Clinical predictors of failing one dose of methotrexate for ectopic pregnancy after in vitro fertilization

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Abstract

Purpose The aim of this study is to investigate the clinical predictors of failure of a single dose of methotrexate (MTX) for management of ectopic pregnancy after in vitro fertilization (IVF).

Methods A retrospective cohort study was performed of women who conceived ectopic pregnancies following fresh or frozen IVF cycles at an academic infertility clinic between 2007 and 2014, and received intramuscular MTX (50 mg/m²). Successful single-dose MTX treatment was defined as a serum beta-human chorionic gonadotropin (hCG) decline $\geq 15\%$ between days 4 and 7 post-treatment. Logistic regression models adjusted for oocyte age, number of embryos transferred, and prior ectopic pregnancy were used to estimate the adjusted odds ratio (OR) (95% confidence interval [CI]) of failing one dose of MTX.

Results Sixty-four patients with ectopic pregnancies after IVF were included. Forty required only one dose of MTX (62.5%), while 15 required additional MTX alone (up to four total

doses, 23.4%), and 9 required surgery (14.1%). By multivariable logistic regression, the highest tertiles of serum hCG at peak (≥ 499 IU/L, OR = 9.73, CI 1.88–50.25) and at first MTX administration (≥ 342 IU/L, OR = 4.74, CI 1.11–20.26), fewer embryos transferred (OR = 0.37 per each additional embryo transferred, CI 0.19–0.74), and adnexal mass by ultrasound (OR = 3.65, CI 1.10–12.11) were each correlated with greater odds of requiring additional MTX and/or surgery.

Conclusion This is the first study to report that in women with ectopic pregnancies after IVF, higher hCG—though well below treatment failure thresholds previously described in spontaneous pregnancies—fewer embryos transferred, and adnexal masses are associated with greater odds of failing one dose of MTX. These findings can be used to counsel IVF patients regarding the likelihood of success with single-dose MTX.

Keywords Ectopic pregnancy · Methotrexate · In vitro fertilization · Human chorionic gonadotropin

Introduction

The risk of ectopic pregnancy following in vitro fertilization (IVF) is estimated at approximately 1.7% [1]. Previously reported risk factors for ectopic pregnancy include prior ectopic pregnancy, tubal surgery, sexually transmitted infections, and current cigarette smoking [2]. Risk factors for ectopic pregnancy associated with the use of assisted reproductive technologies (ART) include tubal factor infertility, fresh embryo transfer, cleavage-stage embryo transfer, and transfer of three or more embryos [3–8].

Among patients diagnosed with ectopic pregnancies following IVF, medical management with methotrexate (MTX) is often first line treatment, as these ectopic pregnancies are usually detected early in asymptomatic, clinically stable

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patients. In studies of MTX for the treatment of ectopic pregnancy (not restricted to an IVF population), successful medical management is attained in roughly 90% of appropriately selected patients; a reported 15–28% of these patients, however, may require more than one dose of MTX due to inadequate hCG declines between days 4 and 7 after the initial dose [9–14].

Previously reported risk factors for failure of medical management with methotrexate, necessitating surgical management, include elevated serum hCG (1300–5000 IU/L), an adnexal mass greater than 4 cm, the presence of fetal cardiac activity, and prior ectopic pregnancy [2, 15–18]. In the few studies specifically assessing risk factors for requiring more than one dose of MTX, higher initial hCG, the presence of fetal cardiac activity, and prior ectopic pregnancy have been identified as risk factors; no previous research, however, has focused on pregnancies conceived using assisted reproduction, which generates an altered hormonal milieu that may affect embryo implantation and development [9, 11, 12, 19, 20]. The higher risk of ectopic pregnancy associated with assisted reproduction likely reflects the higher incidence of tubal disease in the infertility population, as well as the altered physiology and/or the retrograde movement of embryos, which are factors that may also influence response of these ectopic pregnancies to medical management [21].

No studies to date have focused on IVF patients' clinical characteristics and IVF cycle parameters as predictors of failure of single-dose MTX for ectopic pregnancy, which requires additional MTX, prolongs treatment, and contributes to patient anxiety. The purpose of this study was to analyze tubal ectopic pregnancies conceived during fresh or frozen IVF/intracytoplasmic sperm injection (ICSI) cycles over an 8-year period that were treated with MTX, to determine the risk factors for failure of one dose of MTX among this specific group. The findings of this work could inform how patients are triaged and advised regarding treatment of ectopic pregnancy after IVF, allowing for personalized counseling and management.

Materials and methods

Institutional Review Board approval was obtained from our medical center. Records were reviewed for all women who underwent fresh or frozen embryo transfers from 2007 through 2014 at our academic infertility practice ($n = 11,498$). Clinical and laboratory data were extracted from a prospectively maintained departmental database; key parameters (oocyte and recipient ages, obstetrical history, hCG values, imaging results, MTX treatments, surgical reports, and clinical outcomes) were directly verified in the electronic medical record. Ectopic pregnancies were diagnosed by an abnormal hCG trend (hCG rise less than 53% or fall less than

15% in 48 h), with either the absence of ultrasound evidence of intrauterine pregnancy and negative endometrial sampling or definitive ultrasound evidence of tubal ectopic pregnancy, including a yolk sac or embryo in the adnexa [2, 22, 23]. In rare cases, patients refused endometrial sampling in the setting of persistently elevated, abnormally trending hCG measurements, without definitive ultrasound evidence of ectopic pregnancy, and were given methotrexate following counseling regarding this protocol departure.

The hCG measurements were performed at the clinical laboratory of our medical center. This laboratory is inspected by the Joint Commission with Clinical Laboratory Improvement Amendments (CLIA) certification. The electrochemiluminescence immunoassay was run using the Cobas® e601 platform (Roche Diagnostics, Mannheim, Germany). Inter- and intra-assay coefficients of variation (CV) for the assay are less than 5.5%. Results were standardized against the fourth International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (NIBSC) 75/589 [24].

All ectopic pregnancies included in this study were initially treated with a single-dose MTX protocol, with intramuscular MTX (50 mg/m²) administered on day 1. Treatment success was defined as serum hCG decline of 15% or more between day 4 and day 7 after the MTX dose. If the hCG decline was inadequate following the preceding dose, patients were eligible to repeat this cycle three more times, for a total of four doses. Surgical management was pursued in any patient presenting with significant, acute-onset pain or other signs of ruptured ectopic pregnancy, such as complex pelvic free fluid on imaging or an acute decline in hemoglobin [2]. Records with incomplete clinical data or unknown outcome were excluded. Patients who did not receive MTX or were diagnosed with non-tubal ectopic pregnancies (such as ovarian, cervical, or abdominal ectopic pregnancies) by imaging and/or surgery were also excluded, due to the lower efficacy of MTX alone in treating these rare ectopic pregnancies.

Multivariable logistic regression was used to calculate odds ratio (OR) and 95% confidence intervals (CI) of successful management with single-dose MTX. Age of the oocyte producer was included in the model a priori as an exposure variable. The presence of any sized adnexal mass (not readily identified an adnexal cyst, such as a corpus luteum) or free fluid by ultrasound and patient report of pain as reflected in clinical notes were also considered exposures of interest. Covariates tested as primary exposures and also as potential confounders of the relationship with successful treatment included recipient age at the time of embryo transfer, recipient gravidity and parity, prior ectopic pregnancy, infertility diagnosis, day 3 follicle stimulating hormone (FSH) and estradiol (E2) levels, body mass index (BMI), fresh versus frozen cycle, stimulation protocol, use of ICSI, use of assisted hatching, day of embryo transfer, number of embryos transferred, number of

good quality embryos transferred, endometrial stripe thickness at trigger or frozen embryo transfer mapping, first serum hCG, peak serum hCG, and serum hCG and gestational age at the time of first MTX administration.

Good quality cleavage-stage embryos are defined as having at least seven cells, fragmentation scores of 0 (0% fragmentation) or 1 (1–9% fragmentation), and symmetry scores of 1 (perfect symmetry) or 2 (moderate asymmetry) [25]. Good quality blastocysts are defined as hatching or hatched blastocysts with fair or good quality inner cell mass and trophoctoderm [26]. Serum hCG levels were included as continuous variables (per 100 IU/L increase) and in tertiles as defined by the distribution among patients included in this study. Patient race (white, black, Asian, Hispanic, American Indian, or other) and stimulation protocol were included as nominal categorical variables. Stimulation protocols included those using gonadotropin-releasing hormone (GnRH) antagonists, downregulation protocols using GnRH agonists, and poor responder protocols using estradiol priming or low-dose GnRH agonist flare [27–30]. Prior ectopic pregnancy, the presence of tubal factor infertility and/or endometriosis (as adhesions from endometriosis may produce tubal obstruction), fresh versus frozen cycle, use of assisted hatching, use of ICSI, day of embryo transfer (day 3 or day 5), and the presence of an adnexal mass, free fluid, or pain were included as dichotomous variables. Recipient and oocyte age (years), body mass index (kg/m^2), gravidity, parity, day 3 FSH (U/L) and E2 (pg/mL), endometrial stripe thickness (mm), number of embryos transferred, number of good quality embryos transferred, gestational age at the time of MTX (days), and size of any adnexal mass (mm) were included as continuous variables.

Variables that upon addition to the regression model changed the odds ratio for the base model covariates by 10% or greater were retained in the final adjusted model [31]. Only the addition of a history of prior ectopic pregnancy and the number of embryos transferred each changed the effect estimates by more than 10%; as a result, only these were included in the final multivariate regression model as covariates. Of note, as the day of embryo transfer was strongly associated with the number of embryos transferred, sensitivity analyses included the day of embryo transfer; however, this variable did not substantially alter odds ratios of exposures of interest and was not included in the final model. Analyses were performed using Statistical Analysis Software version 9.3 (SAS Institute, Inc.).

Results

Over the time period of this study, endometrial sampling was performed for the diagnosis of pregnancy location in 136 patients (1.2%) without ultrasound evidence of

intrauterine or ectopic pregnancy and abnormal hCG trends. Another 45 patients (0.4%) were diagnosed with ectopic pregnancies based on ultrasound findings and/or patient refusal of endometrial sampling in the setting of persistently elevated, abnormally trending hCG measurements. Please see Fig. 1 for a flow diagram of the final sample selection. Over the study period, 106 ectopic pregnancies occurred among IVF patients who underwent embryo transfer (0.9%). Patients with non-tubal ectopic pregnancies ($n = 9$) or who did not receive MTX as part of their management (by undergoing primary surgical management, $n = 25$), and records with missing data (hCG levels, treatment protocol or outcome, $n = 8$) were excluded, and 64 patients met criteria for inclusion in this study. Of the 64 patients included in this study, 48 were diagnosed using endometrial sampling, and the remaining 16 patients received methotrexate based on ultrasound findings diagnostic of ectopic pregnancy and/or refusal of endometrial sampling in the setting of persistently abnormally trending hCG measurements.

Forty patients were managed with one dose of intramuscular MTX (62.5%), 15 were successfully managed with additional doses of MTX alone (23.4%), and 9 required surgery (14.1%). Thirteen patients required just one additional dose of MTX, while 1 patient required three total doses, and 1 patient was successfully managed with four MTX doses. Two patients' ultrasounds demonstrated fetal cardiac activity in the adnexa; these two patients had serum hCG levels of 1836 and 3360 IU/L at the time of MTX administration. Both patients received one dose of MTX and subsequently developed acute, severe pain and required surgery.

Demographic and IVF cycle characteristics are shown in Table 1. The mean oocyte age among patients successfully treated with a single dose of MTX was 37.5 years, and mean oocyte age among those requiring additional MTX doses and/or surgery was 35.6 years. Among patients requiring only a single dose of MTX and those requiring further medical or surgical management, the mean recipient ages were 37.7 and 36.6 years, respectively, and patients' mean body mass indices were 27.1 and 26.5 kg/m^2 , respectively. Prior ectopic pregnancies were reported in 12.5% of patients in both groups. The majority of cycles were fresh, comprising 82.5% of cycles in patients requiring only single-dose MTX, and 75.0% of cycles in patients requiring further management. The mean number of embryos transferred was 3.0 in patients successfully treated with a single dose of MTX, as compared to a mean of 1.8 in patients requiring multiple doses of MTX and/or surgery. Cleavage-stage (day 3) embryo transfers had been performed in 85.0% of patients requiring only a single dose of MTX and 70.8% of patients requiring further management.

In multivariable regression analysis, higher serum hCG level at the time of the first methotrexate injection, higher peak hCG, fewer embryos transferred, and the presence of an adnexal mass were each associated with greater odds of

Fig. 1 Flowchart of included ectopic pregnancies (EP). EP ectopic pregnancy, hCG human chorionic gonadotropin, MTX methotrexate. 1 Pregnancy of unknown location was defined as a positive serum hCG in the absence of ultrasound evidence of intrauterine or ectopic pregnancy. Abnormal hCG trends were defined as a rise <53% or a fall <15% in 2 days. 2 EP was diagnosed after endometrial sampling by failure to identify villi on pathologic analysis and persistently elevated hCG. 3 Ultrasound diagnosis of ectopic pregnancy required identification of a yolk sac or embryo in the adnexa

requiring further management (additional MTX and/or surgery) (Table 2). Several other exposures were significant or nearly significant in the crude analysis (oocyte age, assisted hatching, presence of free fluid) or had been associated with increased risk of ectopic pregnancy following in vitro fertilization in prior studies (namely tubal factor infertility, endometriosis, cleavage-stage, and fresh embryo transfers and thinner endometrial stripes) but failed to reach the threshold for statistical significance after adjustment. Too few patients required surgery ($n = 9$) to allow for further valid statistical analysis of the odds of requiring additional MTX alone versus surgery.

Every 100 IU/L increase in serum hCG at the time of first MTX dose and at peak increased the odds of failing single-dose MTX by 8 and 7%, respectively. When analyzing serum hCG at the time of the first MTX injection in tertiles, patients with serum hCG in the highest tertile (≥ 342 IU/L) at the time of their first MTX doses had 4.74-fold greater odds of failing management with a single dose of MTX when compared to patients with serum hCG in the first tertile (<126 IU/L, CI 1.11–20.26) (Fig. 2). Similarly, patients with peak serum hCG in the top tertile (≥ 499 IU/L) had 9.73-fold greater odds of failing a single dose of MTX when compared to patients with peak serum hCG in the first tertile (<194 IU/L, CI 1.88–50.25). Patients with adnexal masses had 3.65 greater odds of requiring further management beyond a single dose of MTX (CI 1.10–12.11).

Patients with three or more embryos transferred—all of which were cleavage-stage embryos—had 92% lower odds

of requiring management beyond a single dose of MTX, as compared to patients with just one embryo transferred (OR = 0.08, CI 0.01–0.50). Each additional embryo transferred conferred 63% lower odds of failing management with single-dose MTX (OR = 0.37, CI 0.19–0.74). In a subanalysis of the effect of embryo transfer number stratified by age, patients 35 years and older ($n = 44$, not shown) had 81% lower odds of requiring further management for each additional embryo transferred (OR = 0.19, CI 0.06–0.59), though in patients under 35 years ($n = 20$), the effect was attenuated and not statistically significant (OR = 0.73 per each additional embryo transferred, CI 0.50–1.07, test for heterogeneity = 0.08).

Discussion

Ectopic pregnancy occurs in 1.7% of pregnancies following IVF [1]. Successful medical management is reported to be achieved with MTX in approximately 90% of patients [10, 14]. These reported results, however, do not commonly differentiate between patients requiring one or multiple doses; prior studies have also not focused on risk factors for requiring repeat MTX doses for ectopic pregnancies conceived using IVF. Repeat dosing of MTX is highly clinically relevant by prolonging treatment, heightening patient anxiety, delaying the next treatment cycle, and requiring additional clinic and laboratory visits.

In this study, only 62.5% of patients were successfully managed with a single dose of MTX, well below the 72–85% previously reported among non-IVF populations [9,

Table 1 Demographic and cycle characteristics among 64 patients with ectopic pregnancy after in vitro fertilization (IVF) who received methotrexate (MTX)

Characteristics	Single-dose MTX <i>N</i> = 40	Multiple doses of MTX or surgery <i>N</i> = 24
Race <i>n</i> (%)		
White	27 (67.5)	20 (83.3)
Asian	5 (12.5)	2 (8.3)
Other	8 (20.0)	2 (8.3)
Oocyte age Mean (SD)	37.5 (4.1)	35.6 (5.1)
Recipient age Mean (SD)	37.7 (4.1)	36.6 (3.9)
Carrier BMI Mean (SD)	27.1 (6.8)	26.5 (6.4)
Recipient gravidity Mean (SD)	1.4 (1.5)	1.3 (1.0)
Recipient parity Mean (SD)	0.3 (0.7)	0.4 (0.5)
Prior ectopic <i>n</i> (%)	5 (12.5)	3 (12.5)
Endometriosis <i>n</i> (%)	2 (5.0)	2 (8.3)
Tubal factor infertility <i>n</i> (%)	4 (10.0)	2 (8.3)
Fresh IVF cycle <i>n</i> (%)	33 (82.5)	18 (75)
ICSI used <i>n</i> (%)	17 (42.5)	11 (45.8)
Assisted hatching used <i>n</i> (%)	26 (65.0)	6 (25.0)
Endometrial stripe thickness (mm)	10.7 (3.0)	9.4 (2.9)
Mean (SD)		
Cleavage-stage embryo transfer <i>n</i> (%)	34 (85.0)	17 (70.8)
Number of embryos transferred Mean (SD)	3.0 (1.5)	1.8 (0.8)
Good quality embryos transferred <i>n</i> (%)	32 (80.0)	18 (75.0)
First serum hCG measurement (IU/L)		
Mean (SD)	62.1 (90.2)	67.9 (68.9)
Median (min–max)	30 (5–557)	51.5 (7–324)
Serum hCG at time of MTX (IU/L)		
Mean (SD)	368.3 (577.7)	840.6 (1150.3)
Median (min–max)	155.5 (27–2792)	329 (19–3605)
Peak hCG (IU/L)		
Mean (SD)	505.9 (827.6)	1584.2 (2340.9)
Median (min–max)	283 (40–4736)	494 (36–9146)
Gestational age at time of MTX (days)	42.7 (6.1)	41.5 (5.1)
Mean (SD)		
Adnexal mass <i>n</i> (%)	14 (35.0)	16 (66.6)
Size of adnexal mass (among women with mass on ultrasound) Mean (SD)	1.9 (0.9)	2.4 (1.2)
Presence of free fluid <i>n</i> (%)		
None	30 (75.0)	12 (50.0)
Trace	6 (15.0)	4 (16.6)
Small	4 (10.0)	8 (33.3)
Pain <i>n</i> (%)	0 (0.0)	2 (8.3)

SD standard deviation, *BMI* body mass index, *ICSI* intracytoplasmic sperm injection, *hCG* human chorionic gonadotropin

11–14]. This study also found that in women with ectopic pregnancies after IVF, higher hCG at peak serum and at the time of first MTX dose, adnexal masses, and fewer embryos transferred were associated with greater odds of requiring additional treatment beyond a single dose of MTX.

Serum hCG thresholds conferring the highest risk for failing a single dose of MTX (342 IU/L at the time of first MTX, and peak hCG of 499 IU/L) were surprisingly low, particularly when compared to hCG levels commonly associated with risk of failure of medical management overall (1300–5000 IU/L)

Table 2 Clinical characteristics and odds of failing a single dose of methotrexate (MTX) among women with ectopic pregnancy after in vitro fertilization (IVF)

Characteristic	Multivariable adjusted model OR (95% confidence interval)
Oocyte age (years)	1.03 (0.89–1.19)
Assisted hatching	
Yes	0.35 (0.08–1.52)
No	1.00 (referent)
Structural infertility (endometriosis and/or tubal factor)	
Yes	5.85 (0.72–47.81)
No	1.00 (referent)
Day of embryo transfer	
Day 5	1.92 (0.46–8.11)
Day 3	1.00 (referent)
Frozen embryo transfer	
Yes	1.19 (0.29–4.88)
No (fresh)	1.00 (referent)
Endometrial stripe thickness	0.83 (0.67–1.02)
Gestational age at MTX	0.82 (0.48–1.40)
HCG level at time of first MTX	<i>1.08 (1.002–1.17)</i>
1st tertile (<126 IU/L)	1.00 (referent)
2nd tertile (126–341.9 IU/L)	2.14 (0.46–9.96)
3rd tertile (≥342 IU/L)	4.74 (1.11–20.26)
Peak hCG level	<i>1.07 (1.01–1.13)</i>
1st tertile (<194 IU/L)	1.00 (referent)
2nd tertile (194–498.9 IU/L)	3.97 (0.80–19.72)
3rd tertile (≥499 IU/L)	9.73 (1.88–50.25)
Number of embryos transferred	<i>0.37 (0.19–0.74)</i>
1	1.00 (referent)
2	0.44 (0.11–1.69)
≥3	0.08 (0.01–0.50)
Adnexal mass	
Present	3.65 (1.10–12.11)
Absent	1.00 (referent)
Free fluid	
Present	2.66 (0.81–8.81)
Absent	1.00 (referent)

Significant associations are italicized. OR adjusted odds ratio. Multivariable logistic regression models were adjusted for oocyte age (continuous), number of embryos transferred (continuous), and history of prior ectopic pregnancy (dichotomous); please refer to “Materials and methods” section for criteria for adjustment

[2, 15, 16, 18]. Three studies specifically identifying pre-treatment serum hCG levels predictive of requiring a second dose of MTX in the treatment of ectopic pregnancy (in a non-IVF population) reported mean hCG levels ranging from 2844 to 3275 mIU/mL, again significantly higher than the levels identified in this study [9, 12, 13]. The present finding suggests that hCG thresholds conferring risk for failed treatment with one dose of MTX may be lower in an IVF population,

Fig. 2 Serum hCG at the time of first methotrexate (MTX) dose and odds of failing a single dose of MTX among women with ectopic pregnancy after IVF. Diamonds represent odds ratios; 95% confidence intervals are in parentheses and represented with the bars

which warrants investigation in future studies, and which may have significant implications for patient counseling.

The etiology of the association of adnexal masses with a higher likelihood of requiring additional doses of methotrexate remains to be determined. Adnexal masses greater than 4 cm in diameter have been associated with higher risk of failed medical management of ectopic pregnancy overall, but the mean diameter of adnexal masses in this study was approximately 2 cm. While this issue requires further study, an ectopic pregnancy mass visible by ultrasound may represent a greater trophoblastic tissue burden to be addressed by methotrexate, which may require treatment beyond a single dose.

The protective effect of more embryos transferred was a surprising result, and the biologic basis for finding this is unclear. All patients undergoing transfer of three or more embryos received cleavage-stage embryos, and 82% of these were fresh transfers; fresh cleavage-stage embryos are associated with the highest risk of ectopic pregnancy (as compared to frozen or blastocyst transfer), but a basis for greater ease of treatment of the resulting ectopic pregnancies with methotrexate is lacking [4]. Furthermore, though oocyte producer age was included in multivariate analysis and recipient age did not meet criteria for inclusion, the possibility remains that this finding represents a persistent effect of age, as older patients generally receive more embryos and are at higher risk for ectopic pregnancy [32]. There is not, however, a known association of age and MTX failure. This finding requires further exploration in future studies.

These findings can be used to counsel patients and to identify patients who may benefit from multiple-dose MTX regimens, though the advantage of this approach should be confirmed in prospective studies. Options for more intensive MTX protocols upfront include the two-dose regimen described by Barnhart and colleagues, or a multiple-dose protocol involving up to four MTX doses with leucovorin rescue [2, 10, 33]. Using a more aggressive protocol would allow patients to receive the necessary cumulative MTX dose in a shorter time, though patients may experience more side effects [10]. Future studies should include confirmation of the present results in a larger patient population, and, eventually, a prospective study assessing the efficacy of more aggressive MTX dosing in patients with characteristics predictive of failure of a single dose of MTX.

We acknowledge several limitations of this study. The prospectively maintained ART database has many contributors, which introduces the possibility of data entry errors, though important clinical parameters (namely oocyte and recipient ages, obstetrical history, hCG values, imaging results, MTX treatment, surgical reports and clinical outcomes) were verified directly in the medical record. The study population is small, which may limit applicability to clinical care at other centers. Of note, the small size of the population is likely the result of our center's use of an endometrial sampling protocol to exclude failing intrauterine pregnancies, which has been shown to avoid MTX in two-thirds of patients with suspected ectopic pregnancy [34]. This, in turn, could be considered a strength of the study, as only patients with confirmed ectopic pregnancies were included.

In conclusion, this is the first study describing risk factors for requiring additional MTX beyond a single dose in the management of ectopic pregnancy after IVF. In particular, serum hCG thresholds conferring greater risk of failing one dose of MTX were surprisingly low, as compared to previously published hCG thresholds in non-IVF populations. The findings of this study may help to identify patients at risk for requiring additional doses of MTX in clinical practice, with meaningful implications for patient counseling. These patients may be candidates for a proactive two-dose regimen or multiple-dose regimen, given their increased risk of failure with a single MTX dose [2, 10, 33]. Future studies are needed to determine whether these patients are better served with more aggressive dosing regimens, delivering the necessary cumulative MTX dose in a shorter time, potentially avoiding risk of rupture and surgery and shortening the interval to the next treatment cycle.

Compliance with ethical standards

Ethical statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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Conflict of interest Dr. Ginsburg's disclosures are honoraria and royalties from Up To Date, Springer Inc., and Advance Medical. The other authors (Drs. Brady, Missmer, and Farland) declare that they have no conflicts of interest.

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