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Evaluation of the effect of multi-dose methotrexate therapy on ovarian reserve in ectopic pregnancies: is polycystic ovarian morphology a protective condition for ovarian reserve?

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ABSTRACT

Objectives: The study aims to evaluate the effects of multi-dose methotrexate (MTX) or subsequent salpingectomy on ovarian reserve and explain the conditions that cause the change in serum anti-müllerian hormone (AMH) levels.

Material and methods: Our department had 58 tubal ectopic pregnancy (EP) patients treated with a multiple-dose MTX protocol or subsequent salpingectomy between 2017–2020. Serum AMH level was measured in each patient before the medication and 3–6 months after therapy. Patients' details were recorded and analyzed later.

Results: The mean AMH value decreased in 32 patients (–17.8%), increased in 26 patients (+31.5%) ($p < 0.0001$). In the group with an increase, there was a significantly high number of patients with a polycystic ovary (PCO) condition compared to the other group ($p = 0.0001$). The post-treatment serum AMH levels increased in patients with PCO, whereas those decreased in patients without PCO ($p < 0.001$).

Conclusions: Multiple-dose MTX or subsequent salpingectomy treatment in tubal ectopic pregnancy (EP) patients might not refer to significant differences in patients' AMH levels. Remarkably, post-treatment AMH levels were significantly increased in EP patients with PCO and decreased in those without this condition. PCO may be a protective condition for ovarian reserve.

Key words: anti-müllerian hormone; ectopic pregnancy; methotrexate; ovarian reserve; salpingectomy

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INTRODUCTION

Ectopic pregnancy (EP) occurs when a fertilized egg implants and grows in an extra-uterine location. More than 90% of nearly all EP appear in the salpinx. As the pregnancy develops, it may rupture the salpinx and cause massive internal bleeding, which can be a life-threatening emergency that requires immediate surgery. The incidence of EP is 1–2%; additionally, EP is the leading cause of early pregnancy-related mortality and accounts for 10–15% of all maternal mortality [1–3]. Therefore, the diagnosis, as well as treatment of EP, should be taken seriously. The laparoscopic method is the gold standard for EP diagnosis, yet, clinical examinations can often diagnose EP [1, 2].

The best-known and most common medication used to treat tubal EP is methotrexate (MTX) [3]. Methotrexate is a folic acid antagonist and affects rapidly proliferating cells such as trophoblastic tissue of ectopic pregnancy (EP) [1]. It competitively inhibits folate-dependent biochemical processes, thus inhibiting DNA synthesis [2]. Methotrexate prohibits cellular proliferation, stops the growing EP mass, then extra-uterine pregnancy is absorbed by the body over 4–6 weeks, so excision of the salpinx is usually not required. Accordingly, it is appropriate to try medical treatment first in patients with proper indications. The other treatment preferences for EP include expectant management at a low set of Human chorionic gonadotropin (β-hCG) values and further surgical treatments with salpingectomy, salpingotomy, tubal milking, or fimbriectomy. Reasonably, a salpingectomy is a surgical option that offers a definitive solution in selected tubal EP patients [4]. In practice, clinicians inevitably prefer

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surgery when the procedure of MTX is contraindicated or ineffective in tubal EP management.

Methotrexate regime and surgical treatments for EP may affect ovarian reserve by impairing the proliferation and the blood flow of ovarian cells [1]. The potential effects on ovarian reserve are predictable with anti-müllerian hormone (AMH) serum levels [5]. AMH is synthesized exclusively by granulosa cells in preantral and antral follicles within the ovary, and is considered one of the best predictors of ovarian reserve [6, 7]. In addition, AMH is pointed out as an appropriate biomarker to evaluate ovarian reserve after the EP treatment [1]. Although serum AMH level is independent of menstrual cycle phases and many other factors [8], patients with polycystic ovarian syndrome (PCOS) may have variably high AMH levels. This is due to the increased number of small and dysfunctional follicles in the ovaries of PCOS patients [9]. Also, the AMH was presumed to be an effective serum marker in the confirmation and random diagnosis of undiagnosed PCOS [10]. High AMH levels, especially in PCOS patients, may be associated with a lower probability of conception, and it is significantly closely related to the patients' fertilization outcomes [11, 12]. In addition to all these, another topic on the agenda is the opinion that PCOS and polycystic ovarian morphology (PCOM) [13], which is a frequently used sonographic marker in its diagnosis, are associated with the risk of developing tubal EP [14]. The image of PCOM is more common in the tubal EP population than in women of reproductive age [15]. There are already many studies evaluating the effects of EP treatment with different numbers of MTX doses or salpingectomy on the ovarian response via AMH, some of which are animal experiments and generally include single or two-dose MTX treatment [1, 16–22]. Conversely, none of the previous studies expressed any opinion concerning the polycystic ovary (PCO) condition, which is a risk factor for EP and often associated with it, and has notably different effects on AMH levels.

Objectives

The present study aims to evaluate the effects of multi-dose MTX therapy or subsequent salpingectomy, as different treatment modalities for tubal EP patients, on ovarian reserve by AMH measurements and explain the factors that cause the change in serum AMH levels. Our study has a potentially new perspective on this issue, as it was designed with the multi-dose MTX protocol and considers PCOM in ultrasound.

MATERIAL AND METHODS

This prospective observational study was performed at the Department of Obstetrics and Gynecology of Sahinbey Research and Practice Hospital which belongs to the Faculty of Medicine of Gaziantep University, Gaziantep, Turkey. The study was approved by the Gaziantep University Ethics Committee (Date: 5 February 2020, Ethics committee number: 2020/03) and informed consent was obtained from patients. This current study was carried out together with the ethical standards of the Declaration of Helsinki guidelines. Patient information has been stored in the highly secure digital data recording system of the hospital.

Patient selection criteria and data collection

First, the diagnosis of tubal EP was confirmed by stable or little rising serum β-hCG values and sonographic characteristics. Additionally, for each included patient, the pathology results of the endometrial or surgical specimens supported the diagnosis of tubal EP. The inclusion criteria of patients for the study were tubal EP diagnosis, 18–44 years, no MTX medication contraindications [23], and being eligible for multiple-dose MTX therapy. In addition, the exclusion criteria were emergency laparotomy for tubal EP rupture before starting MTX therapy, plus a history of previous chemotherapy or tuba-ovarian surgery and smoking due to their possible effects on the AMH levels. The study considered current guides [24] about MTX contraindications of EP treatment such as β-hCG values greater than 10.000 IU/L, acute abdomen signs, fetal cardiac activity, tubal rupture diagnosis, and multiple systemic dysfunctions. In addition, previous medical or surgical treatment for EP was a reason for exclusion in patients, but only patients with follow-up treatment were unrestricted for inclusion.

Demographic characteristics, sonographic outcomes, MTX therapy details, and β-hCG and AMH measurements were documented for each patient. The demographic characteristics included the age in years, body mass index (BMI), and anamnesis of PCOS and gestation. Having a PCOM image for each patient was defined by using sonographic parameters such as ovarian volume, number of follicles per ovary, and distribution pattern of follicles. However, since there is no universal consensus on the definition of PCOM, the PCOM description in this study was based on the most commonly cited classification system [13].

In our department, there were a total of 157 tubal EP patients treated with a multiple-dose MTX protocol or subsequent salpingectomy between 2017 and 2020. Consequently, this study included 63 patients who met the above inclusion and exclusion criteria.

Management of the patients with multi-dose MTX Protocol and follow-up method of AMH serum levels

In this study, the treatment protocol of EP was specified based on current approaches [3, 25]. A maximum of 4 doses of MTX, with the highest dose of 80 mg/day, were

injected IM every two days (1 mg/kg dose on the 1st, 3rd, 5th, and 7th day). IM Leucovorin (0.1 mg/kg dose on the 2nd, 4th, 6th, and 8th day) has been used to avoid the side effects of MTX therapy. The baseline β-hCG value without administration of MTX was termed "pre-treatment β-hCG". β-hCG values were measured serially on days 1, 3, 5, and 7 after MTX administration. Moreover, the hemodynamic signs of patients were closely monitored. More than a 15% decrease in serial β-hCG values was a criterion for successful treatment, and these values were termed the "post-treatment β-hCG" value for each patient. Then until it turned negative, a weekly β-hCG test was conducted. Taking serial β-hCG values into account, an increase during ongoing medical therapy or a decrease of less than 15% despite completion of maximum MTX doses, plus a diagnosis of tubal rupture during hospitalization were a criterion for medical treatment failure each. In addition, because all of these failure criteria were an indication for surgery in tubal EP treatment [25], all of the included patients who faced this issue underwent salpingectomy in the study. Accordingly, this final measurement of finished medical treatment for β-hCG was termed the "post-treatment β-hCG" in the current study.

Differences in ovarian reserves were compared using serum AMH levels analyzed with the same kits (Elabscience, Wuhan, China, Catalog No: E-EL-H0317, only single device) available in the hospital hormone laboratory. The pre-treatment AMH value was measured when the patient was diagnosed and hospitalized but not on medication. Although there is no AMH variability between the first trimester and the postpartum period [26], it may show individual and interindividual variability between different phases of the menstrual cycle [27]. Therefore, aiming to minimize the effect of menstrual cycle phases on the post-treatment level of AMH, the measurements were performed on the second day of the second menstrual cycle of each patient, corresponding to a period of 3–6 months after treatment. The pre- and post-treatment serum AMH measurements of patients were termed as "AMH^{first}" and "AMH^{final}", respectively.

Statistical analysis

Initiating the study, the sample size was computed by power analysis using Gpower 3.1 software. The minimum possible number of patients found was 53 of the sample size analysis as per the study by Sahin et al. [22], considering a 95% confidence interval, medium effect size, and an 80% power. Similarly, for the same statistical determinants, the minimum possible number of patients in the analysis based on the data of the first 20 patients included in the study was 56. In addition, the outliers of 63 patients' AMH values were detected using the Grubbs test. Then, the Shapiro-Wilk test was performed to test the compatibility of numerical variables to normal distribution. Normally distributed numerical variables in two independent groups were analyzed with Student's t-test. However, the Mann-Whitney-U test was used to analyze the non-normal data sets. Wilcoxon signed-rank test was used to compare two paired groups for non-parametric variables. The significance of the association between two-ranked numerical variables was tested using the Spearman rank-order correlation coefficient. Categorical variables were tested using the Chi-square test; interchangeably, the Fisher's Exact test was preferred when the Chi-square test did not statistically fulfill the conditions. Alternatively, the McNemar test (also known as the paired or matched Chi-square) was used when comparing the effects of the different treatment methods on results in two groups of patients. The R 3.5.1 statistics program (Mathematics and Statistics Institute, Vienna, Austria) package was used in the statistical analysis. In the study, p value less than 0.05 was considered statistically significant.

RESULTS

The present study started with 63 patients. However, five patients were excluded from the study due to inappropriate AMH values in the outlier analysis. The distribution of 58 patients' AMH values according to the measurement periods is shown in Figure 1. It is noteworthy that there is a decrease in AMH values compared to the median and a slight increase compared to the average. There was no statistically significant difference in changes in AMH values of 58 patients (p = 0.88). Possible reasons for this difference in the median and mean values of AMH are concerned in the discussion section.

The MTX protocol was sufficiently effective in 42 (72.4%) patients concerning the results of the medical treatment protocol. However, 16 (27.6%) patients were treated with salpingectomy in addition to the MTX protocol because the medical treatment failed. There was no significant difference in the changes in serum AMH levels (–12.1% and –3.9%

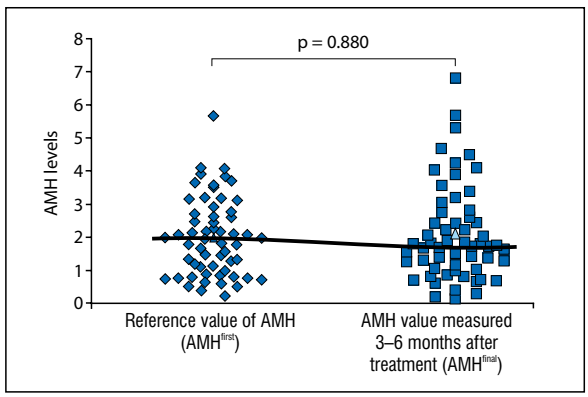

Figure 1. Pre-and post-treatment serum anti-müllerian hormone (AMH) levels of 58 patients

Table 1. Comparison of the pre-and post-treatment anti-müllerian hormone (AMH) values of patients with contrasting changes in AMH levels and those who received different treatments

Groups	AMH^{first} [ng/mL]	AMH^{final} [ng/mL]	p
All patients	1.96 (0.22–5.67)	1.73 (0.13–6.82)	0.88
n = 58 (100%)	2.01 ± 1.18	2.10 ± 1.44	
MTX protocol group (only MTX)	1.97 (0.22–4.06)	1.73 (0.13–6.82)	0.62
n = 42 (72.4%)	1.99 ± 1.11	2.05 ± 1.41	
MTX protocol plus salpingectomy group	1.79 (0.39–5.67)	1.70 (0.30–5.69)	0.33
n = 16 (27.6%)	2.05 ± 1.39	2.25 ± 1.54	
Patients with decreased AMH levels	1.96 (0.50–4.06)	1.63 (0.13–3.92)	**0.0001**
n = 32 (55.2%)	1.96 ± 1.04	1.61 ± 0.92	
Patients with increased AMH levels	1.87 (0.22–5.67)	2.15 (0.40–6.82)	**0.0001**
n = 26 (44.8%)	2.06 ± 1.34	2.71 ± 1.73	

AMH^{first} — pre-treatment serum AMH measurement; AMH^{final} — post-treatment serum AMH measurement (3–6 months after treatment); mean ± SD — mean ± standard deviation; $median^{(min–max)}$ — median and minimum-maximum values; MTX — methotrexate; n (%) — number of patients in the group and percentage. In each column and row, the top value is the "$Median^{(min-max)}$" and the bottom value is the "mean ± standard deviation (SD)"

for median values versus +9.7% and +3% for mean values, respectively) in patients who had only MTX protocol and MTX protocol plus salpingectomy ($p > 0.05$). The mean and median values for AMH^{first} and AMH^{final} in patients treated with either the MTX protocol alone or salpingectomy following the MTX protocol were presented in Table 1.

The AMH values were decreased in 32 (55.2%) of 58 patients, while the AMH values were increased in 26 (44.8%) patients. Patients with and without decreasing AMH levels were compared between their groups according to AMH^{first} and AMH^{final} values. Accordingly, a 17.8% decrease in one group and a 31.5% increase in the other group were computed based on the mean AMH values. There was a statistically significant difference in terms of AMH changes in each group ($p < 0.0001$). So, the comparison of demographic characteristics and sonographic findings of patients with contrasting changes in final serum AMH values are presented in Table 2. Additionally, Table 3 shows both laboratory analysis results and the classification of EP treatment modalities for the same patients.

In the group with an increase in serum AMH levels, there was a significantly high number of patients with a PCOM image on ultrasound or a history of PCOS compared to the other group ($p = 0.0001$). Furthermore, there was no significant difference among them AMH^{first} in the patients with or without a decrease in serum AMH, whereas there was a statistically significant difference among AMH^{final} values ($p = 0.016$). Accordingly, there were statistically significant differences between the two groups with contrasting AMH changes, one group experiencing an increase and the other a decrease, due to both PCOM image in ultrasound and PCOS history. It is known that individual and interindividual high AMH variability in young women who have both PCOM image and PCOS than in more older women without it. This current study used the PCOM criteria in the "Method" section, there was no PCOM image for only one of 19 patients with a history of PCOS, but six patients without a verified history of PCOS had a PCOM image. Correspondingly, this study had a total of 25 patients with PCOS history or sonographic PCOM images. In addition, a simple data analysis of the five patients excluded by outlier analysis showed that their mean age was 25.8 ± 3.70, and the average of AMH^{first} values was 6.15 ± 2.46 ng/mL (mean ± SD). Quite strikingly, all five excluded patients had PCO, and also mean AMH^{final} value was 8.94 ± 2.78 ng/mL (mean±SD), consistent with the higher values predicted for young PCOS patients in previous studies. Within the framework of this information, in this study, the patients were classified to their PCO status and compared with their serum AMH levels due to the estimation that MTX administration may have different effects on changes in AMH levels depending on whether there is PCO or not. Table 4 presented comparisons of patients with and without PCO.

There was no statistically significant difference between AMH^{first} levels in the conditions of whether the patients had PCO or not ($p > 0.05$). On the contrary, there was a statistically significant difference between AMH^{final} levels according to whether the patients had PCO or not post EP treatment ($p < 0.0001$). There were statistically significant differences in AMH changes in each group, with the changes favoring an increase in patients with PCO and a decrease in patients without PCO ($p = 000.1$, $p < 0.0001$ respectively).

Table 2. Comparison of the demographic characteristics and ultrasound signs of patients with contrasting changes in anti-müllerian hormone (AMH) levels

Variables	Patients with a decrease in serum AMH n = 32 (55.2%)	Patients with an increase in serum AMH n = 26 (44.8%)	p
Age (mean ± SD)	31.00 ± 6.41	29.65 ± 5.78	0.37
Gravida (mean ± SD)	3.21 ± 2.19	3.03 ± 1.70	0.96
Parity (mean ± SD)	1.18 ± 1.33	1.34 ± 1.35	0.62
Abortion (mean ± SD)	1.03 ± 1.85	0.69 ± 0.92	0.92
EP history; n [%]	2 (6.2%)	3 (11.5%)	0.64
Abdominopelvic Surgeries (mean ± SD)	0.68 ± 0.89	0.65 ± 0.79	0.98
Endometrial thickness [mm], (mean ± SD)	10.25 ± 4.25	11.19 ± 3.78	0.28
Size of EP mass [mm], (mean ± SD)	18.06 ± 7.90	19.76 ± 8.54	0.50
Free fluid in Douglas [mm], (mean ± SD)	4.78 ± 9.43	5.46 ± 8.46	0.50
EP localization, n [%]			
Right: 41 (70.7%)	22 (68.7%)	19 (73.1%)	0.77
Left: 17 (29.3%)	10 (31.3%)	7 (26.9%)	
PCOM image, n [%]			
Positive: 24 (41.3%)	4 (12.5%)	20 (76.9%)	**0.0001**
Negative: 34 (58.7%)	28 (87.5%)	6 (23.1%)	
PCOS history, n [%]			
Positive: 19 (32.7%)	2 (6.2%)	17 (65.4%)	**0.0001**
Negative: 39 (67.3%)	30 (93.8%)	9 (34.6%)	
BMI [kg/m^2]			
(mean ± SD)	26.18 ± 3.47	27.19 ± 3.82	0.42

BMI — body mass index; EP — ectopic pregnancy; SD — standard deviation; PCOM — polycystic ovary morphology; PCOS — polycystic ovary syndrome. The percentage of the patients in each group was calculated with reference to the number of patients in column labels

Table 3. Comparison of the laboratory results and methotrexate (MTX) therapy dosages of patients with contrasting changes in anti-müllerian hormone (AMH) levels

Variables	Patients with a decrease in serum AMH n = 32 (55.2%)	Patients with an increase in serum AMH n = 26 (44.8%)	p
AMH^{first} [ng/mL], (mean ± SD)	1.96 ± 1.04	2.06 ± 1.34	0.95
AMH^{final} ([ng/mL], (mean ± SD)	1.61 ± 0.92	2.71 ± 1.73	**0.016**
Pre-treatment β-hCG [IU/L], (mean ± SD)	3911.87 ± 3036.32	3620.11 ± 2951.52	0.56
Post-treatment β-hCG [IU/L], (mean ± SD)	2410.90 ± 2078.73	2115.73 ± 1994.61	0.56
MTX dosage [mg], (mean ± SD)	68.46 ± 7.58	66.25 ± 6.72	0.24
Number of MTX administration, n [%]			
Single dose: 9 (15.5%)	5 (15.7%)	4 (15.4%)	0.90
Two doses: 18 (31.0%)	9 (28.1%)	9 (34.6%)	
Three doses: 8 (13.8%)	4 (12.5%)	4 (15.4%)	
Four doses: 23 (39.7%)	14 (43.7%)	9 (34.6%)	
Salpingectomy, n [%]			
Positive: 16 (27.6%)	7 (21.9%)	9 (34.6%)	0.28
Only MTX: 42 (72.4%)	25 (78.1%)	17 (65.4%)	

AMH^{first} — pre-treatment serum AMH measurement; AMH^{final} — post-treatment serum AMH measurement (3–6 months after treatment); SD — standard deviation. The percentage of the patients in each group was calculated with reference to the number of patients in column labels

Table 4. The effects of ectopic pregnancy (EP) treatment on changes in serum anti-mullerian hormone (AMH) levels regarding the patients with or without polycystic ovary (PCO)

Groups	AMH^{first} [ng/mL]	AMH^{final} [ng/mL]	p
Patients with PCO	2.47 (0.39–5.67)	2.6 (0.63–6.82)	**0.0001**
n = 25 (43.1%)	2.39 ± 1.31	3.03 ± 1.60	
Patients without PCO	1.76 (0.22–4.06)	1.38 (0.13–3.20)	**< 0.0001**
n = 33 (56.9%)	1.71 ± 0.98	1.40 ± 0.78	
p	0.057	**< 0.0001**	

AMH^{first} — pre-treatment serum AMH measurement; AMH^{final} — post-treatment serum AMH measurement (3–6 months after treatment). In each column and row, the top value is the "$Median^{(min–max)}$" and the bottom value is the "mean ± standard deviation (SD)"

DISCUSSION

This study was designed to evaluate the possible effects of multi-dose MTX therapy or subsequent salpingectomy on ovarian reserve using serum AMH levels in patients with tubal EP. Moreover, it aimed to declare the potential factors that cause the change in serum AMH levels in treated tubal EP patients.

Both MTX and surgical management used in EP remain contentious topics regarding the conservation of the ovarian reserve. Tetrahydrofolate produced by the enzyme dihydrofolate reductase ensures DNA synthesis and repair and thus proper cell proliferation [1, 2]. MTX is a folic acid antagonist and competitively binds to the enzyme required for tetrahydrofolate synthesis [17]. Thus, MTX inhibits rapidly proliferating cells such as trophoblasts of EP [1–3]. MTX, the most commonly known medication for the medical treatment of EP, does not affect primordial follicles but may pose a threat to developing preantral and antral follicles in the ovary [2, 16]. However, since possibly the transition from primordial follicles to growing follicles could not impair in this case, disruption of follicles might be provisional plus ovarian reserve may be preserved. Regardless, the chemotherapeutic agents are known to reduce the ovarian reserve of reproductive-age women in a dose-dependent manner [28]. Furthermore, in a presented study, MTX has been particularly associated with early ovarian failure in childhood cancer survivors [29]. Another ritual that can affect ovarian reserves in the treatment of EP may be salpingectomy. A salpingectomy typically obliterates the vascular structures such as the ovarian artery in the mesosalpinx localization plus the tubal anastomosis of the uterine artery, hence, partially blocking the ovarian blood cycle [30]. As a result, the ovarian reserve may decrease after salpingectomy. Chan et al. [31] reported that the unilateral salpingectomy reduced the number of ovarian antral follicles in the short term but had no significant effect on ovarian reserve in the long term in EP patients. Patients who underwent salpingotomy, tubal milking, and fimbriectomy were excluded from the current study to aim more clearly to evaluate the effects of a ligated Utero-ovarian anastomosis by salpingectomy on ovarian reserve. Regardless of the final treatment type, 58 patients had already received MTX due to the design in the present study. There was no significant difference between their AMH^{first} and AMH^{final} values of included 58 patients. Nonetheless, there was an increase in the mean value and a decrease in the median value in the post-treatment AMH levels. Analyzing the graph of the AMH distributions, it was detected that AMH levels increased for some patients and decreased for others after treatment. Then, the conditions that may cause AMH values to show these distinctions were concerned. The 16 patients underwent salpingectomy after MTX therapy due to medical treatment failure, plus there were 42 patients receiving only medical treatment, which corresponds to 72.4% multiple-dose MTX treatment success. That is consistent with success rates of 65–94% reported in previous studies [32] and highlighted the effectiveness of the procedure of MTX performed. EP treatment with isolated MTX or subsequent salpingectomy did not cause a significant difference between AMH^{first} and AMH^{final} values in the study. In this respect, this study was consistent with previous studies reporting that MTX or salpingectomy did not cause a decrease in ovarian reserve [17, 18, 20–22]. One of these studies reported that AMH levels tended to increase immediately after, but there was no significant difference or decrease after several months with MTX administration [21]. Again, another study on ovarian response associated with AMH in EP treatment reported the decrease of AMH observed in the 1^{st} month disappeared in the 3^{rd} month [22]. Nevertheless, Uluğ et al. [19] found a significant decline in AMH after multi-dose MTX or salpingectomy surgery in rat models. The individual AMH changes in our included patients displayed different trends as in the previous studies. While AMH tended to decrease in 32 (55.2%) patients, on the contrary, it had an increasing trend in 26 (44.8%) patients. The AMH^{first} values of the patients with decreased or increased AMH were not statistically significantly different from each other, but the mean of those with an increase was slightly higher than the other. On the other hand,

the AMH^{final} values were significantly different between these two groups, with a mean decrease of 17.8% and an increase of 31.5% in AMH, respectively. Age, characteristics of the pregnancy anamnesis, sonographic findings of the EP mass, and BMI did not differ between these groups with different trends of AMH change. Again, the number of MTX administration, drug doses, and the presence of subsequent salpingectomy did not differ among these patients, which was a possible proof that neither of them affects ovarian reserve in our tubal EP population.

The recently increased need for women to have an idea about their future reproductive abilities reveals the importance of evaluating their ovarian reserves. The safest known marker to evaluate functional ovarian reserves in women of reproductive age is serum AMH synthesized from preantral and antral follicles in the ovary [6, 7]. Despite AMH's high reliability, some studies demonstrated the variability of AMH levels in different conditions. Some studies that analyzed non-pregnant women's AMH changes during various stages of menstrual cycles reported a biological variability of approximately 10–20% for serum AMH measurements [9, 27, 33]. The post-treatment AMH measurement was planned on the 2^{nd} day of the 2^{nd} menstrual cycle for each patient, aiming to prevent the possible effects of previously cited fluctuation on the AMH measurements in the present study. On the other hand, the other studies examining pregnancy-associated AMH changes in the literature reported no change in AMH, especially between the first trimester and the maximum 6-month period after pregnancy [26, 34, 35]. In addition, the individual and interindividual high AMH variability are suggested as greater in younger women with PCOM than in older women without PCOM [36]. Also, a high serum AMH level with a PCOM image may indicate PCOS affecting 5–20% of women of reproductive age worldwide [9, 10]. In women with PCOS, the ovaries containing many small antral follicles are 2–3 times larger than the average size. AMH is elevated in serum at preantral and small antral follicle stages and shows highly variable levels in PCOS patients [37]. A study on in vitro fertilization confirmed high serum AMH levels were associated with lower live birth rates of women with PCOS undergoing assisted reproductive technology [12]. Furthermore, a controlled study of ovarian hyperstimulation revealed that women with PCOS had a 3.06 times higher risk of EP than those without PCOS [14]. Also, sonographic PCOM images are associated with an increased relative risk of EP, and the morphology of the ovaries in suspected EP cases can guide early diagnosis, thereby reducing morbidity in patients [15]. The PCO condition was present in 25 of 58 patients (43.1%) in the current study, which was a very high rate relative to an average population but was possibly typical for the EP population. Conspicuously, having a PCOM image or a known history of PCOS was significantly different between patients with the opposite trends of AMH changes in this study. Further, there was a difference between the AMH^{first} values of patients with and without PCO, exhibiting a low level of statistical significance. In fact that the expected difference should have been higher than this, as PCO is known to increase AMH levels. However, not including the AMH values of the five patients excluded from the study due to the outlier analysis may have caused this outcome. All of these excluded patients had PCO, and their elevated AMH values were highly outlied than the study population. The post-treatment mean value of AMH level increased by 26.7% in patients with PCO and decreased by 18.1% in those without PCO as well as there was a statistically significant difference between the pre-and post-treatment AMH values. Accordingly, the presence or absence of PCO in patients treated with EP may have a different effect on the ovarian response of the patients. Numerous studies in the literature evaluating ovarian response with AMH after EP therapy have had variable results, and none of these studies referred to the PCO condition. We think that the reason for these confusional results may be a factor of PCO overlooked in the evaluations. However, due to the lack of a control group consisting of untreated healthy patients with and without PCO and the relatively small number of patients in this study, we emphasize the need for prospective randomized studies involving more patients. Regardless, this new idea may predict more reasonable outcomes for the informed of fertility in the future of patients with EP treated by MTX or subsequent salpingectomy.

CONCLUSIONS

Multiple-dose MTX or subsequent salpingectomy treatment in tubal EP patients might not refer to significant differences in patients' AMH levels. Remarkably, post-treatment AMH levels were significantly increased in EP patients with PCO and decreased in those without this condition. Further studies involving the PCO factor are required to clearly evaluate the effects of MTX or subsequent salpingectomy on ovarian response.

Authorship contributions

FC and AIK carried out the study, performed the medical and surgical procedures, followed up with the patients, and contributed to the first draft of the paper. NBT and FC designed the study, wrote the manuscript, and finalized the paper. FC edited the language. AIK and NBT revised the manuscript critically for important intellectual content. FC analyzed and then interpreted the data. All authors read and approved the final article.

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Conflict of interest

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