

# A Comparative Study of Impacts of Single-dose Systemic Methotrexate and Salpingectomy Surgery Treatments on Ovarian Reserve in Women with Ectopic Pregnancy Using Anti-Mullerian Hormone

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## Abstract

**Background and Objective:** Ectopic pregnancy (EP) is a serious cause of maternal mortality that complicates about 2% of pregnancies. There are two approaches for EP treatments including surgical intervention and methotrexate (MTX). The present study aims to comparatively investigate impacts of single-dose MTX and laparoscopic salpingectomy treatments on ovarian reserve in women with EP using anti-Mullerian hormone (AMH) level as the biomarker of subsequent pregnancy. **Materials and Methods:** This was a prospective randomized clinical trial conducted on 30 patients with EP who referred to two University Hospitals in Shiraz during December 2014–June 2016. The subjects were divided into single-dose MTX group and salpingectomy group. Before the treatments and at 6-month post-treatment follow-up, the serum AMH level was measured using ELIZA method and compared within and between groups. **Results:** The serum AMH level significantly decreased in both groups at 6-month post-treatment compared with the baseline levels ( $P = 0.05$ ). The single-dose MTX group showed more reduction than the surgery group (1.6 vs. 1.2 pg/mL). However, the difference in the post-treatment serum AMH level was not statistically significant between the two groups ( $P > 0.05$ ). **Conclusion:** Both single-dose MTX group and salpingectomy treatments are effective for EP. For patients with less severe EP, it is recommended to administer single-dose MTX than surgical approach.

**Key words:** Ectopic pregnancy, laparoscopic salpingectomy, methotrexate

## INTRODUCTION

Implantation of blastocyst outside the endometrial lining of the uterine cavity will lead to an extra-uterine pregnancy called ectopic pregnancy (EP). This is a serious cause of maternal mortality and morbidity that complicates 1–2% of all first trimester pregnancies in the United States.<sup>[1]</sup> Although the prevalence rate is relatively low, this small proportion disparately accounts for 6% of all pregnancy-related mortalities. This significant maternal mortality necessitates early diagnosis and effective management strategies for EP. EP can develop in different locations outside the uterine cavity, but the most common site for the implantation is within the fallopian tubes. Thanks to the recent advances in diagnostic and imaging modalities during the recent years have facilitated early diagnosis and treatment which

in turn dramatically reduced the EP associated burdens and maternal mortalities.<sup>[2]</sup> Urine and serum beta-human chorionic gonadotropin assays and transvaginal sonography have made earlier diagnosis possible and as a result both maternal survival rates and conservation of reproductive capacity.<sup>[3,4]</sup>

Approximately 95% of EP cases are implantation in the various segment of fallopian tubes that can develop to different types including fimbrial (11%), ampullary (70%), isthmic (12%), or interstitial tubal pregnancy the remaining

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5% of non-tubal ectopic pregnancies implant in the ovaries, peritoneal cavity, cervix, or prior cesarean section scar.<sup>[1]</sup> A classic presentation of EP is characterized by a triad of delayed menstruation, pain, and vaginal bleeding, or spotting.<sup>[5,6]</sup> To reach a definitive diagnosis of EP, several factors are monitored which include physical examinations, transvaginal sonography, serum b-HCG level, serum progesterone level, and diagnostic surgeries including uterine curettage, laparoscopy, or laparotomy surgery. Currently, different therapeutic modalities are administered for tubal EPs, which can be categorized into medications and surgical approaches.<sup>[7,8]</sup>

The main medication is methotrexate (MTX), and the main surgical technique is salpingectomy. Surgical approach has different side effects; therefore, recent studies have aimed to develop efficient and safer protocols of MTX for EP treatment.<sup>[9,10]</sup> MTX is a chemotherapeutic agent which resembles a folic acid antagonist and specifically targets proliferating cells. This agent is an effective compound and widely used for different types of Eps.<sup>[9]</sup>

Several studies have shown that single-dose systemic MTX treatment is an efficient and non-invasive treatment for EP. However, several studies also have reported possible adverse effects of this regimen on fertility through targeting actively dividing cells within the ovaries. Furthermore, the surgical approach could impose a negative effect on ovarian reserve.<sup>[10]</sup> In general, there are controversial findings on the impacts of MTX and surgical treatment on ovarian reserve<sup>[6-8,11-13]</sup>

The best candidate for medical therapy is a patient who is asymptomatic with low initial serum b-HCG level (<5000 u/mL), small EP size (<4 cm), and absent fetal cardiac activity.<sup>[11,12]</sup> For medical treatment, single- and multiple-dose MTX protocol is available. However, in some studies, not all reported a higher success rate for the multidose regimen (93% v/s 88%), but single dose therapy offers simply due to less expense and less intensive post-therapy monitoring and does not require leukoverine rescue.<sup>[13]</sup>

Anti-Müllerian hormone (AMH) is a glycoprotein hormone that is mainly secreted by preantral and small antral follicles in granulosa cells (<4 mm) and exists at relatively constant levels during different phases of the menstrual cycle with little impact on outer determinants.<sup>[14-16]</sup>

AMH is an ideal biomarker for the size of the ovarian follicle pool. The clinical value of serum AMH as a marker of the ovarian reserve has been recently shown in young women underwent treatment for childhood cancer, chemotherapy, radiotherapy, and in the women underwent infertility treatment for ovarian pathophysiology like PCO.<sup>[16]</sup>

Several studies have shown that AMH levels are reliably correlated with the number of oocytes retrieved, quality of embryos, and clinical pregnancy, and live birth rates in

patients who are undergoing *in vitro* fertilization treatment.<sup>[15]</sup> Therefore, AMH level is accepted as a more reliable and objective indicator of ovarian reserve, compared with traditional measures such as FSH level and antral follicle count (AFC).<sup>[16]</sup>

Some studies have reported decreased fertility outcome following EP treatment (17, 18). Job–Job-Spira *et al.* followed up the EP treatment outcomes and reported a post-treatment fertility rate of about 66% among the treated women.<sup>[17]</sup>

The main reasons that can explain the decreased fertility rate after treatment of EP include age higher than 35 years, tubal abnormality, low socioeconomic state, adnexal adhesion, history of ruptured EP, and type of treatment (medication v/s surgery).<sup>[18,19]</sup>

In a prospective randomized clinical trial, Fernandez *et al.* compared the efficacy of MTX treatment with salpingectomy on subsequent fertility in the women with EP and concluded that MTX is as efficient as laparoscopic salpingectomy surgery in improving subsequent fertility.<sup>[20]</sup> However, in other study conducted by Fernandez *et al.*, no significant difference in subsequent fertility was reported between MTX and surgical treatments.<sup>[21]</sup>

Therefore, the present study was aimed to comparatively investigate impacts of single-dose MTX and laparoscopic salpingectomy treatments on ovarian reserve in women with EP using AMH level as the main primary outcome of subsequent pregnancy.

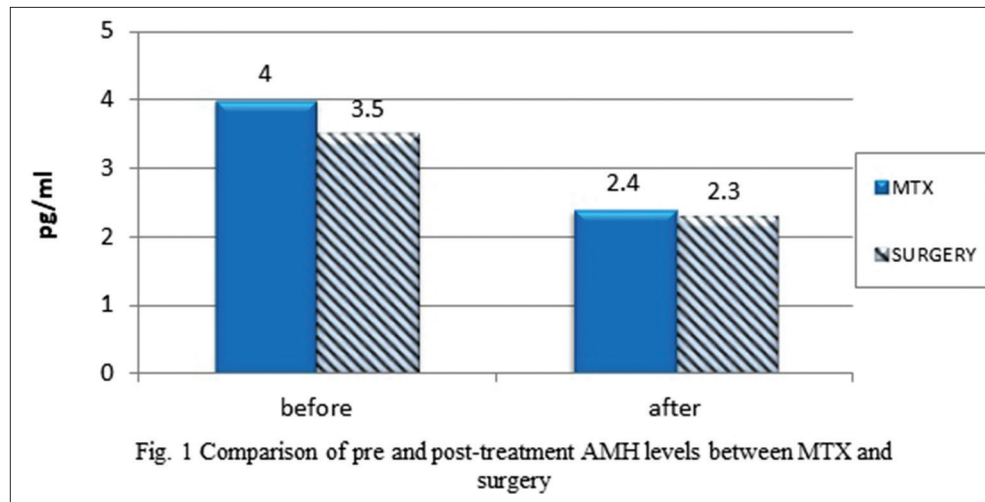
## MATERIALS AND METHODS

This was a prospective randomized controlled trial conducted on patients ( $n = 30$ ) with a definitive diagnosis of EP confirmed by ultrasonography. The subjects of the study were selected among the patients with EP who admitted in the Faghihi and Zeynabiyeh Hospitals, Shiraz, Iran, during December 2014–June 2016 after applying inclusion and exclusion criteria. The subjects were divided into two treatment groups of single-dose MTX and laparoscopic salpingectomy.

The inclusion criteria of the study included pregnant women with age range of 18–45 years old, definite diagnosis of EP, first-trimester pregnancy, patients who need single dose MTX, or laparotomy surgery treatments. The exclusion criteria of the study were previous MTX administration, history of endometriosis, history of chemotherapy or pelvic radiotherapy, previous ovarian surgery, ART or other hormonal therapy, active liver, kidney or lung diseases, NSAIDS consumption, and psychiatric conditions including psychosis or depression, patient with complicated EP, and previous IUD usage.

**Table 1: AMH level before and after treatment**

Measure	Group	Interval	Mean (SD) (pg/mL)	Difference	Average difference	P
AMH	MTX	Before	4 (2.1)	-1.6	+0.5	0.5
		After	2.4 (1.8)			
	Surgery	Before	3.5 (2.0)	-1.2	+0.1	
		After	2.3 (1.6)			

**Figure 1:** Comparison of pre and post-treatment AMH levels between MTX and surgery

### Ethics consideration

All of the experimental procedures of the present study were approved with local Ethics Committee of the Shiraz University of Medical Sciences (Registration code: IR.SUMS.MED.REC.1395.34) that were completely in agreement with the ethical considerations and regulations of human studies set by the Helsinki declaration (1964). After the enrolment of all subjects and before the start of the experimental procedures and data collection, the researchers clearly explained all objectives and possible benefits and side effects of the study to the subjects, and after that, the written consents for participation in the study were signed by all subjects.

### Study protocol

This was a randomized controlled trial conducted on the women with tubal EP confirmed by ultrasonography. The subjects were divided into two treatment groups of single-dose MTX and salpingectomy treatments according to the severity of EP. The patients with less severe EP and no hemodynamic failure were assigned to the MTX treatment and those with more severe EP where the patients should undergo radical or conservative surgery were assigned to the salpingectomy treatment.

Before the treatments and at 6-month post-treatment follow-up, the subjects were assessed using serum AMH level according to the following protocol: 3 ml of blood sample

was collected from an antecubital vein from each subject. The collected blood samples were immediately refrigerated at 4°C, and within 1 h of blood sampling, the serum samples were processed into aliquots and frozen at -70°C until shipped to the laboratory affiliated with the hospitals for the AMH level assessments using ELAISA method. In this study, the main primary outcome for assessment of the ovarian reserve was serum AMH measured by ELIZA technique.

### Statistical analysis

The collected data were analyzed with a statistical package of SPSS (WINDOWS, version 18) using independent sample *t*-test and Chi-square test. The AMH before and 6 months after treatment in both groups were compared within and between groups using independent sample *t*-test and Chi-square test of SPSS software version 18 for windows.  $P < 0.05$  was considered statistically significant.

## RESULTS

During 1 year period, 30 patients entered the study. Mean age of the study population was 29.3 years old, and the two groups showed no significant difference in age ( $P > 0.05$ ).

Serum AMH level decreased significantly in both groups 6 months after treatment especially in the patients

who received single-dose MTX. However, there was no significant difference in the post-treatment serum AMH level between the two groups ( $P > 0.05$ ) [Table 1 and Figure 1].

## CONCLUSION

This randomized clinical trial compared the impact of the serum AMH level is an appropriate biomarker for assessment of ovarian reserve. In this prospective clinical trial, we observed that both treatments of single-dose MTX and salpingectomy surgery in the patients with EP reduced the serum AMH level, but the difference was not significant.

In contrast to our finding, Ulug and Oner demonstrated that single-dose MTX treatment yields a better outcome in the fertility capacity of the patients than the surgical approach.<sup>[22]</sup> Xi *et al.* demonstrated that salpingectomy had no significant effect on ovarian response during IVF cycle.<sup>[23]</sup> The study conducted by Hill *et al.* showed no significant difference between MTX and surgery groups in term of basal FSH level, Estradiol, ovarian volume, and AFC.<sup>[24]</sup>

Conducting further randomized controlled trials with large sample size is needed to definitively address this issue in EP patients who may be more vulnerable to the side effects of the conventional treatments for ovarian reserve. In conducting large sample size, international efforts are needed to arrange for the conduct of multicenter clinical trials with large sample size to address the issue of side effects and therapeutic efficacy of single-dose MTX and salpingectomy surgery for patients with EP.

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