

# The Effect of Administration of Methotrexate For Ectopic Pregnancy On Ovarian Reserve

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## ABSTRACT

Methotrexate (MTX) has an essential role in the treatment of ectopic pregnancy, and high success rates have been achieved. MTX acts on rapidly proliferating cells like ovarian germinal cells. The aim was to elucidate whether the administration of MTX has a significantly adverse effect on ovarian reserve.

165 patients were included and divided into 3 groups. 38 patients having MTX treatment (50mg/m<sup>2</sup>) were group1, 45 patients having surgical treatment (laparoscopy or salpingostomy/salpingectomy by laparotomy) were group2, and 82 patients who admitted to gynecology outpatient clinic were group3, the control group of the study. The patients who had surgery because of rupture following MTX treatment were not included. The patients were investigated for ovarian reserve test 3 months after having negative b-hCG test. Basal levels of hormones FSH, E2 measured, and by transvaginal USG, we counted the number of antral follicles and measured ovarian volume.

The average age of all patients was 29.6±3.8; in group1 30.1±3.9, in group2 30±3.6, in group3 29.2±3.9 years. There is no statistical difference between groups (p: 0.31). FSH level was 7±1.7IU/L in group1, 7.4±1.6IU/L in group2, and 7±1.4IU/L in group3. There is no difference between groups (p: 0,77). Estradiol level was 44.5±24.2 pg/mL, 45.8±17.6 pg/mL, 48.5±16.4 pg/mL in groups respectively. There is no statistical difference between groups (p: 0.09). There is also no statistically significant difference between groups for basal antral follicle numbers and ovarian volumes (p: 0.11, p: 0.16, respectively).

In unruptured ectopic pregnancies, MTX treatment has advantages for cost-effectivity and morbidity. There is no effect of MTX treatment on ovarian reserve.

**Keywords:** Ectopic pregnancy, ovarian reserve, methotrexate

## Introduction

Ectopic pregnancy is one of the important preventable reasons for maternal mortality in the first trimester of pregnancy, and its frequency has increased in recent years. The rise in the incidence of pelvic infection, the increase in the use of intrauterine devices, surgical treatment of infertility, and the widespread use of assisted reproductive techniques are thought to be the main reasons for this increment (1). The other possible explanation for the increase in ectopic pregnancy rates is the more sensitive measurements of the human chorionic gonadotropin levels, along with the common use of transvaginal sonography and laparoscopy had allowed for an early and definitive diagnosis of the disease (2). In addition to surgical treatment, especially by way of advanced early diagnosis methods, medical treatment of ectopic pregnancy has an essential role in treatment of ectopic pregnancy, and high success rates have been achieved

by methotrexate (MTX) administration. The MTX treatment in case of non-ruptured ectopic pregnancy has many advantages over surgical treatments. Less tubal damage and lower cost can be considered (3). MTX acts on rapidly proliferating cells by disrupting cellular replication and proliferation. Ovarian germinal cells may also be adversely affected by this condition (4). In recent years preservation of ovarian reserve has become very important for reproductive-aged women; because of delaying in childbearing by couples and advanced maternal age. Thus this study aimed to elucidate whether administration of MTX treatment in case of ectopic pregnancy has a significantly adverse effect on ovarian reserve.

## Materials and Methods

This study was conducted in Zeynep Kamil Research and Education State Hospital in Istanbul, Turkey. The

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Received: 21.03.2021, Accepted: 01.03.2023

study was designed as a cross-sectional study. Patients who admitted to the Gynecology Service with the diagnosis of ectopic pregnancy between May 2013 and September 2013 and who underwent MTX treatment and surgical treatment, laparoscopic or laparotomy salpingostomy or salpingectomy, were included in this study, and patients that have no infertility problem and who applied to Gynecology Polyclinic at the same time were involved as a control group. Women with a history of ovarian surgery and disease that may adversely affect ovarian reserve over 35 years were excluded from the study (endometriosis, smoking, etc.). The institutional review board approved the study (dossier number:2014/74), and all participating patients gave written informed consent.

In ectopic pregnancy, administration of MTX treatment is used based on the following criteria:

- $\beta$ -HCG concentration below 5000 mIU/mL
- The patient is hemodynamically stable
- No fetal cardiac activity
- Ectopic pregnancy less than 4cm
- Make sure of patient's compliance with follow-up treatment

The criteria for applying surgical treatment (salpingostomy or salpingectomy) to patients diagnosed with ectopic pregnancy are:

- Hemodynamic instability
- Presence of fetal cardiac activity
- Ectopic pregnancy above 4cm
- $\beta$ -HCG concentration below 5000 mIU/mL

Demographic data, biochemical parameters, age, and smoking status of the participants were recorded. Moreover, all of the patients in the study were of the same age period. 165 patients were included and were divided into three groups: patients who received MTX (50 mg/m<sup>2</sup>) treatment (group 1, n=38), patients who underwent surgical treatment laparoscopic or laparotomy with salpingostomy or salpingectomy (group 2, n=45), and control group (group 3, n=82). In the control group, all patients had regular menstrual cycles, and none of them had ovarian surgery history or ovarian diseases that may cause a decrease in ovarian reserve.

After liver and renal function tests, IM MTX was administered at a dose of 50mg/m<sup>2</sup>. In order to evaluate the success of MTX treatment, serum  $\beta$ -HCG concentrations of the patients were measured after MTX administration on the 4th and 7th days. After medical treatment from the day of the 4th to the 7th day, a decrease of 15% or more in  $\beta$ -HCG levels was considered an effective treatment. If  $\beta$ -

HCG decrease was less than 15%, multiple-dose MTX treatment was applied (7 patients). Then weekly  $\beta$ -HCG values were measured until the negativity of  $\beta$ -HCG values was confirmed.

Depending on tubal damage level, patients underwent laparoscopically or laparotomy, salpingostomy, or salpingectomy. Salpingectomy was performed by laparoscopy or laparotomy for hemodynamically unstable patients or with signs of tubal rupture. MTX treatment was not implemented for patients who underwent surgical treatment.

After the treatments mentioned above modalities, the ovarian reserve of all patients was evaluated three months after their  $\beta$ -HCG levels reached negative values. Ovarian reserve tests were performed on the third day of the menstrual cycle, by measuring the serum FSH E2 level and by transvaginal ultrasonography calculating ovarian volume and antral follicle count. On the third day of the cycle, FSH and E2 levels were measured and recorded with the Beckman Coulter Unigel DX 1 800 Access Immunoassay system. Basal antral follicle count and ovarian volume were evaluated and recorded by the same operator with TVUSG (GE Medical Systems Logic 5 Pro). Ovarian volume was calculated and registered with the formula ( $V: A*B*C*0,52$ ).

Statistical tests were performed using SPSS version 15 software. The compatibility of the variables to the normal distribution was examined using visual and analytical methods. Descriptive analyzes were given using the median and interquartile range (IQR) for non-normally distributed variables. Since none of the variables showed a normal distribution, the Kruskal-Wallis test was used to analyze the three groups. The total type-1 error level was determined as 5% for statistical significance.

## Results

When the demographic characteristics of the patients were investigated, the mean age was  $29.6 \pm 3.8$  years. The mean age was  $30.1 \pm 3.9$  in the MTX treatment group,  $30 \pm 3.6$  in surgical treatment patients, and  $29.2 \pm 3.9$  in the control group. There was no difference between the groups regarding age ( $p=0.31$ ). The participants' mean body mass index (BMI) is  $24.6 \pm 2.8$ . The mean BMI was  $24.8 \pm 3.7$  in the group receiving MTX treatment,  $25 \pm 2.8$  in the surgical treatment group, and  $24.2 \pm 2.3$  in the control group. There was no statistically significant difference between the groups regarding BMI ( $p=0.48$ ).

The average dose of MTX used in the study was  $84.5 \pm 3.8$ mg. The maximum dose of MTX used is 200mg, and the minimum dose of MTX is 50mg.

**Table 1:** Average Age, Ovarian Volume, Hormone Profile, and Antral Follicle Count of The Groups

	MTX treatment (n=38)	Surgical treatment (n=45)	Control Group (n=82)	P value	MTX treatment (n=38)
Age (y)	30.1±3.9	30±3.6	29.2±3.9	0.31	Age (y)
BMI (kg/m <sup>2</sup> )	24.8±3.7	25±2.8	24.2±2.3	0.48	BMI (kg/m <sup>2</sup> )
Total Basal AFC	13.6±3	13.2±2.7	14.1±3.2	0.11	Total Basal AFC
Right ovarian volume (mm <sup>3</sup> )	8±2.6	9.1±3	8.1±2	0.16	Right ovarian volume (mm <sup>3</sup> )
Left ovarian volume (mm <sup>3</sup> )	9.1±4.1	8.9±5	7.6±1.8	0.11	Left ovarian volume (mm <sup>3</sup> )
FSH (IU/L)	7±1.7	7.4±1.6	7±1.4	0.77	FSH (IU/L)
LH (IU/L)	5.7±3.3	6.8±4.8	6.2±1.2	0.06	LH (IU/L)
E2 (pg/ml)	44.5±24.2	45.8±17.6	48.5±16.4	0.09	E2 (pg/ml)

\*Values are presented as mean ± SD unless otherwise stated

MTX: methotrexate, AFC: antral follicle count, FSH: follicle stimulating hormone, LH:luteinizing hormone, E2: estradiol, SD:standard deviation p <0.05

Seven patients (18%) received two doses of MTX treatment (7/3. Among 45 patients who underwent surgical treatment, salpingectomy was performed in 15 (33%) patients, and salpingostomy was performed in 30 (67%) patients.

Basal FSH levels measured on the third day of the menstrual cycle were in the group receiving MTX: 7±1.7 IU/L and was 7.4 ±1.6 IU/L in the surgically treated group and 7±1.4 IU/L in the control group. No statistically significant difference was determined between study groups in terms of basal FSH levels. Basal E2 levels were in the MTX group, 44.5 ±24.2 pg/mL, 45.8±17.6 pg/mL, and 48.5±16.4 pg/mL in the surgical and control groups. There was no difference between the groups regarding basal E2 levels (p=0.09). Basal antral follicle count was 13.6±3 in the group receiving MTX, in the group who underwent surgery, 13.2±2.7, and 14.1±3.2 in the control group. There was no difference between the groups regarding basal antral follicle count (p=0.11). In addition, the ovarian volume of patients was the same between the study groups (p=0.11, Table 1).

## Discussion

It is well known that ectopic tubal pregnancy has adverse effects on fertility (5). Tubal damage due to ectopic tubal rupture and surgical intervention to the tuba negatively impacts fertility (5). Age, contralateral tubal condition, and history of infertility are the most critical determinant factors of the future fertility potential of women with a history of ectopic pregnancy. Minimizing tubal damage is not only crucial for the prevention of recurrence of ectopic

pregnancy; but also essential to maintaining fertility. Early diagnosis of ectopic pregnancy reduces tubal damage and increases the success of medical treatment. Methotrexate is the most commonly used drug in the medical treatment of ectopic pregnancy. MTX is a drug that impairs DNA repair and inhibits the enzyme dihydrofolate reductase (4). It mainly affects the S phase of cell division, and also its effect covers all stages of cell division. The impact of MTX is more pronounced on rapidly dividing cells such as gastrointestinal cells, bone marrow, and primordial follicles of the ovary. Since ovarian germinal cells are always prone to proliferate, there is a concern that, theoretically, the ovarian reserve may be affected by MTX therapy.

It has been reported that the follicle pools of women who received chemotherapy because of various malignancies decrease in a dose-dependent manner. In addition, menopausal cases were detected due to MTX therapy as a chemotherapeutic agent for gestational trophoblastic diseases (6). Partridge A. H. et al. have evaluated the ovarian reserve of patients who received combined chemotherapy, including alkaline agents, due to the early stage of breast cancer. Despite the interruption of menstrual cycles during chemotherapy being determined in 80% of patients, ovarian suppression developed in only 10% of patients (7). While menstrual cycle regularity is a good indicator of good ovarian reserve, a menstrual irregularity was not detected in any patient who received MTX treatment in our study. Also, the menstrual cycles of patients were regular.

Boots C.E. et al. analyzed 66 infertile patients who received MTX treatment for ectopic pregnancy in

terms of ovarian reserve and IVF results before and after MTX treatment. This retrospective cohort study concluded that there was no difference in basal FSH levels, basal antral follicle count, and oocyte retrieval count before and after MTX treatment. But only after MTX treatment higher dose of gonadotropin was required; however, it was concluded that there was no difference in the number of pregnancies obtained. This study concluded that administration of MTX treatment due to ectopic pregnancy did not affect IVF results and ovarian reserve (8).

In another prospective study by Uyar İ. et al., the effect of a single dose of MTX therapy applied for ectopic pregnancy on the ovarian reserve was investigated (9). Eighty-two women with a history of ectopic pregnancy and eighty healthy women were involved in the study; Of 82 patients with a history of ectopic pregnancy, 49 were treated with a single dose of MTX, and 33 were treated surgically. The study participants were divided into three groups: the control group, the surgical group, and the MTX group. The patients were subjected to ovarian reserve tests and examined for ovarian reserve. No significant difference was detected between the groups regarding ovarian volume, basal FSH levels, basal antral follicle count, and ( $p>0.05$ ).

In another study which was conducted by Mc Laren J. F. et al., 48 patients who were treated with MTX and followed a controlled ovarian hyperstimulation protocol applied; On the 3rd day of the cycle, FSH levels, pregnancy rates, and the number of oocytes obtained (IVF results) were evaluated, with the values in the first 180 days after MTX treatment and after 180 days. It was concluded that, although the number of oocytes retrieved from patients who had controlled ovarian hyperstimulation within the first 180 days after MTX treatment was less, It was determined that there was no difference in the number of oocytes retrieved after 180 days of treatment. As a result of this study, it was concluded that the negative effect of MTX treatment on oocyte production is time-dependent and reversible (10).

Ohannessian A. et al., 2014 have published a meta-analysis that includes IVF results of patients who were administered MTX treatment due to ectopic pregnancy and received posttreatment infertility treatment. The meta-analysis comprised seven observational studies and 329 patients. This meta-analysis evaluated patients' basal FSH levels, total stimulation time, IVF treatment results, the number of oocytes, E2 levels on the day of B-HCG administration, and gonadotropin dose. No statistically significant difference was found ( $P>0.05$ ) in the oocyte retrieval number before and after MTX treatment. As a result, it was concluded that MTX

administration because of ectopic pregnancy has no adverse effect on the infertility treatment, which will be applied in the later period of MTX treatment (11).

Antimüllerian Hormone (AMH) is produced by granulosa cells of preantral and small antral follicles. The number of antral follicles is correlated with the size of the remaining follicle pool, and serum AMH levels decrease progressively and become undetectable in the near-menopausal period. It has been found that diminished AMH levels are associated with a decreased ovarian reserve and poor ovarian response. Oriol B. et al. investigated the ovarian reserve of 25 patients who developed ectopic pregnancy after IVF treatment and single-dose MTX administration. In the study, AMH levels of 25 patients were recorded at the time of diagnosis of ectopic pregnancy and after MTX treatment. Then IVF treatment was applied to the patients in the future. No significant difference was found in serum AMH levels before and after MTX treatment ( $p>0.05$ ). At the same time, no statistically significant difference was detected in terms of IVF results before and after MTX treatment. As a result of the study, it has been concluded that a single dose of MTX treatment had no negative effect on ovarian reserve (12). In another study by Benian A. et al., the effect of multidose MTX treatment on serum AMH levels was investigated. MTX treatment on days 1, 3, 5, and 7 have been implemented; Serum AMH levels were recorded after each MTX treatment. Pre-treatment mean serum AMH level was 102.4 ng/mL; levels 1, 3, 5, 7 days after MTX administration, serum AMH levels were: 70.6 ng/mL, 136.1 ng/mL, 121.2 ng/mL and 104.7 ng/mL respectively. In terms of serum AMH levels between days, It was found that there was no statistically significant difference. In conclusion, this study concluded that multidose MTX treatment did not affect serum AMH levels (13).

Our study has several limitations. Among the limitations, our study was retrospectively designed, and serum AMH levels were not evaluated in our research. However, it can still be used as a useful ovarian reserve marker to measure the antral follicles count and FSH, E2 levels on the 2nd and 3rd day of the menstrual cycle. In addition, unlike other studies in the literature, we also evaluated ovarian volume in our study.

In conclusion, as determined in our study, MTX administration for ectopic pregnancy has no negative effect on ovarian reserve. However, further studies are needed that will also include the long-term ovarian reserve of the patients.

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