Systemic methotrexate to treat ectopic pregnancy does not affect ovarian reserve

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Objective: To evaluate whether methotrexate (MTX) compromises ovarian reserve and future reproductive outcome in women undergoing assisted reproductive technology (ART), when it is used as first-line treatment for ectopic pregnancy (EP).
Design: Prospective, observational study.
Setting: University-affiliated private IVF unit.
Patient(s): Twenty-five women undergoing IVF-ICSI who were treated with MTX (1 mg/kg IM) for an EP after ART.
Intervention(s): Evaluation of reproductive outcome and serum anti-Müllerian hormone (AMH) levels. Serum AMH was evaluated before administering MTX and ≥ 1 week after the resolution of the EP. Reproductive outcome was evaluated by comparing subsequent IVF-ICSI cycles after EP resolution.
Main Outcome Measure(s): Serum AMH levels, cycle length, gonadotropin dose required, peak serum E_2 level, oocytes collected, and embryos obtained.
Result(s): Serum AMH levels before MTX were not statistically significantly different from those after treatment (3.7 ± 0.3 ng/mL vs. 3.9 ± 0.3 ng/mL). Patients undergoing a subsequent cycle after systemic treatment for EP had similar cycle durations (10.3 vs. 10.8 d), gonadotropin requirements (2,775 vs. 2,630.3 IU), peak E_2 levels (1,884.3 vs. 1,523.6 pg/mL), number of oocytes retrieved (12.1 vs. 10.5), and total number of embryos obtained (7.1 vs. 6.5).
Conclusion(s): Single-dose MTX is a safe first-treatment choice that does not compromise future reproductive outcomes in women who are diagnosed with EP after ART. (Fertil Steril® 2008;90:1579–82. ©2008 by American Society for Reproductive Medicine.)

Key Words: Ectopic pregnancy, methotrexate, AMH, ovarian reserve

Ectopic pregnancy (EP) is still the leading cause of pregnancy-related death in the first trimester (1). The incidence of EP is 1%–2% but increases threefold in women who are undergoing assisted reproductive technology (ART) because of tubal pathology, high steroid hormone levels that impair tubal function, and the higher number of embryos available (2). Currently, EP can be diagnosed accurately at very early stages by using transvaginal ultrasound and serum hCG measurements. Outpatient medical treatment with systemic, single-dose methotrexate (MTX) has become the most accepted treatment alternative to surgical options (3, 4).

Methotrexate is a folic acid antagonist that competitively binds to the enzyme dihydrofolic acid reductase, which converts dihydrofolate to tetrahydrofolate, the active form of folate. Without tetrahydrofolate, DNA synthesis and repair as well as cellular replication are impaired, with actively proliferating cells being most sensitive to the effects (5). Although it is used mainly to treat cancer, trophoblastic disease, psoriasis, or rheumatoid arthritis, MTX has been used to successfully treat EP since 1982 (6). Because germinal cells continuously are proliferating, MTX could reduce ovarian reserve. Methotrexate is considered low-risk chemotherapy for fertility preservation because the menstrual cycles are maintained. However, fertility should be evaluated not only by the presence of regular menses but by ovarian reserve. The effects of a single dose of MTX on ovarian reserve have not been studied elsewhere.

Anti-Müllerian hormone (AMH) is one of the best markers of ovarian reserve that has been used recently because it is independent of the menstrual cycle (7) and has higher intercycle and intracycle reproducibility than markers previously used (8). The purpose of this study is to evaluate whether a single dose of MTX for treatment of ectopic pregnancy compromises ovarian reserve. First, we evaluated AMH in women who were diagnosed with EP before and after treatment with MTX; second, we analyzed cycle outcome before and after MTX to determine whether the ovarian response was affected by medical treatment for EP.

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MATERIALS AND METHODS

From January 2005 to November 2006, 25 patients diagnosed with EP after treatment for infertility at our institution were included in the study, from a total of 59 EPs that were diagnosed in the study period. The diagnosis of EP was suspected when no intrauterine sac was visualized by transvaginal ultrasound, even if there were no significant abnormalities in the adnexa, and serum β-hCG was >1,000 IU/L (9). To confirm the diagnosis, a serial β-hCG determination was performed. In a normal, ongoing intrauterine pregnancy, beta-hCG concentrations should increase at least 66% every 48 hours, before reaching 1,000 IU/L (10).

Patients were eligible for single-dose MTX (1 mg per kg of actual body weight) treatment for EP if they were asymptomatic and hemodynamically stable, had the ability and willingness to comply with posttreatment monitoring, had β-hCG of <5,000 IU/L, had no free fluid of >50 mL in the Douglas pouch, had adnexal mass (if visible) of <3 cm, and had no embryonic heartbeat on ultrasound examination (11). All patients gave written informed consent, and the study was approved by our institutional review board. Follow-up of these patients consisted of serial β-hCG levels until those dropped to <10 IU/L.

After EP resolution, patients underwent another IVF-ICSI cycle, which was monitored and managed according to the standardized clinical protocol that was reported elsewhere (12). One to 3 embryos were transferred on day 3. Data regarding baseline clinical characteristics are described in Table 1.

Anti-Müllerian hormone was measured in patients' sera both before the administration of MTX and ≥1 week after EP resolution (β-hCG of <10 IU/L). Mean time between cycles was 226.4 ± 23.8 days. Blood was collected by peripheral venipuncture, the serum was separated from cells by centrifugation, and samples were frozen at –20°C until assayed. Immunoreactive AMH concentrations were determined by using ELISA (Inmunotech; Beckman Coulter, Villepinte, France) according to the manufacturer's instructions. All samples were tested in the same assay and performed in duplicate. The intra-assay and interassay coefficients of variation were 12.3% and 14.2%, respectively.

Data were expressed as the mean ± SEM. Student's *t*-test and the χ^2 test were used, as appropriate. A *P* value of <.05 was defined as statistically significant. Statistical analysis was performed by using SigmaStat for Windows, version 2.0 (Jandel Scientific Corporation, San Rafael, CA).

RESULTS

As shown in Figure 1, serum AMH levels were not affected by single-dose MTX treatment for EP. Mean serum AMH values in patients diagnosed with EP before MTX administration were comparable to those observed in patients ≥1 week

TABLE 1

Patient and cycle outcome characteristics.

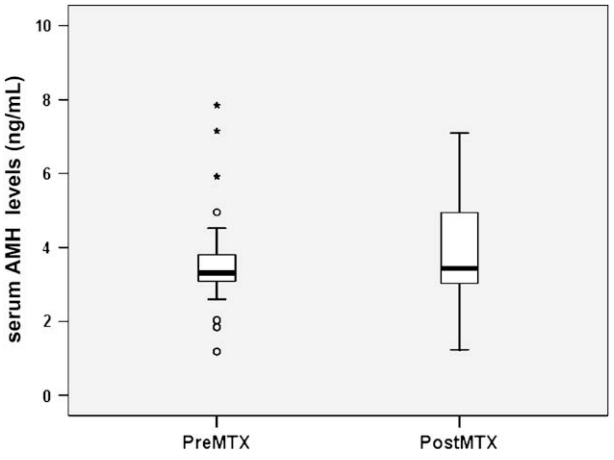
Parameter	Before MTX (n = 14)	After MTX (n = 14)
Mean (min-max) age in y	33 (29–38)	
Mean time between cycles (d)	226.4 ± 23.8	
Indications for ART (%)		
Tubal	31.5	
Male	36.8	
Failure of intrauterine insemination	42.1	
No. of days of stimulation[a]	10.3 ± 0.4 (8–13)	10.8 ± 0.4 (9–14)
Total dose of gonadotropins (IU)	2,775 ± 282.5	2,630.3 ± 145
Serum E_2 day of hCG (pg/mL)	1,884.3 ± 223.7	1,523.6 ± 242.8
No. of oocytes retrieved[a]	12.1 ± 2.1 (4–30)	10.5 ± 1.8 (2–25)
Metaphase II oocytes (%)	82.6	80.9
Fertilization rate (%)	70.7	71.4
Total no. of embryos[a]	7.1 ± 1.3 (2–18)	6.5 ± 1.3 (2–17)
Mean no. of embryos transferred[a]	2.2 ± 0.1 (2–3)	2.3 ± 0.2 (1–3)
Biochemical pregnancy rate, % (no. of events)	NA	8.3 (1)
Clinical pregnancy rate, % (no. of events)	NA	33.3 (4)
Miscarriage rate, % (no. of events)	NA	0 (0)
Ectopic pregnancy rate, % (no. of events)	100 (14)	8.3 (1)
Implantation rate (%)	NA	21.4

Note: NA = not applicable.
[a] Data are mean ± SEM (minimum–maximum).

Oriol. Single-dose methotrexate and ovarian reserve. Fertil Steril 2008.

FIGURE 1

Serum AMH levels before and after treatment with MTX. For each *box*, the lower and upper limits represent quartiles 25 and 75, respectively. *Thick lines*, median values; *upper and lower whiskers*, 95% confidence interval; *circles*, outlier values; *asterisks*, extreme values.



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after EP was resolved (3.7 ± 0.3 ng/mL vs. 3.9 ± 0.3 ng/mL, *P*=.138). No treatment failures were recorded, and surgery was not required to resolve any of the diagnosed EPs.

Of the 25 patients evaluated, 14 had already repeated an IVF-ICSI cycle. Indications for ART treatment are summarized in Table 1. Mean time between cycles was 226.4 days. Table 1 also shows the cycle outcome before and after MTX, with no significant differences in any of the parameters evaluated, including total dose of gonadotropins required (2,775 vs. 2,630.3 IU, *P*=NS), peak E_2 level (1,884.3 vs. 1,523.6 pg/mL, *P*=NS), number of oocytes retrieved (12.1 vs. 10.5, *P*=NS), and total number of embryos obtained (7.1 vs. 6.5, *P*=NS).

DISCUSSION

Single-dose MTX is the most accepted alternative to surgical treatment of EP. Although it inhibits DNA synthesis and cell division, which may affect growing preantral follicles, MTX at the doses and intervals that are used for EP treatment did not have any deleterious effect on ovarian reserve, as evaluated by serum AMH levels or by outcomes in subsequent IVF-ICSI cycles.

As an antimetabolite, MTX appears to affect growing preantral and antral follicles but not primordial follicles in the ovaries (13). Although MTX is an agent that is associated with low or no risk for infertility, female fertility may be compromised by any treatment that decreases the number of early follicles (14). Different chemotherapy regimens have been shown to cause occult ovarian failure, or even premature ovarian failure, because of massive destruction of the ovarian reserve (15). Women undergoing ART may already have a compromised ovarian reserve, limiting their chances to conceive a child. Therefore, it is reassuring to show that the effects of MTX, after it is used as a medical treatment for an EP, does not affect or further compromise a woman's future reproductive potential.

Growing evidence suggests that AMH, a glycoprotein exclusively produced by the ovarian granulosa cells of the preantral and antral follicles, is a unique biomarker of ovarian follicular reserve (16). Hence, we chose AMH as a surrogate marker of putative ovarian damage that is induced by MTX. Anti-Müllerian hormone is the best endocrine marker for assessing the age-related decline of reproductive capacity (17) and the effect of chemotherapy on ovarian reserve (18), has high intracycle and intercycle reproducibility (8), and can be evaluated at any stage of the menstrual cycle (7). When we compared serum levels of AMH before and after MTX, we did not observe any significant variation, suggesting that a single low dose of MTX does not damage the pool of preantral follicles.

From a clinical perspective, we were able to evaluate 14 IVF-ICSI cycles that were performed in women who had already been treated with MTX after a mean waiting time of 7.5 months (226.4 d). As suggested by the AMH tests, we did not find any differences in the amount of gonadotropins required, the peak E_2 levels, the number of oocytes retrieved, or the total number of embryos obtained. The implantation rate remained within the expected range. Thus, we conclude that the single-dose MTX protocol can be used safely as a treatment for EP, because it does not compromise the outcome of future cycles.

There appears to be some hCG control of AMH production. During the luteal phase after COH, AMH serum concentrations decrease after hCG administration, probably because of follicular luteinization, and rise again 7 days later, once the effect of hCG has vanished, probably reflecting luteal follicular development (19). This is in accordance with the effect of hCG on AMH production in the testis, which is mediated by the T increase (20). However, contradictory data appeared recently, showing how hCG administration may increase androgen intrafollicular concentrations as well as AMH concentration (21). It appears that serum AMH levels increase with pregnancy, suggesting a fetoplacental contribution and a possible biological role for this molecule in early pregnancy (22). However, EP in our study was diagnosed so early that it is difficult to assess whether the early trophoblast had any impact on serum AMH levels. In fact, we are not aware of any publications investigating AMH concentrations and pregnancy at such an early stage.

The decision of how to treat EP is based on careful consideration about which technique is least likely to compromise reproductive potential. A recent review confirmed that laparoscopic salpingostomy, multiple-dose MTX, or single-dose MTX all result in similar reproductive performance after

treatment with respect to successful resolution, tubal patency rate, subsequent intrauterine pregnancy rate, or subsequent ectopic pregnancy rate (23). Medical treatment of EP has taken precedence over surgical treatment in most early cases and has had a tremendous impact on women because of its high success rate (86%–94%) (24). This conservative approach, using systemic, single-dose MTX, is a simple, noninvasive, first-line medical treatment for EP (25).

For many years, pragmatic experience with subsequent fertility in women treated with single-dose MTX has shown no deleterious influence of the antimetabolite. In fact, a recent publication showed clinical data confirming this empiric approach (26). Our research is of interest because no one has investigated this question elsewhere, taking into consideration not only the woman's reproductive future but also her ovarian reserve. We have shown that single-dose MTX has no effect on ovarian reserve, considering a clinically relevant difference of 25% in the serum value of AMH levels, at a power of 80% and an alpha value of 5%.

In conclusion, single-dose MTX treatment for EP does not compromise ovarian reserve in terms of serum AMH levels and does not alter subsequent IVF-ICSI cycle outcomes. It is a simple, highly efficient, and safe regimen for treatment of EP in very early stages in ART patients. Our data confirm that at the doses and intervals used in the medical treatment of EP, MTX does not compromise the reproductive future of the patient.

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