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Ovarian reserve and subsequent ART outcomes following methotrexate therapy for ectopic pregnancy and pregnancy of unknown location

Micah J. Hill, DO^a, Janelle C. Cooper, MD^b, Gary Levy, MD^a, Connie Alford, MD^a, Kevin S. Richter, PhD^c, Alan H. DeCherney, MD^a, Charles Katz, MD^a, Eric D. Levens, MD^c, and Erin F. Wolff, MD^a

^aProgram in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

^bWashington Hospital Center, Washington, DC

^cShady Grove Fertility Reproductive Science Center, Rockville, MD

Abstract

Objective—It is unclear whether the stimulated state of the ovaries as part of ART results in an increased vulnerability to the effects of methotrexate. The objective of this study was to assess ovarian reserve following methotrexate treatment for ectopic pregnancy or pregnancy of unknown location after ART.

Design—Retrospective cohort study.

Setting—Large ART practice.

Patients—Methotrexate or surgery following ART.

Interventions—None.

Main Outcome Measures—Follicle stimulating hormone (FSH), antral follicle count (AFC), and oocyte yield were compared between subjects treated with methotrexate and surgery. Secondary outcomes were clinical pregnancy and live birth.

Results—There were 153 patients in the methotrexate group and 36 patients in the surgery group. Neither group demonstrated differences in ovarian reserve or oocyte yield comparing before and after treatment values. The change in ovarian reserve and oocyte yield after treatment were similar between the two groups. The number of doses of methotrexate was not correlated with changes in ovarian reserve, indicating no dose-dependent effect. Time between treatment and

Correspondence: Micah J. Hill, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 10 Center Drive, Bethesda, MD. 301-496-5800, hillmicah@mail.nih.gov..

Conflict of interest

None

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repeat ART was not correlated with outcomes. Live birth in subsequent cycles was similar in the two groups.

Conclusions—Ovarian reserve and subsequent ART cycle outcomes were reassuring following methotrexate and surgical management of ectopic pregnancy. An adverse impact of methotrexate was not detected in this large fertility cohort as has been previously described.

Keywords

methotrexate; salpingectomy; ART; ovarian stimulation; ectopic; pregnancy of unknown location

Introduction

Methotrexate is a chemotherapeutic agent commonly used in the treatment of ectopic pregnancy and pregnancies of unknown location. Methotrexate acts as a di-hydrofolate reductase inhibitor and targets rapidly proliferating cells (1, 2), raising the concern that methotrexate treatment might adversely affect ovarian follicles and result in diminished future fertility (3, 4). Adverse effects on ovarian follicles and fertility have been clearly demonstrated with other chemotherapeutic agents (5, 6). Despite this potential concern, several studies have shown that methotrexate treatment of naturally occurring ectopic pregnancies does not adversely affect ovarian reserve or future spontaneous fertility (7-9). This reassuring data has come from studies of patients who naturally conceived an ectopic pregnancy, were treated with methotrexate, and had subsequent spontaneous fertility. However, it is biologically plausible that the ovaries of patients undergoing assisted reproductive technology (ART) are at an increased risk of ovarian damage from chemotherapy (10).

Ectopic pregnancy is more common with infertility patients (11) and accounts for 4.9% of maternal deaths in developed countries (12). Ovarian stimulation for ART results in increased size and blood flow to the ovaries (13, 14). Angiogenic factors, such as vascular endothelial growth factor, are highly secreted in the stimulated ovary (15, 16). The altered ovarian physiology from exogenous gonadotropin stimulation could result in increased uptake and exposure to chemotherapeutic agents (10). Additionally, infertile patients are often older and demonstrate lower ovarian reserve than spontaneously fertile patients (17). It is biologically plausible the patients requiring ART may have ovaries and follicles that are more susceptible to the damaging effects of chemotherapy when compared to spontaneously fertile women. If there was an adverse effect of methotrexate on later fertility potential this would be of particular concern to ART patients as they are already being treated for fertility problems and would want to avoid treatment that might further interfere with their attempts to conceive.

The current published data comparing methotrexate treatment with surgical treatment of abnormal pregnancies following ART are limited to small cohort studies of fewer than 100 patients (18, 19). While some studies have documented no adverse effect on ovarian reserve from methotrexate (4, 18-20), this finding is not always consistent. McLaren *et al.* demonstrated a decrease in oocyte yield in patients who underwent repeat ovarian stimulation and ART within 180 days of methotrexate treatment, but not those who had repeat ART 180 days after methotrexate (3). This finding indicates a potential time-dependent negative effect of methotrexate on folliculogenesis. Additionally, the current literature evaluating methotrexate following ART cycles is limited to studies assessing single-dose regimens (3, 4, 19, 20). To our knowledge, the effect of multi-dose methotrexate on ovarian reserve following ART cycles has not been evaluated. The objective of this study was to compare ovarian reserve and subsequent oocyte yield in ART patients treated with

surgery or methotrexate for ectopic pregnancy or pregnancy of unknown location in a larger cohort of patients. The secondary objective was to assess the effect of multi-dose methotrexate on ovarian reserve.

Methods

Study Design

This was a retrospective cohort study of all fresh autologous ART patients treated for ectopic pregnancy or pregnancy of unknown location at Shady Grove Fertility Reproductive Science Center from 2004-2010. The retrospective review and analysis of data collected during routine clinical care was Institutional Review Board approved.

Patients

The charts of all patients who underwent fresh autologous ART were screened and any patients treated with methotrexate or surgery for a diagnosis of ectopic pregnancy were included in the study. Pregnancies of unknown location without evidence of an intrauterine gestation were included as ectopic pregnancies. Pregnancy of unknown location included patients with multiple plateaued quantitative hCG values (values not increasing over 50% in 48 hours) or with hCG values >2000 and no ultrasonographic evidence of intrauterine gestation.

Outcomes

The primary study outcomes were basal antral follicle count (AFC), basal serum follicle stimulating hormone (FSH) levels, and oocyte yield from ART. Secondary outcomes were subsequent clinical pregnancy and subsequent live birth. Clinical pregnancy was defined as a transvaginal ultrasound with confirmed fetal cardiac activity. Live birth was defined as a living infant born after 23 weeks gestation. Additional data was collected on variables which might affect response to methotrexate and included multiple doses of methotrexate, patient age, the diagnosis of diminished ovarian reserve, and time between treatment and repeat ART cycles.

Statistics

Normally distributed data were expressed as means with standard deviations. Non-parametric data were expressed as medians and range. Chi square or Fisher's exact test were used to compare dichotomous outcomes. Students T test or Mann Whitney U test were used to compare baseline demographics and AFC, FSH, and oocyte yield between the groups. Paired Students T test or Wilcoxon Ranked Sum Test was used to compare ovarian reserve yield in patients pre- and post-treatment within each group. Students T test or Mann Whitney U test was used to compare changes in oocyte yield from pre- to post-treatment values between the treatment groups. ANCOVA was used to compare changes in ovarian reserve between the groups while controlling for patient age and the time duration between treatment and repeat ART cycles. ANOVA was used to compare changes in ovarian reserve in patients receiving one, two, or three treatment courses of methotrexate and changes in ovarian reserve between patient age groups. Univariate regression was used to examine dose effects of the number of methotrexate courses needed on ovarian reserve. Less than efficiency curves were used to examine the effect of time after methotrexate treatment and oocyte yield. Statistical analysis was performed using SPSS software (IBM, Armonk, NY). *P* values of <0.05 were considered statistically significant.

Results

There were over 23,000 fresh autologous ART cycles at Shady Grove Fertility Reproductive Science Center from 2004-2010. Out of these cycles, there were 153 patients treated with methotrexate and 36 patients treated with surgery for a diagnosis of ectopic pregnancy or pregnancy of unknown location. Patients with combined methotrexate therapy and surgical treatment ($n=27$) were excluded from the primary analysis but were included in a sub-analysis. The time between treatment cycles, patient age, parity, infertility diagnosis, and basal FSH were similar between the two groups (Table 1).

Neither the methotrexate or surgery group demonstrated changes in ovarian reserve after treatment (Fig 1). The methotrexate group had basal FSH values of 6.9 [2.4-14.2] before and 7.2 [2.3-16.3] after treatment ($P=0.86$), AFC values of 12 [1-53] before and 13 [1-60] after treatment ($P=0.86$), and oocyte yield of 14 [2-36] before and 14 [0-35] after treatment ($P=0.67$). The surgery group had basal FSH values of 7.3[2.6-16.0] before and 7.9 [5.1-10.4] after treatment ($P=0.53$), AFC values of 10 [3-50] before and 10 [4-45] after treatment ($P=0.68$), and oocyte yield of 13 [3-31] before and 12 [3-31] after treatment ($P=0.16$). There were no significant differences between the methotrexate and surgical groups in FSH, AFC, and oocyte yield in pre- and post-treatment values (Table 1). Similarly, the change in ovarian reserve from pre- to post-treatment values was similar between the two groups for basal FSH (MTX: +0.4 versus surgical: +0.3, $P=0.66$), AFC (MTX: -0.5 versus surgical: +0.4, $P=0.36$), and oocyte yield (MTX: -0.3 versus surgical: -0.7, $P=0.15$). Clinical pregnancy and live birth in the ART cycles following treatment were similar between the two treatment groups (Table 1).

Patient age was negatively correlated with AFC and oocyte yield ($P<0.001$ for both). The number of days between the initial ART cycle and subsequent cycle was negatively correlated with the change AFC ($P<0.001$) as would be expected with aging, but not with the change in oocyte yield or FSH. In ANCOVA controlling for patient age and days between treatment cycles, there remained no significant differences in the treatment groups in FSH, AFC, and oocyte yield.

Other chemotherapeutic agents demonstrate a dose-response effect on ovarian reserve (21). To examine if there was a dose-dependent effect of methotrexate on ovarian reserve, the number of doses required to treat the patient was examined. There were 58 patients requiring one dose, 74 requiring two doses, and 21 patients requiring three doses of methotrexate. ANOVA demonstrated no differences in the changes in FSH, AFC, or oocyte yield between the three dosing groups of methotrexate (Table 2). In univariate regression, the number of doses of methotrexate was not correlated with the change in FSH ($P=0.78$), the change in AFC ($P=0.54$), or the change in oocyte yield ($P=0.26$) after treatment.

Methotrexate has previously been shown to have a negative effect on oocyte yield in repeat ART cycles performed within 180 days of methotrexate (3). In univariate regression sensitivity analyses, oocyte yield and time between repeat ART treatment was not correlated ($P=0.19$). There was no difference in the change in oocyte yield in patients repeating ART in <180 days versus those >180 days after treatment (<180 days: -0.3 versus >180 days: +0.8, $P=0.31$). Similarly, the total number of oocytes retrieved after methotrexate was similar (<180 days: 13.9 versus >180 days: 14.7, $P=0.47$). To control for patients who had a long duration between ART cycles (365-1742 days) which could result in an age related decline in oocyte yield, oocyte yield was compared in patients repeating ART in <180 days versus 180-365 days. There was no difference in the change in oocyte yield between these two groups (-0.3 versus 0.6, $P=0.40$). Visual inspection of scatter plots comparing oocyte yield and time between repeat ART treatment demonstrated no significant effect of time to

treatment ($R^2 = 0.012$) and restricting the scatter plot to patients who underwent repeat ART cycles within 365 days demonstrated no effect of time to treatment on oocyte yield ($R^2 = 0.0001$) (Fig. 2). Finally, changes in oocyte yield were evaluated via less than efficiency curves to determine if there were other time frames between methotrexate and repeat ART cycle in which decreased oocyte yield was seen (Supplemental Fig. 1). There was no time frame below which a reduction in oocyte yield was achieved. Even patients with the shortest time period to a repeat ART cycle of <100 days ($n=5$) demonstrated no reduction in oocyte yield (Supplemental Fig. 1). Similar scatter plot analysis of changes in FSH and AFC did not demonstrate any negative effect of methotrexate at 180 days (Supplemental Fig. 2 and 3).

The effect of methotrexate administration on oocyte yield was examined by patient age groups in order to evaluate if older patients demonstrated greater sensitivity to potential effects of methotrexate on ovarian reserve. There were no differences in oocyte yield post-methotrexate ($P=0.96$) or in the change in oocyte yield when comparing patient age groups ($P=0.94$) (Supplemental Table 1). In univariate linear regression analysis, age was not correlated with the change in FSH ($P=0.58$), AFC ($P=0.18$), or oocyte yield ($P=0.26$).

The effect of methotrexate administration on oocyte yield was also examined in patients with diminished ovarian reserve (DOR), defined as oocyte yield of ≤ 3 oocytes in the initial ART cycle (cite), to evaluate if patients with DOR demonstrated greater sensitivity to potential effects of methotrexate on ovarian reserve. As expected, patients with DOR ($n=5$) demonstrated significantly lower yield in their post-methotrexate ART cycle (DOR: 4.0 ± 3.1 oocytes versus Normal Responders: 14.4 ± 6.5 oocytes, $P=0.001$). However, the change in oocyte yield after methotrexate was similar between patients with DOR and those who were normal responders (DOR: $+1.0$ versus Normal Responders: -0.4 , $P=0.66$). Although limited by small numbers in the sub-analyses, no negative effect of methotrexate was seen in older patients or those with a diagnosis of DOR.

Patients who underwent both methotrexate treatment and subsequent surgery were then analyzed to determine if the combined therapy was detrimental to ovarian reserve. The methotrexate + surgery group had basal FSH values of 8.0 ± 4.6 before and 8.5 ± 4.1 after treatment ($P=0.74$), AFC values of 12.2 ± 9.1 before and 14.5 ± 9.9 after treatment ($P=0.32$), and oocyte yield of 13.6 ± 5.1 before and 13.6 ± 6.3 after treatment ($P=0.93$). ANOVA comparison of the patients receiving both methotrexate + surgery compared to the methotrexate and surgical groups only, there were no differences in the changes in FSH ($P=0.50$), AFC ($P=0.16$), or oocyte yield ($P=0.78$). In the ART cycle following treatment, patients in the methotrexate + surgery group had a clinical pregnancy rate (44%) and live birth rate (30%) which was statistically similar to the outcomes of patients in both the methotrexate and salpingectomy only groups.

Discussion

It has been postulated that patients exposed to controlled ovarian stimulation and ART may be at higher risk for adverse effects of methotrexate (10). McLaren *et al.* reported an adverse effect of methotrexate that persisted for 180 days on subsequent ART oocyte yield, resolving after that time period (3). There is biologic plausibility for a 180 day window of harm, given both the potential washout period of methotrexate (22) and the time frame of ovarian folliculogenesis (3, 23). We performed multiple analyses of our data using regression analysis, scatter plots, comparisons of means, and less than efficiency curves to look for linear and non-linear effects and found no evidence to support a time-dependent effect of methotrexate on oocyte yield, despite having three times the number of patients in our study, which makes it unlikely that our study was underpowered to detect a difference if it existed. It is possible that the older age of patients in the McLaren *et al.* study might

explain the different findings, as the average patient age in the McLaren *et al.* study was 36.7 versus 34.3 in the present study. However, neither study demonstrated a specific negative effect on age and ovarian response following methotrexate. It is possible that the smaller number of patients (n=48) in the McLaren *et al.* study made that analysis more sensitive to out-lying data points. Finally, McLaren *et al.* did not have a surgical control group for comparison and were evaluating only the effect of methotrexate.

Other studies examining fertility after ectopic pregnancies in patients treated with assistive reproductive technology have also been reassuring. Only two prior trials have directly compared methotrexate and surgical management of ectopic pregnancies resulting from an ART cycle (18, 19). Neither of these trials demonstrated differences in FSH, AFC, ovarian volume, oocyte yield, or ongoing pregnancy rates in the subsequent cycles after treatment between the two groups. Two trials evaluating the changes in ovarian reserve in methotrexate patients without a surgical comparison group were also similar to the present data and showed no adverse effect (4, 20). Our data on patients treated following ART is similar to the published data concerning spontaneous pregnancy after ectopic pregnancies treated with methotrexate. In a large multicenter randomized trial, Fernandez *et al.* recently reported two year spontaneous pregnancy rates of 67% following methotrexate treatment compared to 71% in patients treated with conservative surgery (7). This data is similar to another retrospective cohort study of patients who were treated with methotrexate for ectopic pregnancy, where the follow-up spontaneous pregnancy rate was 57.5% after one year and 66.9% after two years (8).

The other main finding of the present study was the absence of a dose-dependent effect of methotrexate on ovarian reserve. Prior studies evaluating methotrexate following ART cycles have been limited to assessing single-dose regimens (3, 4, 19, 20). Given that other chemotherapeutic agents demonstrate negative dose-response relationships with ovarian reserve (21), it was important to evaluate if the findings pertaining to single-dose methotrexate also apply to patients who requires multiple doses. It is reassuring that the data demonstrated no adverse effect on ovarian reserve in patients requiring two or three doses of methotrexate for resolution of their abnormal pregnancies. The lack of any detectable dose-dependence is further evidence of a lack of any association between methotrexate use and ovarian reserve damage.

The primary strength of the present study is in the larger sample size of patients undergoing treatment for abnormal pregnancy after ART. The relatively low incidence of ectopic pregnancy has made evaluating its management in ART patients challenging. Prior studies comparing methotrexate to surgery in the ART patient population were limited to 58 and 82 patients (18, 19). In a large center performing over 23,000 fresh autologous ART cycles during the study period, we were able to identify 226 confirmed or suspected ectopic pregnancies. The sample size of the present data significantly increased the power to detect a potential negative effect of methotrexate on ovarian reserve. We also performed sub-analyses based on time to repeat ART cycle, age, and DOR and were unable to identify patient groups who are at risk from methotrexate. The current study also followed patients for a longer time frame to repeat ART cycle (up to 1780 days), which allowed examination of treatment effects over a much longer period. Finally, this is the first study we are aware of to evaluate for a dose-dependent effect of methotrexate by evaluating patients receiving one, two, and three courses of treatment. The larger sample size and multiple additional analyses are reassuring that there is not a negative effect of methotrexate on ovarian reserve in ART patients. A potential weakness of the paper is the retrospective design. We cannot exclude subtle or undetected biases between the patient groups, biases that may affect the results of the study. We also cannot exclude that some small changes in ovarian reserve occurred that were too small to be detected by the power of this study. The large majority of these patients

had ovarian reserve values within the normal thresholds of ovarian reserve and it is unclear if changes in ovarian reserve within the normal range would have clinical relevance. Additionally, we included ART patients with both ectopic pregnancy and pregnancy of unknown location, which is similar to prior literature (18, 20). We are not aware of evidence or biologic rationale to suggest that ovarian reserve would be differentially affected in patients with an ultrasound proven ectopic as compared to those with an unknown location. Compared to ectopic pregnancies, pregnancies of unknown location tend to have lower hCG quantitative values and ultrasound findings that are indeterminate. However, one would anticipate the ovarian response to chemotherapeutic agents to be similar regardless of the location of the gestation. Further, this data does provide some reassurance for aggressive treatment of pregnancy of unknown location with methotrexate without concern about subsequent ovarian reserve; early diagnosis and intervention has been credited with the sharp decline in maternal deaths over the last 30 years from ectopic pregnancy(24).

In conclusion, this study found no difference between the use of methotrexate and surgery to treat ectopic pregnancy and pregnancy of unknown location in ART patients. Randomized controlled trial data from the DETMER trial demonstrates similar reassuring data in patients with spontaneous ectopic pregnancies (7). In the absence of randomized controlled data for ART patients, this present data reinforces the majority of the existing reassuring data concerning methotrexate use in ART patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A. Surgical treatment **B. Methotrexate treatment** **Figure 1.**

Box and whisker plots of pre- and post-treatment oocyte yield, AFC, and FSH values in the surgical and methotrexate groups. Boxes represent the inter-quartile range and the line within the boxes represent the median. The vertical lines protruding from the box extend to the minimum and the maximum values of the data set, as long as these values do not differ from the median by more than one and half times the inter-quartile range. Outliers from this range are represented by dots. There were no statistically significant differences between pre- and post-treatment values within either group.

Figure 2.

Scatter plots of the change in oocyte yield from pre- to post-treatment values plotted against the time between ART cycles in day. A- includes all patients in the methotrexate group out to 1780 days. B- includes only patients in the methotrexate group having a repeat ART cycle with 365 days.

Table 1

Comparison of age, days between ART cycles, and pre- and post-treatment measures of ovarian reserve in the methotrexate and surgical groups.

	Methotrexate (n=153)	Surgery (n=36)	<i>P</i> value
Age	34.3 ± 4.5	35.8 ± 4.3	0.06
Parity	.35 ± 0.21	.40 ± 0.22	0.42
Days between ART cycles	158 [92-1742]	159 [61-870]	0.16
FSH pre-treatment (IU/L)	6.9 [2.4-14.2]	7.2 [2.6-16.0]	0.24
FSH post-treatment (IU/L)	7.2 [2.3-16.3]	7.9 [5.1-10.4]	0.65
Mean change in FSH (IU/L)	+0.4	+0.3	0.86
AFC pre-treatment	12 [1-53]	10 [3-50]	0.10
AFC post-treatment	13 [1-60]	10 [4-45]	0.29
Mean change in AFC	-0.5	+0.4	0.36
Oocyte yield pre-treatment	14 [2-36]	13 [3-31]	0.12
Oocyte yield post-treatment	14 [0-35]	12 [3-31]	0.07
Mean change in oocyte yield	-0.3	-0.7	0.15
Clinical Pregnancy (%) ^a	68 (44%)	14 (39%)	0.58
Live Birth (%) ^a	53 (35%)	13 (33%)	0.99

Data expressed as mean ± SD or median [range] for parametric and non-parametric data respectively.

^aPregnancy outcomes in the ART cycle following treatment.

Table 2

Changes in FSH, AFC, and oocyte yield from pre- to post-treatment values compared in based on the number of doses of methotrexate received with ANOVA analysis.

	1 Dose (n=58)	2 Doses (n=74)	3 Doses (n=22)	ANOVA <i>P</i> value
FSH change	+0.4	+1.0	−0.6	0.30
AFC change	0.0	−1.2	+0.4	0.52
Oocyte yield change	−0.7	+0.1	+0.5	0.64