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Review

Multiple-dose and double-dose versus single-dose administration of methotrexate for the treatment of ectopic pregnancy: a systematic review and meta-analysis

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KEY MESSAGE

This meta-analysis compares the three protocols for the treatment of ectopic pregnancy. The aim was to explore which regimen is appropriate for patients with ectopic pregnancy. The double-dose regimen was an efficient and safe alternative to the single-dose protocol.

A B S T R A C T

In this systematic review and meta-analysis, the effectiveness and safety among different dosage of methotrexate protocols for the treatment of unruptured tubal ectopic pregnancy was evaluated. Six studies of randomized contorlled trials were identified through searches conducted on *PubMed*, *Embase* and *Cochrane Library* between January 1974 and March 2016. The overall success rate of multiple-dose protocol was similar to the single-dose protocol (RR 1.07, 95% CI 0.99 to 1.17, I^2 = 0%). The difference between double-dose and single-dose groups was not significant (RR 1.09, 95% CI 0.98 and 1.20, I^2 = 0%). The incidence of side-effects of double-dose regimen was similar with single-dose regimen. Side-effects, however, are more common in multiple-dose regimen (RR 1.64, 95% CI 1.15 to 2.34, P = 0.006, I^2 = 0%). This meta-analysis indicated that the incidence of side-effects of multiple-dose protocol was significantly higher than single-dose protocol, and the success rates between them were similar. The double-dose regimen was an efficient and safe alternative to the single-dose protocol. Further high-quality researches are needed to confirm our findings and to develop the optimal protocol.

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Introduction

Ectopic pregnancy is a gynaecologic acute abdominal disease and an important cause of maternal mortality in early pregnancy (Agdi and Tulandi, 2009). The rate of ectopic or extrauterine pregnancy is 1.3–2% (Lozeau and Potter, 2005). Methotrexate, a folinic acid antagonist, has been used as first-line therapy for haemodynamically stable patients with ectopic pregnancies (Stovall, 1995; ACOG practice bulletin. Medical management of tubal pregnancy. Number 3, December 1998. Clinical management guidelines for obstetrician gynecologists. American College of Obstetricians and Gynecologists, 1999; Lipscomb et al., 2000). Systemic methotrexate (MTX), involving multiple-dose, single-dose and double-dose protocols, have been reported for the treatment of haemodynamically stable ectopic pregnancy (American College of Obstetricians and Gynecologists, 2008). Consensus has not been achieved, however, on which protocol is optimal (Hajenius et al., 2007).

The multiple-dose regimen involves the administration of four intramuscular methotrexate doses alternating with intramuscular leucovorin rescue factor (Lipscomb et al., 2000). The single-dose protocol includes only a one-time administration of intramuscular methotrexate, then the serum HCG values are observed on day 4 and day 7; if the serum HCG level reduction is less than 15%, a second dose of methotrexate is required (Stovall et al., 1991; ACOG practice bulletin. Medical management of tubal pregnancy. Number 3, December 1998. Clinical management guidelines for obstetrician gynecologists. American College of Obstetricians and Gynecologists, 1999). This protocol has been developed in an attempt to reduce the incidence of side-effects after a multiple-dosing regimen, eliminating the need of leucovorin rescue factor, and to increase the convenience of administration (Barnhart et al., 2003). The double-dose protocol (also called 'two-dose' protocol) includes the administration of two methotrexate doses on day 0 and day 4, which was developed in an attempt to combine the efficacy and safety of the multiple-dose and single-dose regimens (Barnhart et al., 2007).

No meta-analysis, however, has compared the treatment success rates and side-effects rates of the three protocols. Therefore, we conducted this meta-analysis to explore which regimen is appropriate for patients with ectopic pregnancy.

Materials and methods

Study design

This systematic review and meta-analysis strictly followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines 2009 (Altman et al., 2009).

Search strategy

Relevant studies were identified by searching *PubMed*, *Embase* and *Cochrane library* for studies published between January 1974 and March 2016. The following key words were used: 'methotrexate' or 'MTX', 'ectopic pregnancy' or 'tubal pregnancy' and 'dose'. The reference lists of all publications were hand-searched to identify missing relevant publications. Two authors (CY, YG) independently conducted the search, and reviewed titles, abstracts and full manuscripts.

Table 1 – Study eligibilty criteria.

Population	Patients diagnosed with ectopic pregnancy.
Intervention	Standard single-, double- or multiple-dose methotrexate protocols applied for the treatment of ectopic pregnancy.
Comparison	Double-dose versus single-dose methotrexate protocols; multiple-dose versus single-dose methotrexate protocols.
Outcomes	Risk ratios of overall success events and side-effects.
Study design	Randomized control trials.

Eligibility criteria

The study selection criteria are presented in **Table 1**.

Study selection

Trials were selected according to the eligibility criteria. Only studies with randomized design were included. The meeting abstracts fulfilling the criteria were also included. Case series, retrospective or non-randomized trials were excluded. This process was carried out by two authors independently.

Data extraction

Two authors independently extracted the following data from each included study: first author's last name, year of publication, number of patients, size of the ectopic pregnancy, serum HCG concentration, presence or absence of fetal cardiac activity, overall success rate and incidence of side-effect. Any disagreements were resolved by consultation with a third author.

Assessing the risk of bias and grading the quality of evidence

For randomized controlled trials, the Cochrane Collaboration's tool was used to assess the risk of bias (Higgins and Green, 2011), and the GRADE system was used to assess the grades of evidence (Atkins et al, 2004). The assessment for the risk of bias was strictly conducted according to the guidelines in the Cochrane handbook. Two authors independently reviewed the studies and assigned a value of 'low', 'uncertain' or 'high' to six domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias.

The GRADE system identified the following four grades to rate the quality of evidence (Schuiiemann and Oxman, 2009): (1) high: further researches are unlikely to change the estimate of the effects; (2) moderate: further researches are likely to influence the estimate of the effects; (3) low: further researches are very likely to change the estimate of the effects; and (4) very low: the estimate of the effects is very uncertain.

Outcome measures

The primary outcome measure was the treatment success, which was defined as a higher than 15% reduction of serum HCG between day 4 and day 7 (single- and double-dose group). For multiple-dose protocol, the treatment success was defined as a 15% decrease of serum HCG in 48 h or after administration of four doses of MTX. The above definitions were regarded as treatment success whether or not a

continued drop of serum HCG to undetectable levels was observed. Re-interventions (surgical or medical) for either clinical symptoms or inadequately declining serum HCG levels were considered as treatment failures. The incidence of side-effects was included as a secondary outcome measure in the pooled results.

Statistical analysis

The heterogeneity among included studies was evaluated graphically using forest plots and statistically using the I^2 statistic. A fix-effects model was applied for low heterogeneity. Otherwise, the random-effects model was used. RevMan 5.3 (Cochrane, Collaboration, Oxford, UK) was applied to generate figure of the 'Risk of bias graph and risk of bias summary'. The GRADE profiler software (available at: http://www.gradeworkinggroup.org) was used to assess the grades of evidence. A pooled risk ratios (RR) with 95% confidence intervals (CI) was used to assess the dichotomous outcome data. $P < 0.05$ was considered statistically significant.

Results

Study selection and characteristics

The search strategy yielded 335 studies. Of these, a total of 15 articles were found to be relevant by examining the abstracts and titles. The literature search results are represented in **Figure 1**. Nine original articles were excluded because three studies were retrospective analyses (Gungorduk et al., 2011; Guven et al., 2007; Mergenthal et al., 2013), one was non-randomized trial (Lipscomb et al., 2005), one lacked the control group (Balci et al., 2010), the data of a conference abstract was not correct (Golmohammadlou et al., 2012), the multiple-dose regimen was not standard in two studies (Fakheri et al., 2014; Klauser et al., 2005), and in one study, no extractable data were available (Zargar et al., 2008). Therefore, the total number of studies included in the meta-analysis was six (Alleyassin et al., 2006; Guvendag Guven et al., 2010; Hamed et al., 2012; Saadati et al., 2015; Song et al., 2016; Tabatabaii Bafghi et al., 2012). All of the six studies were randomized controlled trials. The main characteristics of all of the studies are presented in **Table 2**.

Risk of bias and grades of evidence

The results of the bias risk assessment for six randomized controlled trials are presented in **Figure 2**, and the grades of evidence are shown in **Table 3** and **Table 4**. Two RCTs (Alleyassin et al., 2006; Tabatabaii Bafghi et al., 2012) offered better descriptions of blinding of participants and personnel and blinding of outcome assessment. The main bias of the other trials was the lack of blinding or the lack of describing whether they were double-blind or open-label trials.

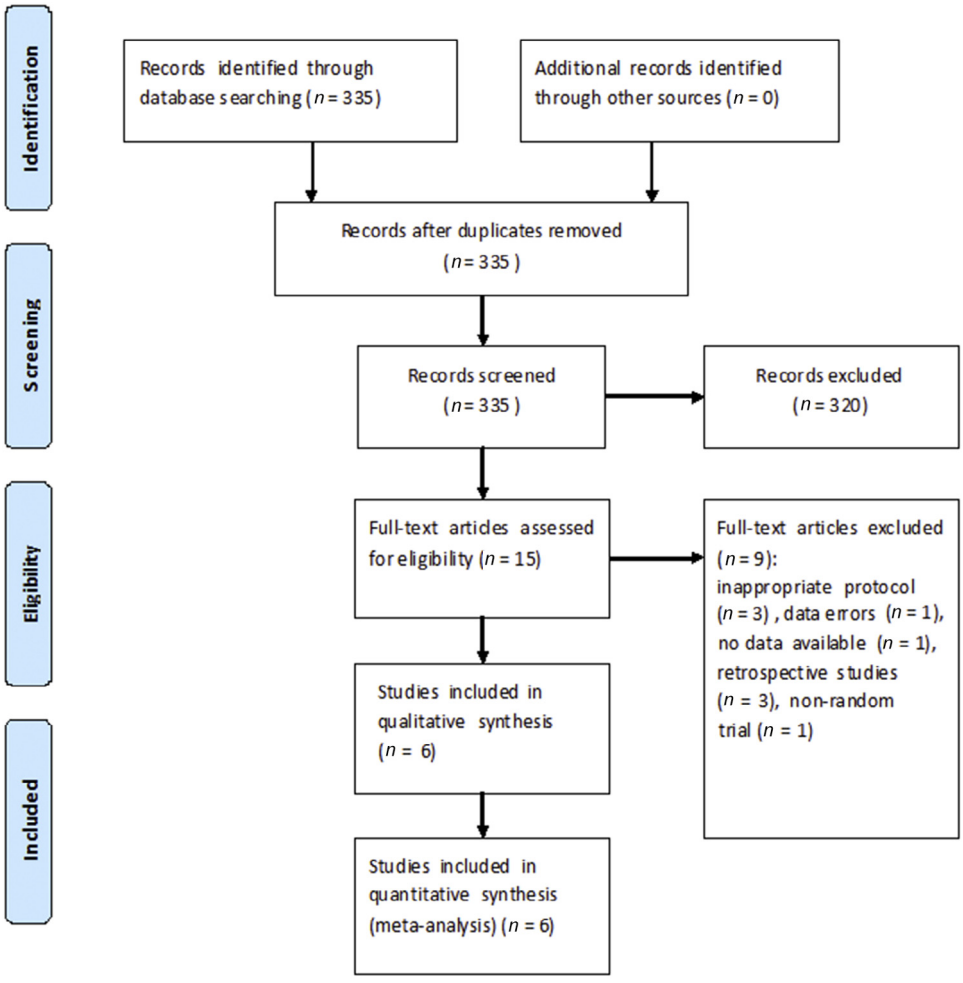


Figure 1 – PRISMA four-phase flow diagram of search yield, screening and inclusion steps.

Table 2 – Main characteristics of the studies.

Study	Study design	Treatment		Inclusion criteria	Exclusion criteria	Main outcomes
		Single-dose group	Double-dose group			
Hamed et al., 2012	RCT	50 mg/m^2 methotrexate IM on day 0 (n = 78).	50 mg/m^2 methotrexate IM on days 0 and 4 (n = 79).	<4 cm; serum HCG <15000 IU/L; no cardiac activity; stable haemodynamic condition.	Non-adnexal ectopic pregnancy; clinically suspected tubal rupture; free fluid extending beyond the Douglas pouch on TVS; laboratory tests showing possible deleterious effects of methotrexate treatment on organ functions.	Success rate; side effects; treatment duration.
Saadati et al., 2015	RCT	50 mg/m^2 methotrexate IM on day 0 (n = 38).	50 mg/m^2 methotrexate IM on days 0 and 4 (n = 38).	Serum HCG <15000 IU/L; no cardiac activity; stable haemodynamic condition.	Women had a history of liver and kidney disease, blood dyscrasia or problems with breastfeeding	Success rate; side effects.
Song et al., 2016	RCT	50 mg/m^2 methotrexate IM on day 0 (n = 46).	50 mg/m^2 methotrexate IM on days 0 and 4 (n = 46).	<4 cm; serum HCG <15000 IU/L; no cardiac activity; stable haemodynamic condition; tubal pregnancy, except interstitial pregnancy.	Heterotrophic pregnancy or persistent tubal pregnancy; clinically or TVS suspected tubal rupture; laboratory tests showing possible deleterious effects of methotrexate treatment on organ functions.	Success rate; side effects; treatment satisfaction; cost; days off work or school.
		Single-dose group	Multiple-dose group			
Alleyassin et al., 2006	RCT	Methotrexate 50 mg/m2 IM, day 1 (n = 54).	Methotrexate 1 mg/kg IM, days 1, 3, 5, 7 Leukovorin 0.1 mg/kg IM, days 2, 4, 6, 8 (n = 54).	<3.5 cm; serum HCG <15000 IU/L; no cardiac activity; stable haemodynamic condition.	Clinically or TVS suspected tubal rupture.	Success rate; side effects.
Guvendag Guven et al., 2010	RCT	Methotrexate 50 mg/m^2 IM, day 1 (n = 62).	Methotrexate 1 mg/kg IM, days 1, 3, 5, 7 Leukovorin 0.1 mg/kg IM, days 2, 4, 6, 8 (n = 58).	<3.5 cm; stable haemodynamic condition; no history of previous tubal surgery; serum HCG levels reaching a plateau or increased by ≤50% in 48-h intervals.	Women had a history of liver and kidney disease; not willing to participate in the study.	Success rate; side effects.
Tabatabaii Bafghi et al., 2012	RCT	Methotrexate 50 mg/m^2 IM, day 1(n = 35).	Methotrexate 1 mg/kg IM, days 1, 3, 5, 7 Leukovorin 0.1 mg/kg IM, days 2, 4, 6, 8 (n = 35).	≤4 cm; serum HCG <15000 IU/L; no cardiac activity; stable haemodynamic condition.	Evidence of bleeding shown by laparoscopic surgery and TVS.	Success rate; side effects; fertility.

IM, intramuscularly; RCT, randomized controlled trial; TVS, transvaginal sonography.

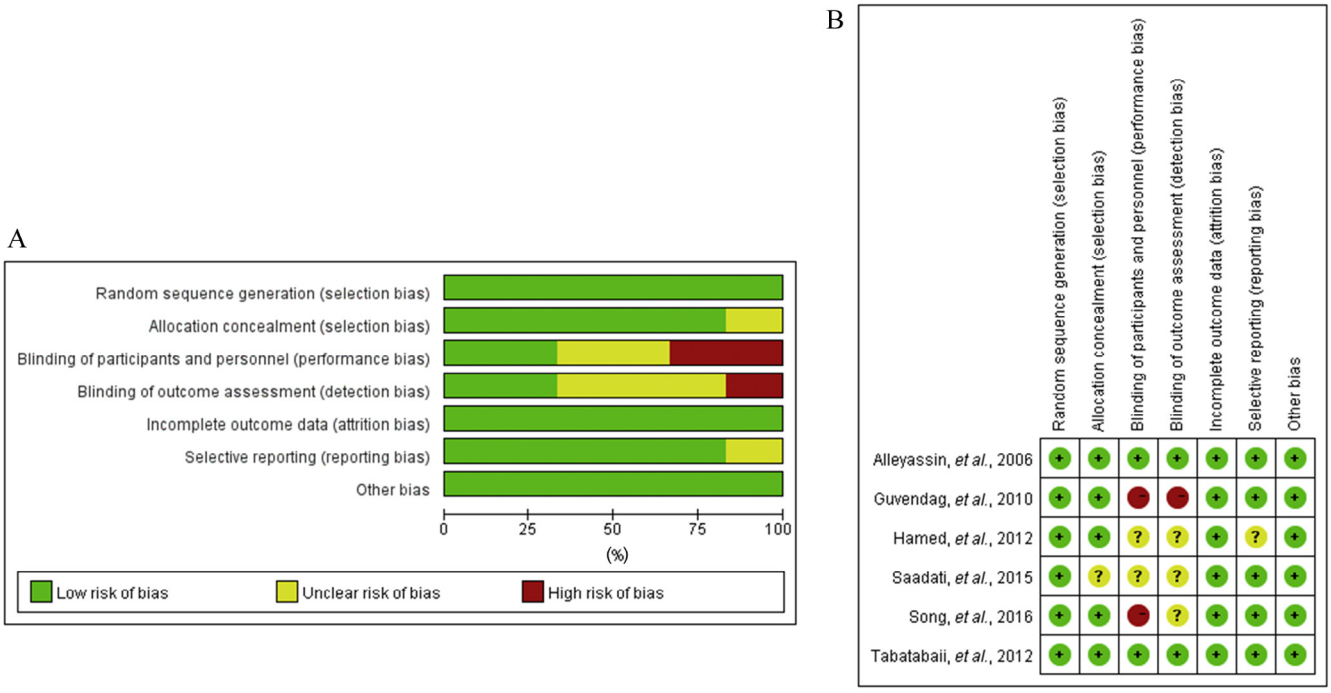


Figure 2 – Risk of bias graph (A) and risk of bias summary (B) based on review authors' judgments about each risk of bias item.

Primary outcomes

Overall success rate

The meta-analysis of pooled results is shown in **Figure 3**. Three RCTs (Hamed et al., 2012; Saadati et al., 2015; Song et al., 2016), involving 325 haemodynamically stable patients with an unruptured ectopic pregnancy, compared the overall success rate of double-dose protocol to single-dose protocol. A non-significant trend towards a higher treatment success after the administration of double-dose methotrexate was found (RR 1.09, 95% CI 0.98 to 1.20, I^2 = 0%). Three trials (Alleyassin et al., 2006; Guvendag Guven et al., 2010; Tabatabaii Bafghi et al., 2012), involving 298 women, compared the overall success rate of multiple-dose versus single-dose group. The combined results of three ranodmized controlled trials (Alleyassin et al., 2006; Guvendag Guven et al., 2010; Tabatabaii Bafghi et al., 2012) showed that the success rate of multiple-dose protocol was similar with single-dose protocol (RR 1.07, 95% CI 0.99 to 1.17, I^2 = 0%).

Secondary outcomes

Incidence of side-effects

The meta-analysis pooled results are shown in **Figure 4**. Three randomized controlled trials (Hamed et al., 2012; Saadati et al., 2015; Song et al., 2016), involving 325 women, compared the incidence of side-effects between double-dose and single-dose protocol. The combined results suggested a non-significant trend towards a higher incidence of side-effects after the administration of double-dose methotrexate (RR 1.33, 95% CI 0.92 to 1.94, I^2 = 0%). The combined results of three ranodmized controlled trials (Alleyassin et al., 2006; Guvendag Guven et al., 2010; Tabatabaii Bafghi et al., 2012), involving 298 women, showed that the incidence of side-effects of multiple-dose protocol was significantly higher than single-dose protocol (RR 1.64, 95% CI 1.15 to 2.34, P = 0.006; I^2 = 0%,).

Discussion

To date, therapeutic options for ectopic pregnancy are surgery, medical treatment or expectant management. Systemic MTX treatment has been accepted as a cost-effective alternative to laparoscopy for haemodynamically stable patients. The single-dose protocol was developed to minimize side-effects, improve convenience and to reduce overall costs (Tabatabaii Bafghi et al., 2012). The double-dose protocol was an attempt to combine the efficacy and safety of the multiple-dose and single-dose protocols, which was first introduced by Barnhart et al. in 2007.

To the best of our knowledge, this is the first meta-analysis to compare the overall success rate and incidence of side-effects of the three protocols. The results of the studies indicated that the incidence of side-effects of multi-dose protocol are significantly higher than single-dose protocol, which in double-dose regimen are similar with single-dose regimen. The success rates between multi-dose protocol and single-dose protocol, as well as between the double-dose regimen and single-dose regimen are similar. Although the difference was not significant, the success rate in the subgroup of patients with an initial serum HCG level of over 5000 IU/L (Song et al., 2016) was higher in the double-dose group than in the single-dose group (80.0 versus 58.5%). Moreover, the success rate of double regimen was significantly higher in patients with serum HCG levels between 3600 and 5500 IU/L (P = 0.03) and approaching significantly higher in patients with an ectopic mass diameter between 2.7 and 3.5 cm (P = 0.055) (Hamed et al., 2012).

Table 3 – GRADE profile evidence of the included studies for single-dose methotrexate versus double-dose methotrexate.

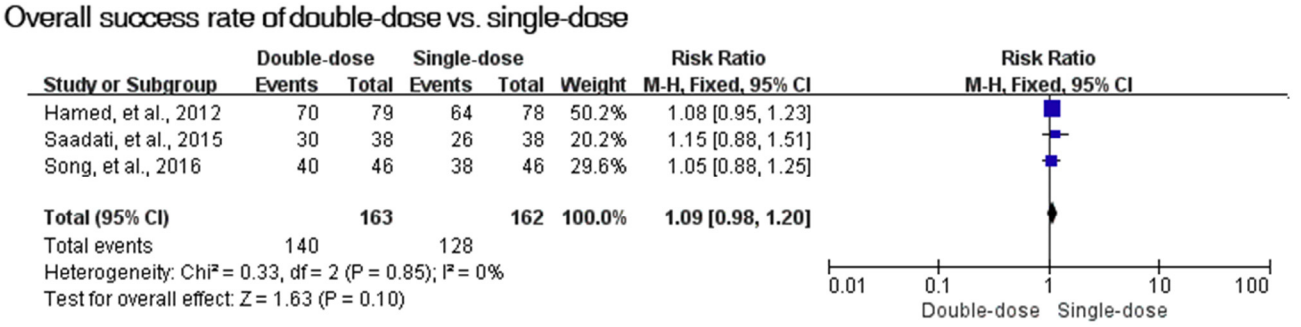
Quality assessment							Number of patients		Effect		Quality[b]	Importance
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Single-dose methotrexate	Double-dose methotrexate	Relative (95% CI)	Absolute (95% CI)		
Overall success rate												
3	Randomized trials	Serious[a]	Not serious	Not serious	Not serious	None	119/162 (73.5%)	137/163 (84.0%)	RR 1.09 (0.98 to 1.20)	76 more per 1000 (from 17 fewer to 168 more)	⊕⊕⊕◯ Moderate	Critical
Side-effects												
3	Randomized trials	Serious[a]	Not serious	Not serious	Not serious	None	35/162 (21.6%)	47/163 (28.8%)	RR 1.33 (0.92 to 1.94)	95 more per 1000 (from 23 fewer to 271 more)	⊕⊕⊕◯ Moderate	Critical

[a] Three studies had bias in blinding; one study had bias in allocation concealment.
[b] According to scale ⊕⊕⊕⊕ high quality ⊕⊕⊕◯ moderate quality. ⊕⊕◯◯ low quality. ⊕◯◯◯ very low quality.
CI, confidence interval; RR, risk ratio.

Table 4 – GRADE profile evidence of the included randomized controlled trials for single-dose methotrexate versus multiple-dose methotrexate.

Quality assessment							Number of patients		Effect		Quality[a]	Importance
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Single-dose methotrexate	Multiple-dose methotrexate	Relative (95% CI)	Absolute (95% CI)		
Overall success rate												
3	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	127/151 (84.1%)	133/147 (90.5%)	RR 1.07 (0.99 to 1.17)	63 more per 1000 (from 9 fewer to 154 more)	⊕⊕⊕⊕ High	Critical
Side effects												
3	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	34/151 (22.5%)	54/147 (36.7%)	RR 1.64 (1.15 to 2.34)	235 more per 1000 (from 55 more to 492 more)	⊕⊕⊕⊕ High	Critical

[a] According to scale ⊕⊕⊕⊕ high quality ⊕⊕⊕◯ moderate quality. ⊕⊕◯◯ low quality. ⊕◯◯◯ very low quality.
CI, confidence interval; RR, risk ratio.



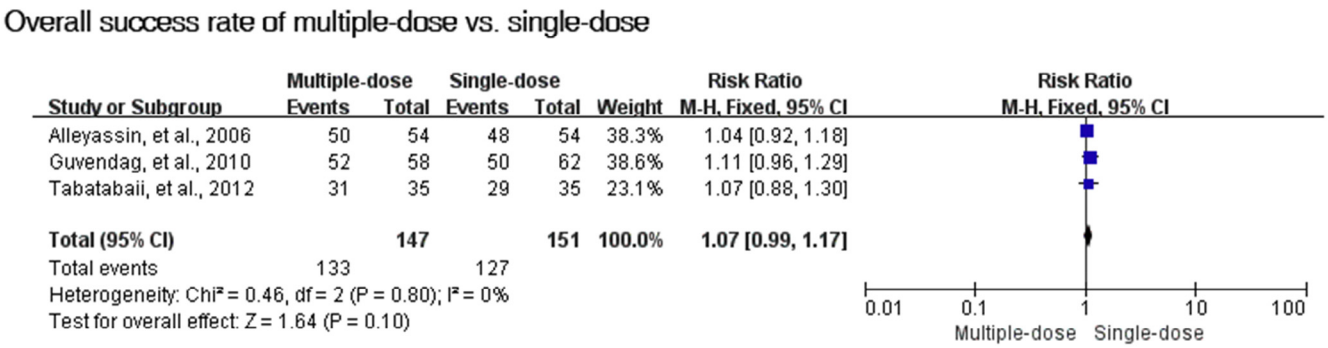
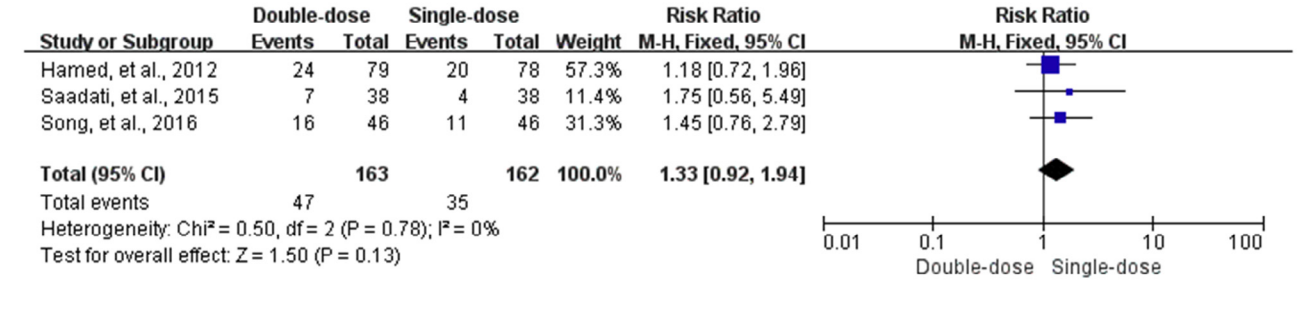


Figure 3 – Meta-analysis results of overall success rates.

A previous meta-analysis found that the multiple-dose regimen was slightly more effective than single-dose regimen (Barnhart et al., 2003), which included 26 case series. No studies, however, directly compared the two medical regimens. The studies in a meta-analysis should include patients, intervention/exposure, control, outcome and study design (PICOS principle), otherwise, the results should be interpreted with caution. In this meta-analysis, the data were from six randomized controlled trials, which directly compared multiple-dose



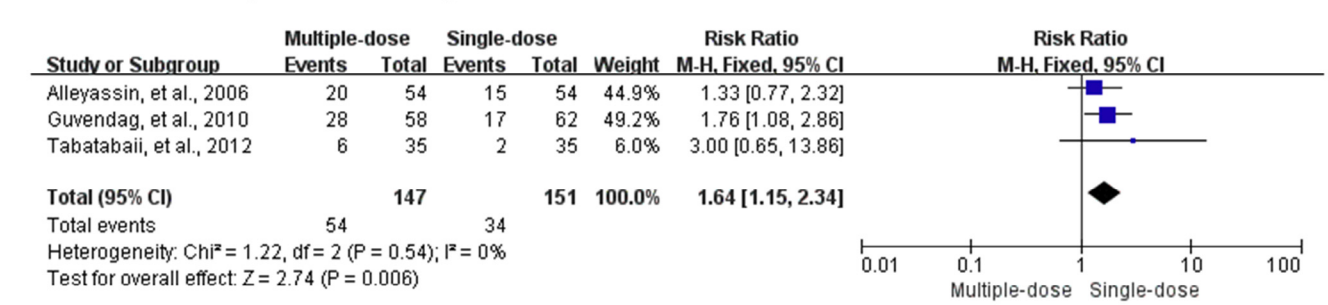


Figure 4 – Meta-analysis results of incidence of side-effects.

and double-dose regimen with single-dose regimen. The pooled result of three randomized controlled trials (Alleyassin et al., 2006; Guvendag Guven et al., 2010; Tabatabaii Bafghi et al., 2012) suggested the overall success rate of multiple-dose protocol was similar with single-dose protocol, which was inconsistent with the result of Barnhart et al. (2003). Another previous meta-analysis (Mol et al., 2008) included only two studies (Alleyassin et al., 2006; Klauser et al., 2005), which compared single-dose versus multiple-dose MTX. No significant difference in treatment success was found. The multiple-dose regimen in Klauser et al. (2005), however, was not standard, it only included the administration of three methotrexate doses without leucovorin.

The greatest strength of our meta-analysis is the number of patients included and the low heterogeneity of all of the studies retained. Moreover, all of the studies included are RCTs. We used the Cochrane Collaboration's risk of bias graph to assess the risk of bias and the GRADE system to assess the grading of evidence, which are more objective in evaluating the bias risk and the evidence grading of studies. The results for the risk of bias revealed that the overall bias of the included RCTs was moderate. The GRADE system suggested that the overall grading of evidence in the double-dose versus single-dose group was 'moderate', and the multiple-dose versus single-dose group exhibited a 'high' rating.

In conclusion, treatment with single-dose MTX has fewer side-effects, and the success rate is similar with multiple-dose protocol. The double-dose MTX protocol was found to be an efficient and safe alternative to the single-dose MTX protocol. In women with pretreatment serum HCG level between 3600 and 5500 IU/L or with an ectopic mass diameter between 2.7 and 3.5 cm, administration of double dose is recommended. Furthermore, multicentre, randomized clinical trials with larger sample sizes are warranted to confirm the results.

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