GTNs in the world, but these studies have been sparse.[7,11,12]

In our prospective study, we evaluated AMH evolutions during chemotherapy in patients with GTN treated with MTX regimen and determined its association with some factors.

Materials and Methods

Study populations

This prospective study was carried out from August 2021 to August 2022 at Hanoi Obstetrics and Gynecology Hospital. This study was approved by the Institutional Review Board (IRB) of Hanoi Obstetrics and Gynecology Hospital (IRB No 3159/QD/BVPS/ TTDT CDT issued on December 15, 2021).

Inclusion criteria were as follows: (1) All patients who are under 40 years old diagnosed of low-risk GTN treated with MTX regimen, (2) no history of chemotherapy, (3) no evidence of endocrine disorders (hyperprolactinemia, Cushing's syndrome, hypothyroidism and hyperthyroidism).

Exclusion criteria were a history of previous surgery on ovarian or pituitary.

Procedure

These women were screened for eligibility by research doctors. After screening, they were provided with a full Patient Information Sheet, Consent Form and invited to a complete discussion with investigators about the study. All patients signed written informed consent before the study.

A detailed physical examination, including pelvic examination, taking blood test and transvaginal sonography, were performed for each patient. The level of serum human chorionic gondatropin ß (ß hCG) was measured for all patients in order to diagnose according to the International Federation of Gynecology and Obstetrics (FIGO).[13] All patients were scored according to the FIGO staging system.[14] Those in low-risk group were chosen into the study and treated with MTX regimen.[15] In our study, the used MTX regimen for those patients is eight-day methotrexate regimen (1 mg/kg intramuscular (IM), days one, three, five and seven) with folinic acid rescue (days two, four, six and eight), repeated 14 to 16 days.

A blood sampling for evaluating AMH levels were performed for each patients at diagnosis (sample S0), after 1st, 2nd, 3rd chemotherapy cycle (samples S1, S2, S3, respectively). After each cycle, serum AMH were measured before the first day of the following cycle. AMH levels were measured using an automatic chemiluminescens immunoassay analyzer (Access 2). was analyzed according to Access 2.

Statistical Analysis

Data were collected and analyzed using SPSS 20.0 software (The Statistical Package for Social Sciences). Qualitative variables were represented as numbers and percentages. Quantitative variables were represented as median, minimum, and maximum values. The Wilcoxon tests were used to detect significant variations in the tested parameters at different time points. The box and whisker plots were also used to display quantitative data. The Spearman coefficient was used for correlation studies. A p-value <0.05 was considered to represent a statically significant difference.

Results

During the study, we followed 35 participants with low-risk GTN with uterine preservation and those were treated with Methotrexate regimen.

Basal serum AMH level and AMH levels during chemotherapy

Table 1 shows serum AMH levels at diagnosis and after each course of chemotherapy. At dignosis, the median basal AMH level was 2.87 ng/mL (0.96 – 7.9 ng/mL). After each cycle, serum AMH levels decreased gradually (1.16, 0.91, and 0.41 ng/mL). There was a significant difference between AMH level at diagnosis and after each cycle ($p < 0.001$) (Fig. 1)

Table 1. AMH levels at diagnosis and after each course of chemotherapy

AMH levels	Median (ng/mL)	Min-Max	p
At diagnosis S0	2.87	0.96-7.9	-
S1	1.16	0.06-7.69	p1/0 < 0.001
S2	0.91	0.01-5.89	p2/0 < 0.001
S3	0.41	0.01-3.56	p3/0 < 0.001
p2/1 < 0.001 p3/1 < 0.001 p3/2 = 0.0001			

p1/0, p2/0, p3/0 AMH level after the first, second and third chemotherapy cycle vs at diagnosis, respectively. p2/1 AMH level after the 2nd chemotherapy vs 1st. p3/1 AMH level after the 3rd chemotherapy vs 1st. p3/2 AMH level after the 3rd chemotherapy cycle vs 1st (Wilcoxon test).

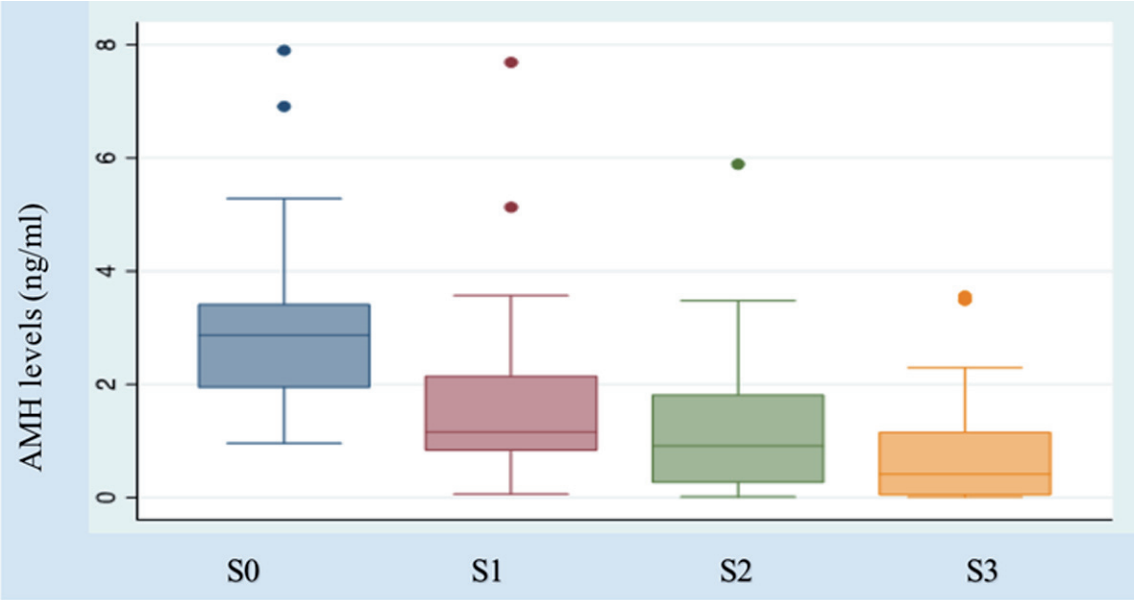

Fig. 1. AMH levels at diagnosis and after each course of chemotherapy
S1: AMH level at diagnosis. S1-3: AMH level after 1st, 2nd, 3rd chemotherapy cycle

Cumulative variation of AMH during chemotherapy

Table 2 shows the magnitude of the AMH level decline after each chemotherapy cycle. The degree of AMH decrease after 1st, 2nd, 3rd chemotherapy cylcles were 51.2%, 69.4% and 84.6%, respectively and this difference is statistically significant with $p < 0.001$. The magnitude of AMH level decline after 3rd cycle was greater than 2nd and 1st cycle. In the same way, the magnitude of AMH level decline after 2nd cycle was greater than 1st ($p < 0.01$) (Fig. 2).

Effect of age, basal AMH level on AMH variation

According to Fig. 3A, there was a significant correlation between basal AMH level and patient's age at diagnosis ($r = -0.598$, $p = 0.0001$). Median AMH level was significantly lower in women > 30 years than in women aged 20-30 or < 20 years old. But there was no significantly difference in median AMH level between the women aged 20-30 group and the women < 20 years old group (Fig. 3B).

A negative correlation was found between the cumulative AMH variation after 2nd chemotherapy cycle and basal AMH ($r = -0.551$, $p = 0.003$). Similarly, a significantly higher cumulative decrease rate after 3rd cycle was observed in patients with "low" basal AMH levels ($r = -0.426$, $p = 0.027$). However, the magnitude of AMH level decline after 1st chemotherapy cycle was not signigicantly different from basal AMH level ($p = 0.093$) (Fig. 4). No significant associations were found between the cummulative variation of AMH level during the study and age, βhCG at diagnosis.

Menstrual status

During chemotherapy, 21 patients had abnormal menstrual flow (60.0%). Of these, 2 had amenorrhea (5.7%), 6 had decreased flow (17.2%). The prevalence of chemotherapy-related menorrhagia was 37.1% (n=13) (Table 3). Menstrual status during chemotherapy was not associated with basal AMH level ($p = 0.539$), variation of AMH level ($p = 0.363$).

Table 2. Cumulative variation of AMH during chemotherapy

	dAMH (%)	Median (%)	Min - Max	p
After 1st cycle	**dAMH1 = D1**	51.2	2.7-91.8	-
After 2nd cycle	**dAMH2 = D2**	69.4	17.8-99.0	p* < 0.001
After 3rd cycle	**dAMH3 = D3**	84.6	20.2-99.7	p** < 0.001
p*** = 0.007				

D1,D2, D3: Cumulative variation of AMH level after 1st, 2nd, 3rd chemotherapy cycles, respectively
Di = dAMHi = ([AMH0] – [AMHi])/[AMH0]x100%
*the magnitude of AMH level decline after 2nd cycle vs 1st . ** the magnitude of AMH level decline after 3rd cycle vs 1st. *** the magnitude of AMH level decline after 3rd cycle vs 2nd (*Wilcoxon test*)

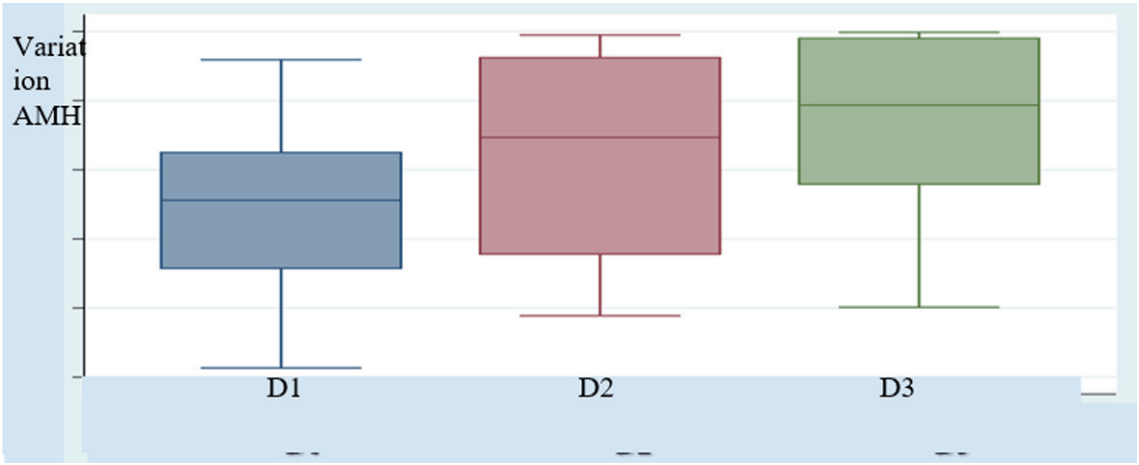

Fig. 2. Cumulative variation of AMH level during chemotherapy
D1,D2, D3: Cumulative variation of AMH level after 1st, 2nd, 3rd chemotherapy cycles.

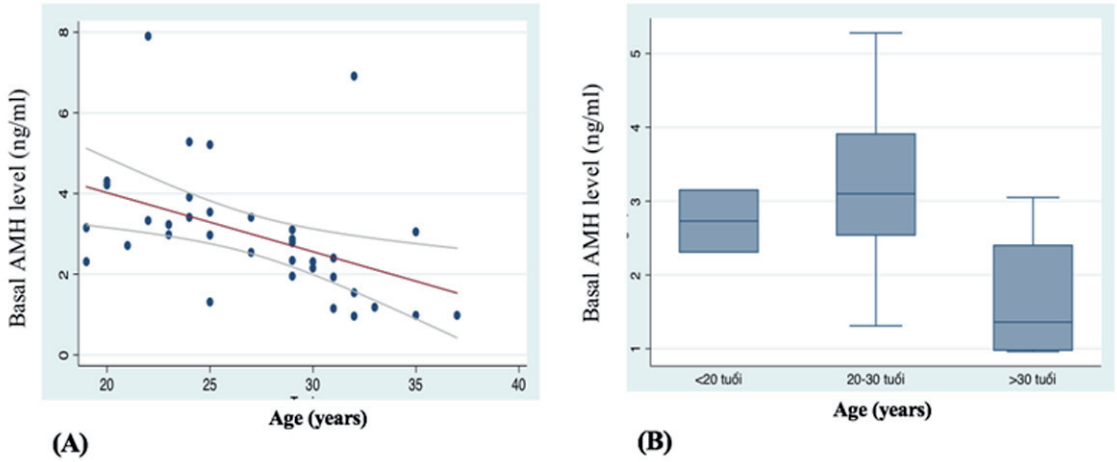

Fig. 3. Correlation between age and basal AMH level
A): correlation between basal AMH level and patient's age at diagnosis
B): Distribution of AMH level according to age groups

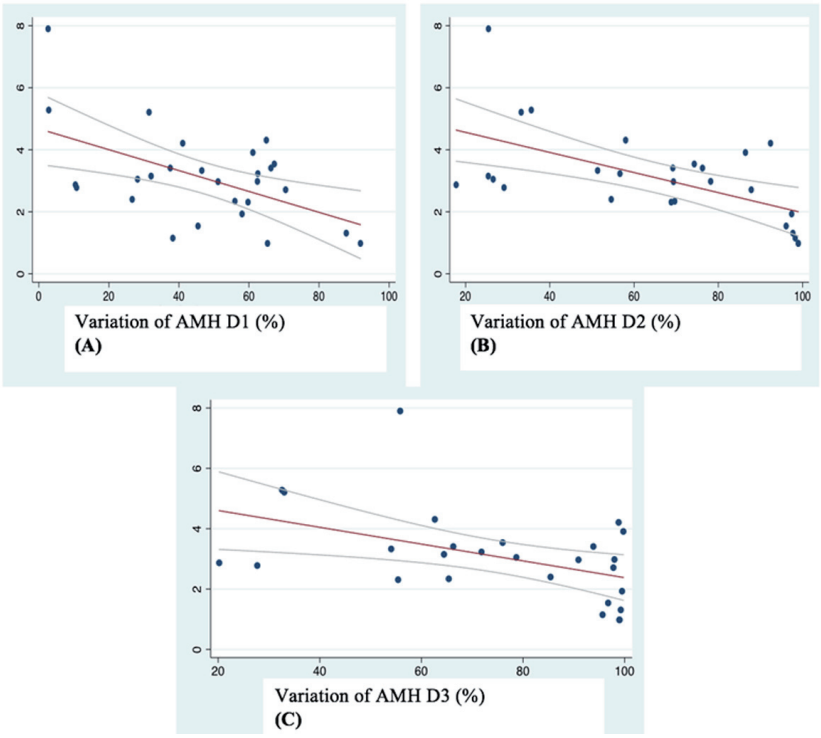

Fig. 4. Correlation between AMH variation and basal AMH level
A, B, C: Correlation between basal AMH level and the cumulative AMH variation after 1st, 2nd, 3rd chemotherapy cycle (Spearman coefficient)

Table 3. Menstrual status during chemotherapy

	MTX regimen		
	S0 n(%)	S1 (n/%)	S3 n(%)
Amenorrhea	0 (0.0)	0 (0.0)	2 (5.7)
Decreased flow	0 (0.0)	3 (8.6)	6 (17.2)
Menorrhagia	8 (22.8)	16 (45.7)	13 (37.1)
Menstrual irregularities	3 (8.6)	2 (5.7)	0 (0.0)
Normal menstrual flow	24 (68.6)	14 (40.0)	14 (40.0)

Discussion

In this observational prostective study, we assessed AMH evolution from the time of diagnosis to 3rd chemotherapy cycle in 35 reproductive-age women with GTN who received MTX regimen. At diagnosis, the median basal AMH level was 2.87 ng/mL (0.96 – 7.9 ng/mL). We found that serum AMH levels decreased significantly after each couse of chemotherapy in all patients ($p < 0.001$). The median AMH levels afer 1st, 2nd, 3rd chemotherapy cycles were 1.16, 0.91 and 0.41 ng/mL (Table 1). Xiaoning Bi et al. reported that serum AMH levels decreased after 3 chemotherapy cycle (3.27 vs. 1.70 ng/mL).[16] Dezellus et al. showed that serum AMH levels decreased after 5th chemotherapy cycle and declined significantly in women with breast cancer who underwent chemotherapy (0.68 ± 3.01 ng/mL vs. 4.19 ± 4.84 ng/mL).[6] Similarly, Iwase also reported that serum AMH levels were lower in patients with GTN who received chemotherapy than in patients with hydatidiform mole who did not undergo chemotherapy (0.32-3.94 ng/mL vs. 0.77-6.53 ng/mL, $p = 0.002$).[17] Chemotherapy agents are known to have rapidly impact on dividing cancer cells which are in mitosis (G, S, G2 and M stages). Therefore, anticancer treatment can effect on cells either by directly or indirectly inducing DNA damage, or by overactivation and subsequent depletion of cells.[18] In particular, MTX is a commonly used chemotherapeutic agent known to degenerate cells through binding and competitive inhibition of dihydrofolate reductase, which is necessary for DNA biosynthesis and subsequent cellular production.[9] Therefore, treatment with MTX can lead to inhibited cell proliferation or apotosis.[19] However, because of its non-selectivity, MTX also impairs normal (non-cancerous) cell function and causes long-term damage to healthy tissue (bone marrow cells, gastrointestinal mucosa,...). Thus, these agents are also toxic to granulosa cells and cortical cells of the ovary, especially cells in division process.[10] The degree of reduction depends on the type of chemical, length of exposure time and the cumulative doses. This leads to decline AMH levels rapidly and steadily in all patients during chemotherapy.

Both the serum AMH concentrations and the magnitude of its decline during chemotherapy are used as a surrogate for changes in serum AMH levels in patients with GTN treated MTX regimen, but the absolute value of serum AMH levels changed in many studies, depending on laboratory techniques and racial factors. Therefore, the magnitude of the AMH levels decline seems to reflect changes in serum AMH levels more accordantly. The median degree of AMH decrease after 1st, 2nd, 3rd chemotherapy cylcles were 51.2%, 69.4% and 84.6%, respectively and this difference is statistically significant with $p < 0.001$. Xiaoning Bi et al. showed that the magnitude of AMH levels decline in GTN patients treated single-agent and combination chemotherapy group after 3 chemotherapy cycles were 27.57% and 61.80%, respectively, and the difference was statistically significant with $p = 0.0004$.[16] Dezellus et al. reported that the cumulative AMH variation was 50.9% (95% CI 45.4-56.4%) after the first chemotherapy cycle and 97.3% (95% CI 95.9-98.7%) after 5th cycles.[6] Furthermore, Anderson et al found a 55% decrease on average after only one cycle.[20] The magnitude of AMH levels decline in our study was lower than that Bi's study. This result may support the hypothesis that a stronger and deeper reduction of serum AMH levels in combination chemotherapy group has been demonstrated in many previous studies. However, our study only evaluated patients treated with MTX regimen, we recommended that future studies should evaluate both single-agent and combination chemotherapy groups to clarify these hypothesis. Figure 2 represents the ascending decline in serum AMH level after each course of chemotherapy, demostrating that the dose of chemotherapy also influences the degree of gonadotoxic.

In our study, basal AMH level had a negative correlation with patient age at diagnosis. Dezellus et al. showed that basal AMH level was highly negative correlated with advancing age.[6] The median AMH level in women 20-30 years of age was the highest and the median AMH level were significantly lower in the group of patients > 30 years old than in the group < 30 years old. In this regard, Kelsey demontrated that age is the most important factor for variations in individual ovarian non-growing follicle populations, and there were strong correlations between AMH levels and both non-growing follicle populations for age ranges before and after peak AMH level (mid-twenties).[21]

There was a negative correlation between the magnitude of AMH level decline and the basal AMH level (Figure 4). In which, the degree of AMH levels decrease after first cylce had a significantly negative correlation with basal AMH level. This result are similar to those of Dezellus.[6] Therefore, it is found that the magnitude of AMH levels decline depended on the basal AMH concentration. The lower the basal AMH level is , the more serum AMH level after chemotherapy decreases.

After each cylce of chemotherapy, the prevalence of menorrhagia in our study increased 2 times (16/35 cases, 45.7%). After 3 courses of chemotherapy, the rate of amenor-

rhea was 2/35 (5.7%); 6 cases had decreased flow (17.2%); 13/35 cases had menorrhagia (37.1%) and 14/35 cases had normal menstrual flow (40%). This has shown that MTX also affects the menstrual status, because it has significant impacts on the division process of oocytes, leads to decrease in ovarian reverse. However, we has not yet found a correlation between AMH variation and menstrual status. Consequently, the menstrual status cannot predict the changes in ovarian reserve during and after chemotherapy because of the small sample size, which constitutes a limitation of the present study.

Conclusion

AMH levels decreased rapidly and steadily during chemotherapy. Although AMH cannot be alone used to represent for future fertility, it is a reliable biomarker for the degree of ovarian reverse decline during and after chemotherapy. Methotrexate is a gonadotoxic, reduces ovarian reserve and the cumulative variation has a positive correlation with the cumulative dose of chemicals. However, our data are limited to the 3 courses of chemotherapy and it is recommended that the need for further studies strongly support the degree of the impact of chemicals as well as the ability to recovery ovarian reserve after chemotherapy. Evaluating the relationship between pre- and post-chemotherapy AMH levels with reproductive outcomes in patients with GTN could be meaningful to consider methods for the preservation of ovarian function or fertility prior to the intiation of chemotherapy.

Data Sharing Statement

The datasets used and analyzed during the current study are available from corresponding author on reasonable request.

Ethical Approval

This study was approved by the Institutional Review Board (IRB) of Hanoi Obstetrics and Gynecology Hospital (IRB No 3159/QD/BVPS/ TTDT CDT issued on December 15, 2021). Individual patient consent for inclusion in the study was obtained. Before the study, written informed consent was provided to all patients after explanation off the purpose of this study. Patients had the right to discontinue at any time during the study.

Disclosure

The authors declare that they have no conflicts of interest in this work.

References

1. Lurain JR. Gestational trophoblastic tumors. Semin Surg Oncol. 1990; 6(6):347-53. doi: 10.1002/ssu.2980060610. PMID: 2175931
2. Kohorn EI. Negotiating a staging and risk factor scoring system for gestational trophoblastic neoplasia. A progress report. J Reprod Med. 2002 Jun; 47(6):445-50. PMID: 12092012
3. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. Lancet. 2010 Aug 28;376(9742):717-29. doi: 10.1016/S0140-6736(10)60280-2. Epub 2010 Jul 29. PMID: 20673583.
4. Waterland RA, Jirtle RL. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition. 2004 Jan;20(1):63-8. doi: 10.1016/j.nut.2003.09.011. PMID: 14698016
5. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril. 2015 Mar;103(3):e9-e17. doi: 10.1016/j.fertnstert.2014.12.093. Epub 2015 Jan 10. PMID: 25585505
6. Dezellus A, Barriere P, Campone M, et al. Prospective evaluation of serum anti-Müllerian hormone dynamics in 250 women of reproductive age treated with chemotherapy for breast cancer. Eur J Cancer. 2017 Jul;79:72-80. doi: 10.1016/j.ejca.2017.03.035. Epub 2017 Apr 29. PMID: 28463758
7. Solheim O, Tropé CG, Rokkones E, et al. Fertility and gonadal function after adjuvant therapy in women diagnosed with a malignant ovarian germ cell tumor (MOGCT) during the "cisplatin era". Gynecol Oncol. 2015 Feb;136(2):224-9. doi: 10.1016/j.ygyno.2014.12.010. Epub 2014 Dec 12. PMID: 25511159
8. Anderson RA, Themmen AP, Al-Qahtani A, et al. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Hum Reprod. 2006 Oct;21(10):2583-92. doi: 10.1093/humrep/del201. Epub 2006 Jul 4. PMID: 16820385
9. Chabner BA, Allegra CJ, Curt GA, et al. Polyglutamation of methotrexate. Is methotrexate a prodrug? J Clin Invest. 1985 Sep;76(3):907-12. doi: 10.1172/JCI112088. PMID: 2413074; PMCID: PMC423952
10. Beane OS, Darling LE, Fonseca VC, et al. Disparate Response to Methotrexate in Stem Versus Non-Stem Cells. Stem Cell Rev Rep. 2016 Jun;12(3):340-51. doi: 10.1007/s12015-016-9645-9. PMID: 26815725; PMCID: PMC4880537
11. Fréour T, Barrière P, Masson D. Anti-müllerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy. Eur J Cancer. 2017 Mar;74:1-8. doi: 10.1016/j.ejca.2016.12.008. Epub 2017 Jan 28. PMID: 28135602
12. Zamani M, Mohsenpour F, Torkzaban F, et al. The Effects of Chemotherapy on the Levels of Serum Anti-Müllerian Hormone in Patients with Gestational Trophoblastic Neoplasia. J Reprod Infertil. 2023 Jan-Mar;24(1):43-48. doi: 10.18502/jri.v24i1.11908. PMID: 36919051; PMCID: PMC10008131
13. Kohorn E. Practice bulletin No. 53--Diagnosis and treatment of gestational trophoblastic disease. Obstet Gynecol. 2004 Dec;104(6):1422; author reply 1422-3. doi: 10.1097/01.AOG.0000147669.68914.f6. PMID: 15572508
14. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. Int J Gynaecol Obstet. 2002 Jun;77(3):285-7. doi: 10.1016/s0020-7292(02)00063-2. PMID: 12065144.
15. Lawrie TA, Alazzam M, Tidy J, et al. First-line chemotherapy in low-risk gestational trophoblastic neoplasia. Cochrane Database Syst Rev. 2016 Jun 9;2016(6):CD007102. doi: 10.1002/14651858.CD007102.pub4. PMID: 27281496; PMCID: PMC6768658
16. Bi X, Zhang J, Cao D, et al. Anti-Müllerian hormone levels in patients with gestational trophoblastic neoplasia treated with different chemotherapy regimens: a prospective cohort study. Oncotarget. 2017 Dec 6; 8(69):113920-113927. doi: 10.18632/oncotarget.23027. PMID: 29371957; PMCID:

PMC5768374
17. Iwase A, Sugita A, Hirokawa W, et al. Anti-Müllerian hormone as a marker of ovarian reserve following chemotherapy in patients with gestational trophoblastic neoplasia. Eur J Obstet Gynecol Reprod Biol. 2013 Apr;167(2):194-8. doi: 10.1016/j.ejogrb.2012.11.021. Epub 2012 Dec 27. PMID: 23274042.
18. Woods D, Turchi JJ. Chemotherapy induced DNA damage response: convergence of drugs and pathways. Cancer Biol Ther. 2013 May;14(5):379-89. doi: 10.4161/cbt.23761. Epub 2013 Feb 4. PMID: 23380594; PMCID: PMC3672181.
19. Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases. Arthritis Res. 2002;4(4):266-73. doi: 10.1186/ar419. Epub 2002 Mar 19. PMID: 12106498; PMCID: PMC128935.
20. Anderson RA, Rosendahl M, Kelsey TW, et al. Pretreatment anti-Müllerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer. Eur J Cancer. 2013 Nov;49(16):3404-11. doi: 10.1016/j.ejca.2013.07.014. Epub 2013 Aug 19. PMID: 23968732; PMCID: PMC3807650
21. Kelsey TW, Anderson RA, Wright P, et al. Data-driven assessment of the human ovarian reserve. Mol Hum Reprod. 2012 Feb;18(2):79-87. doi: 10.1093/molehr/gar059. Epub 2011 Sep 20. PMID: 21933846