

Efficacy of low-molecular-weight heparin on the outcomes of in vitro fertilization/intracytoplasmic sperm injection pregnancy in non-thrombophilic women: a meta-analysis

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Key words

Low-molecular-weight heparin, in vitro fertilization/intracytoplasmic sperm injection, non-thrombophilia, pregnancy outcomes, randomized controlled trial

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Xiu-Ying Yang and Yang Xu contributed equally to the study.

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Abstract

Introduction. The aim of our study was to evaluate the effect of low-molecular-weight heparin on pregnancy outcomes in women without thrombophilia during in vitro fertilization/intracytoplasmic sperm injection treatment. **Material and methods.** We searched Pubmed, Web of Science, Embase, Cochrane and CNKI (from inception to 2 February 2018). Our study identified randomized controlled trials or quasi-randomized controlled trials comparing low-molecular-weight heparin subcutaneous treatment with no treatment or only luteal support control. The outcomes included live birth rate, clinical pregnancy rate and miscarriage rate. **Results.** Five trials, including 935 women receiving in vitro fertilization/intracytoplasmic sperm injection treatment, were included in meta-analyses. There were 458 women receiving low-molecular-weight heparin and 477 in the control group. No significant differences for live birth rate, clinical pregnancy rate and miscarriage rate were found between the low-molecular-weight heparin and control groups. Of them, four trials reported live birth rate as an outcome and the risk ratio was 1.13 (95% confidence interval 0.88–1.43, $p = 0.34$). All five trials reported clinical pregnancy rate as an outcome, the risk ratio was 1.08 (95% confidence interval 0.87–1.32, $p = 0.47$). Three trials reported miscarriage rate and the risk ratio was 0.58 (95% confidence interval 0.30–1.10, $p = 0.09$). In women with two or more failed in vitro fertilization/intracytoplasmic sperm injection cycles, the risk ratio of live birth rate was 1.15 and the risk ratio of clinical pregnancy rate was 1.17. In women with three or more failed in vitro fertilization/intracytoplasmic sperm injection cycles, the risk ratios of live birth rate and clinical pregnancy rate were 1.36 and 1.35, respectively. **Conclusions.** Our results suggested that low-molecular-weight heparin had no effect on pregnancy success rate in non-thrombophilic women undergoing in vitro fertilization/intracytoplasmic sperm injection treatment. However, to justify the use of low-molecular-weight heparin in clinical practice, multicenter trials are still necessary.

Abbreviations: CPR, clinical pregnancy rate; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; LBR, live birth rate; LMWH, low-molecular-weight heparin; MR, miscarriage rate; RCT, randomized clinical trial; RR, risk ratio.

Introduction

Recent innovations in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment have provided considerable hope to couples with fertility complications. However, the pregnancy success rate of IVF/ICSI remains very low (1). IVF/ICSI treatment is influenced by various factors, such as age, history of recurrent miscarriage, type of fertility problems, quality and number of embryos, lifestyle, type of protocol, endometrial receptiveness and thrombophilia. A number of therapeutic interventions, such as low-molecular-weight heparin (LMWH)/heparin and aspirin, have been used to improve the success of IVF/ICSI (2,3).

The LMWHs are a class of anticoagulant agents with an average molecular weight <8000 Da. Studies showed that LMWH improves the pregnancy outcomes by modulating many physiological processes required for blastocyst adherence, implantation and trophoblast invasion (2,3). LMWH can be given subcutaneously, causing a more controllable anticoagulant effect and is associated with fewer side effects than other anticoagulant agents. LMWH might also promote placental angiogenesis during the first and second trimesters of pregnancy and promote the expression of VEGF soluble receptor-1 during the first trimester (4,5). Previous favorable effects of LMWH in women with thrombophilia precipitated the use of this drug in those without thrombophilia. However, studies on the effect of LMWH as an adjunct to IVF/ICSI treatment have shown conflicting results. Several studies (6–9) suggested that LMWH has beneficial effects in women with recurrent implantation failure, whereas some other studies showed no evidence of benefits (10,11).

The aim of this systematic review and meta-analysis was to evaluate the role of LMWH subcutaneous administration in pregnancy outcomes in non-thrombophilic women undergoing IVF/ICSI. The outcomes included live birth rate (LBR), clinical pregnancy rate (CPR) and miscarriage rate (MR).

Material and methods

The review protocol was established by two investigators (YXL, YXY) before commencement. This review considered randomized controlled trial studies conducted on the efficacy of LMWH therapy in women undergoing IVF/ICSI without thrombophilia. Information regarding patients, such as number of IVF cycles, age and pregnancy outcomes, were also acquired. We followed the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) in conducting this systematic review (see Supporting Information, Table S1 and S2).

Sources

To identify relevant literature we searched the following online databases: Medline (1950–February 2018), EMBASE (1980–February 2018), Cochrane Central Register of Controlled Trials (2003–February 2018), Web of Science (1990–February 2018), China National Knowledge Infrastructure (CNKI, 1979–February 2018), and Japan Scholarly and Academic Information Navigator (CiNii, 2006–February 2018). There was no geographic restriction. The search strategy was performed without any language restriction, i.e. included all non-English articles with relevance. The search in the CiNii was performed in Japanese, and the search in the CNKI database was performed in Chinese language. Medical subject headings and free words were used to identify the citations for addressing the research question. The focused research questions followed PICO (population, intervention, control, and outcomes). The reference lists of all published articles were searched to collect articles related to the subject (search strategy is shown in the Supporting Information, Table S2).

Study identification and data extraction

Our study focused exclusively on the LMWH subcutaneous administration in non-thrombophilic women. We identified studies that evaluated the effect of LMWH in women undergoing IVF/ICSI on live birth, clinical pregnancy and miscarriage rates. We included randomized clinical trial (RCT) and quasi-RCT studies that compared the use of LMWH (intervention) with placebo or no treatment (control) in women undergoing IVF/ICSI without thrombophilia. Populations with or without recurrent implantation or IVF failures were included. In all included studies, LMWH was given from ovulation induction, oocyte retrieval or at embryo transfer until the detection of fetal heart, 9–12 weeks of pregnancy, or for the entire period of the pregnancy.

Studies were selected in a two-stage process. First, the titles and abstracts were screened independently by two reviewers (YXL, YXY) to meet the predefined criteria. Second, each full-text paper was read to decide whether the inclusion or exclusion criteria were applied (YXL, YXY, CF). In cases of duplicate publication, the most recent or

Key Message

Low-molecular-weight heparin may not improve in vitro fertilization outcomes in non-thrombophilic women.

complete manuscripts were used. Any relevant references from the full manuscripts were checked. Any disagreement regarding the inclusion or exclusion criteria was consulted and arbitrated by a third reviewer (GHD and XY).

Of the potentially eligible studies, we included five studies in the total and subgroup analyses. We excluded studies for the following reasons: no placebo control group (for example, aspirin as control); no results related to pregnancy outcomes (for example, pregnancy complications); inclusion of women with thrombophilia (7,12) or other characteristics (for example, age) that would influence the final analysis.

Two reviewers (YXL and YXY) independently searched the relevant publications and extracted relevant data from the validated articles. We defined clearly the data to ensure a standardized data extraction. The quality of each study was assessed using the Jadad scale (13). Two reviewers (CF and XY) completed the quality assessment. We designed a protocol and an EXCEL template according to the criteria for each variable to ensure consistency and meet the objective of the data, and the data were recorded comprehensively. The eligibility of each participant was verified before the data were included. The participants who did not meet the inclusion criteria or did not include the relevant outcomes were excluded. Analyses of the data in the trials were repeated twice to verify the results before their inclusion in the meta-analysis.

Outcome measures

The outcomes of the eligible studies comprised composite outcomes. The primary outcome include LBR, and the secondary outcomes included the CPR and MR. LBR was defined as the number of live births divided by the number of women in a group. MR was defined as the number of miscarriages divided by the number of women in a group before 20 weeks of gestation. CPR was defined as the observation of gestational sac on ultrasound at 5–7 weeks of gestation. Side effects are adverse maternal or fetal outcomes, including serious bloody discharge, preeclampsia during the gestational period and intrauterine growth restriction. These definitions were applied in the included studies and used to calculate the outcomes in this review.

Risk of bias assessment

The risk of bias was assessed for each study by YXL and YXY independently using the Cochrane Collaboration Risk of Bias Tool (14). The criteria of bias included selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. Review authors' judgments were categorized as "low risk," "high risk" or "unclear risk" of bias. All judgments were given by discussion.

Statistical analysis

Risk ratios (RRs) and the related confidence intervals (CIs) were calculated for each outcome included in the study separately. Presence of publication bias was explored using funnel plots of effect size against standard error. Heterogeneity of the exposure effects was evaluated graphically using forest plots. The data were pooled using a fixed-effects model (15). The REVIEW MANAGER software (RevMan, version 5.3 for Windows, Oxford, UK; The Cochrane Collaboration) was used for statistical analysis. A p value ≤ 0.05 was considered statistically significant for all analyses.

Results

Study characteristics

A flowchart of our search strategy is shown in Figure 1. After comprehensive assessment of the full-text articles, five papers were included in this review. The characteristics of all included studies are listed in Table 1. All included studies were RCTs or quasi-RCTs, and evaluated the effect of LMWH on the treatment outcomes of IVF. No significant differences in heterogeneity were found between the included studies. The results of the studies were then pooled and used for meta-analysis. In total, 935 IVF/ICSI-treated women were included in the two groups: 458 women received the LMWH treatment and 477 women received the control treatment.

LMWH therapy

All the included studies in this meta-analysis used subcutaneous administration of LMWH in IVF/ICSI-treated women. Studies administered LMWH treatment before the beginning of the stimulation phase of the cycle (10), i.e. on the day of oocyte retrieval (11,16,17) or on embryo transfer day (8). Drug was stopped either with a negative pregnancy test or continued till a positive pregnancy test (8), 2 weeks (17), 9 weeks (16), 12 weeks (11), or until delivery (10). In these clinical trials, the previous assisted reproductive technology number varied from zero (10,16) to at least two times (11,17) or three times (8,11,17).

The dosage of four studies fell in the range of 2500–6400 IU daily (8,10,16,17), and one study used 1 mg/kg/day, which was approximately equal to 100 IU/mg (11). Two studies (11,17) used enoxaparin (MW 4500), one study (16) used dalteparin sodium (MW 5000), one (10) used parnaparin (MW 5000), and one (8) used LMWH calcium injection (MW 3600–5000).

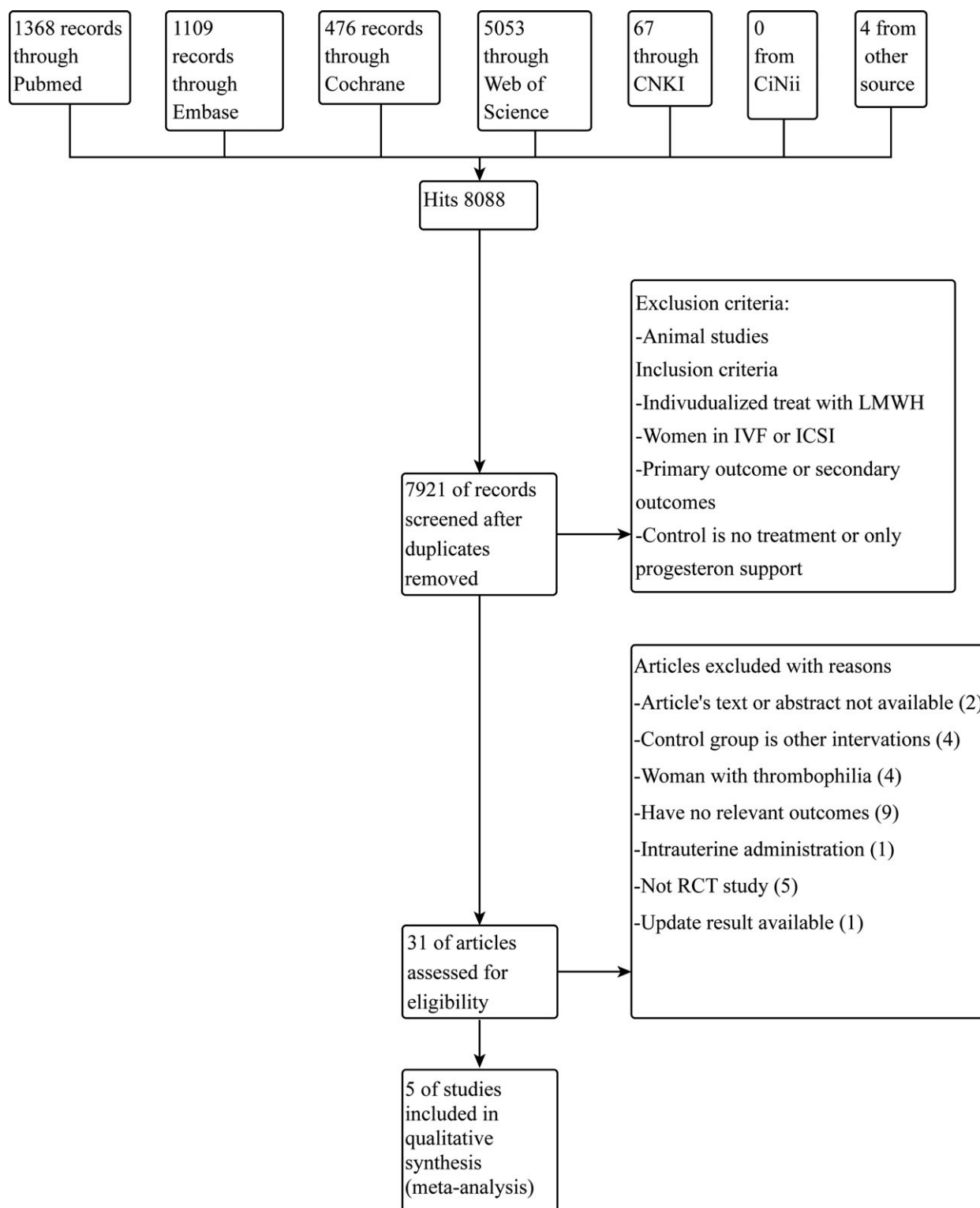


Figure 1. Flowchart of selection procedure according to PRISMA guidelines. LMWH, low-molecular-weight heparin; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; RCT, randomized controlled trial.

Table 1. Baseline characteristics and treatment of women in the included studies.

Study (country)	Design	Participants	Intervention	Control	Outcomes
Lodigiani <i>et al.</i> 2017 (Italy) (10)	RCT, single center (<i>n</i> = 266)	With: Idiopathic or primary infertility. Without severe thrombophilia, antiphospholipid autoantibodies and abnormal platelet count	LMWH, 4250 IU <60 kg, 6400 IU >60 kg, daily subcutaneous, starting from ovulation induction until delivery in ART. Age: 18–40 years (<i>n</i> = 135)	No treatment. Age: 18–40 years (<i>n</i> = 131)	LBR, CPR, IR, MR
Xiong <i>et al.</i> 2015 (China) (8)	RCT, single center (<i>n</i> = 147)	With: three or more failed IVF/ICSI cycles. Without: coagulation disorders (d-dimer test >1.5 mg/L), uterine abnormalities, tubal effusion; combined medical diseases (thyroid dysfunction, etc.)	LMWH, 4100 IU were administered from ET, until detection of the fetal heart. Age: 34.89 ± 2.49 years (<i>n</i> = 65)	Luteal phase support. Age: 35.05 ± 2.79 years (<i>n</i> = 82)	CPR, IR
Berker <i>et al.</i> 2011 (Turkey) (17)	Quasi-RCT, single center (<i>n</i> = 219)	With: two or more failed implantations. Without: Coagulation disorders. (Mutations of factor V Leiden, prothrombin gene, methylene tetrahydrofolate reductase gene, and abnormal levels of anti-cardiolipin immunoglobulin G, immunoglobulin M, lupus anticoagulant, anti-thrombin, protein C and protein S). Uterine abnormalities	Enoxaparin sodium, 4000 IU (40 mg) subcutaneously from the day of oocyte retrieval to 12th week of pregnancy. Age: 31.3 ± 4.9 years (<i>n</i> = 110)	No treatment. Age: 31.2 ± 5 (20–44) years (<i>n</i> = 109)	LBR, CPR, IR, MR
Noci <i>et al.</i> 2011 (Italy) (16)	RCT, single center (<i>n</i> = 153)	With: First IVF/ICSI cycle. Without: Coagulation disorders (both acquired and inherited thrombophilia). Hormonal or uterine abnormalities	Dalteparin sodium, 2500 IU/day (from the day of oocyte retrieval to week 9 of pregnancy). Age: 34.7 ± 3.6 years (<i>n</i> = 73)	Control group received only progesterone. Age: 35.1 ± 3.1 years (<i>n</i> = 80)	LBR, CPR, IR, MR
Urman <i>et al.</i> 2009 (Turkey) (11)	RCT, single center (<i>n</i> = 150)	With: two or more failed IVF/ICSI cycles; Without: Coagulation, hormonal or immunological disorders. Obvious causes of implantation failure; Uterine abnormalities	LMWH, 1 mg/kg/day (from the day after oocyte retrieval to week 12 of pregnancy). Age: 34.0 ± 5.0 years (<i>n</i> = 75)	No treatment. Age: 34.8 ± 5.8 years. (<i>n</i> = 75)	CPR, LBR, IR, MR

ART, assisted reproductive technology; CPR, clinical pregnancy rate; ET, embryo transfer; ICSI, intracytoplasmic sperm injection; IR, implantation rate; IVF, in vitro fertilization; LBR, live birth rate; LMWH, low-molecular-weight heparin; MR, miscarriage rate; RCT, randomized clinical trials.

Study population

The participants in this review were from Italy, Turkey and China, respectively. According to our exclusion criteria, a study that uses heparin instead of LMWH as treatment was excluded in this meta-analysis (18). Two studies (7,12) that included women with thrombophilia were excluded. One study (12) was excluded due to evaluation of *M2/ANXA5*-positive couples, a gene that was closely related to thrombophilia. A study that investigated the intrauterine injection of LMWH was also excluded

(19). The characteristics of excluded studies are listed in Table 2. The age range of women included in this meta-analysis was 18–47 years.

Quality assessment and publication bias

The studies were accessed according to the quality assessment (Figure 2). A risk of bias assessment was completed. Overall, three studies (10,11,16) strictly followed the RCT rule for blinding the participants, personnel or outcome assessment. These publications were at a low risk of bias

Table 2. Baseline characteristics and treatment of women in the excluded studies.

Study (country)	Design	Participants	Intervention	Control	Exclusion criteria
Siristatidis <i>et al.</i> 2017 (Greece, Egypt) (31)	RCT, three-center ($n = 115$)	With: two or more failed fresh IVF/ICSI cycles; Without: coagulation and/or autoimmune disorders	LMWH and prednisolone at start of stimulation. Age: 35.1 ± 0.7 years ($n = 57$)	No treatment. Age: 34.1 ± 0.7 years ($n = 58$)	Treatment is not relevant (LMWH and prednisolone); Retrospective cohort study
Shirlow <i>et al.</i> 2017 (Australia) (32)	Retrospective cohort study, multi-center ($n = 13\ 372$)	With: IVF/ICSI women	Thirteen adjuvants (inralipid, steroids, melatonin, coenzyme Q10, Filgrastim, estosterone, DHEA, growth hormone, antibiotics, hCG infusion, aspirin, noxaparin/heparin, and dopamine agonists) ($n = 1904$) ANXA5 M2 positive were enlisted to the cohort and treated with a prophylactic dose of LMWH on achieving embryo transfer. Age: 36 years ($n = 103$)	No treatment ($n = 11\ 468$)	Not RCT; thrombophilia included
Fishel <i>et al.</i> 2016 (UK, Ireland) (12)	Observational cohort study, multi-center ($n = 206$)	With: IVF/ICSI women, ANXA5 haplotype	Injected with intrauterine LMWH (enoxaparin sodium) during mock ET, just after OPU (2–5 days before ET). Age: 28.80 ± 5.17 years ($n = 59$)	No treatment. Age: 36 years ($n = 103$)	Thrombophilia included
Kamel <i>et al.</i> 2016 (Egypt) (19)	RCT, single center ($n = 114$)	With: IVF/ICSI women. Without: both acquired and inherited thrombophilia.	Injected with similar volume of tissue culture media. Age: 28.15 ± 4.79 years ($n = 55$)	Injected with a similar volume of tissue culture media. Age: 28.15 ± 4.79 years ($n = 55$)	local intrauterine LMWH injections
Hamdi <i>et al.</i> 2015 (Iran) (18)	RCT, single center ($n = 100$)	With: two or more failed implantations; Without: thrombophilia, hormonal immunologic disorders	Heparin 5000 IU were administered for 15 days from the day of oocyte pick up. Age: 32.46 ± 5.14 years ($n = 50$)	Luteal phase support. Age: 30.9 ± 4.71 years ($n = 50$)	Treatment is not relevant (Heparin)
Tormene <i>et al.</i> 2015 (Italy) (33)	RCT, single center ($n = 81$)	With: two or more failed implantations. Without: coagulation disorders	LMWH, dalteparin 5000 IU/day. Age: mean age 39 years ($n = 33$)	No treatment. Mean age: 40 years ($n = 48$)	Only IR as outcome
Grandone <i>et al.</i> 2014 (Italy) (6)	prospective comparative study, single center ($n = 1107$)	With: two or more failed IVF/ICSI cycles	Administer LMWH (enoxaparin or nadroparin at prophylaxis doses: 4000 IU, 3800 IU and respectively, subcutaneously, once per day by self-injection), aspirin or combined with LMWH ($n = 210$)	No treatment ($n = 21$)	Not RCT
Siristatidis <i>et al.</i> 2013 (Greece) (34)	Comparative study, single center ($n = 52$)	With: two or more failed IVF/ICSI women. Without: thrombophilia, hormonal immunologic disorders	LMWH at a dose of 1 mg/kg/day were initiated on the first day of injections until the pregnancy test. Age: 37.1 ± 4.9 years ($n = 19$)	No treatment. Age: 41.6 ± 3.8 years ($n = 18$)	Not RCT, Age different between groups.
Lodigiani <i>et al.</i> 2011 (Israel) (9)	Retrospective observational analysis, single center ($n = 569$)	With: two or more failed implantation. Without: both acquired and inherited thrombophilia, two or more failed implantations	LMWH was administered at a prophylactic dosage (i.e. enoxaparin 40 mg daily or nadroparin 80/100 IU/kg or dalteparin 80/100 IU/kg once daily), starting the day before COH until the day of β -human chorionic gonadotropin testing ($n = 512$)	No treatment ($n = 57$)	Not RCT
Qublan <i>et al.</i> 2008 (Jordan) (7)	RCT, single center ($n = 83$)	With: three or more failed IVF/ICSI women, thrombophilia	Enoxaparin 40 mg/day subcutaneous injections. Age: 29 ± 6.3 years ($n = 42$)	No treatment. Age: 29.2 ± 6.1 years ($n = 41$)	With thrombophilia

COH, controlled ovarian hyperstimulation; DHEA, dehydroepiandrosterone; ET, embryo transfer; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IR, implantation rate; IVF, in vitro fertilization; LMWH, low-molecular-weight heparin; OPU, ovum pick-up; RCT, randomized clinical trials.

according to the randomized trials score after quality assessment. The other two studies were either at medium (17) or high (8) risk after quality assessment. These two publications either did not strictly follow the RCT blinding rule or did not report sufficient information for the assessment. In total, the included studies were assessed with a relatively moderate bias.

Funnel plot for all the analyses suggested no evidence of publication bias. The outcomes of pregnancy were considered to be objective and unlikely to be influenced by masking. All studies either had no funding (8) or clearly described the involvement of funding agencies and reported no conflicts of interest.

Primary outcome: live birth rate. Four studies reported LBR as an outcome (10,11,16,17). The total risk ratio using a fixed-effects model was 1.13 with 95% CI 0.88–1.43 ($p = 0.34$, Figure 3a). Three other studies showed relatively favorable results for LBR in women receiving LMWH treatment compared with placebo or no treatment control, and none of these studies showed significant differences. This meta-analysis showed no significant differences in LBR between LMWH-treated and control groups. Because the outcomes of pregnancy are closely related to number of IVF/ICSI cycles, women with failed IVF/ICSI cycles usually had poorer reproductive outcomes. We further divided the participants into subgroups

according to the number of IVF/ICSI cycles. The risk ratio results showed no significant difference in women with two or more failed IVF/ICSI cycles (RR 1.15; 95% CI 0.84–1.58, $p = 0.38$, Figure 3b). Meanwhile, pooled risk ratios in women with three or more failed IVF/ICSI cycles showed a relative improvement in LBR, but without significant difference (RR 1.36; 95% CI 0.82–2.26, $p = 0.24$, Figure 3c).

Secondary outcomes. Five studies reported CPR as an outcome. Two studies reported a significant increase in CPR (8,16). However, this meta-analysis found no significant differences in CPR (RR 1.08; 95% CI 0.88–1.32, $p = 0.47$, Figure 4a). Three studies compared CPR in women with different numbers of IVF/ICSI cycles. However, the risk ratio results showed no significant difference in women either with two or more failed IVF/ICSI cycles (RR 1.17; 95% CI 0.90–1.51, $p = 0.23$) or with three or more failed IVF/ICSI cycles (RR 1.35; 95% CI 0.93–1.96, $p = 0.12$, Figure 4b,c). The quality of evidence was assessed at low heterogeneity.

Three papers (10,11,16) reported the MR. This meta-analysis showed less of a trend for MR in the LMWH group, but showed no significant difference (RR 0.58; 95% CI 0.30–1.10, $p = 0.09$, Figure 5).

Age was another main factor that influenced the success rate of IVF/ICSI. Two studies reported women with different ages (8,10), and the participants were divided

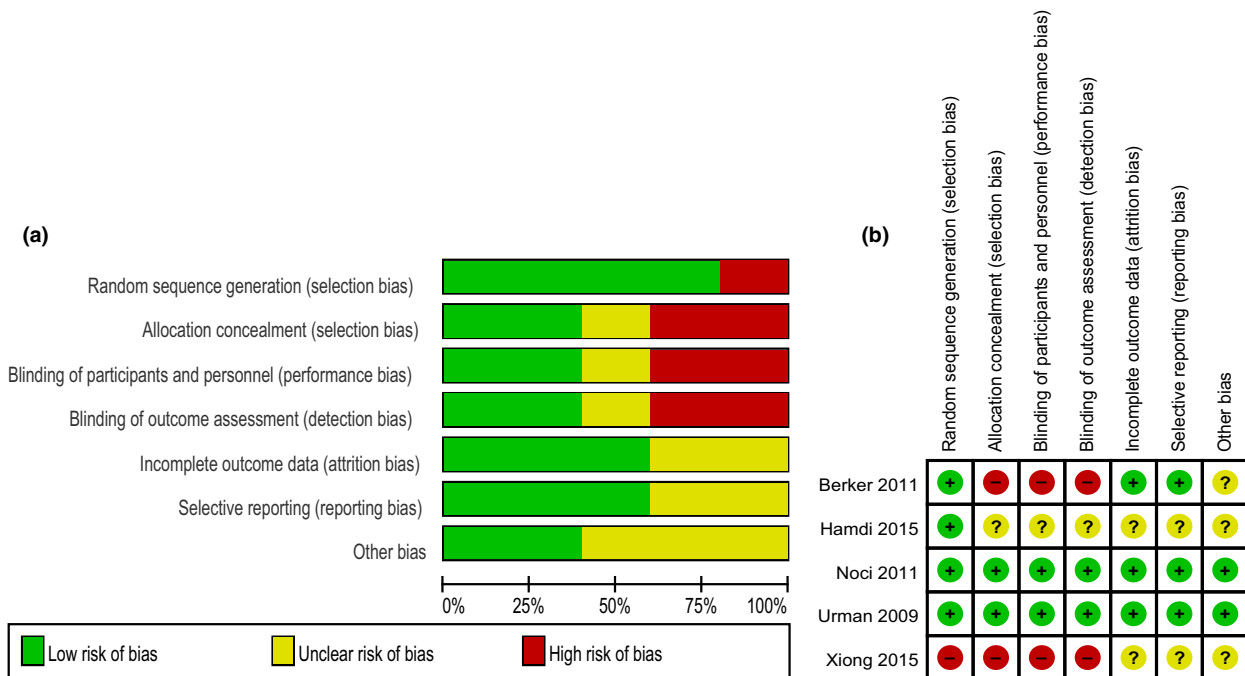


Figure 2. Summary of risk of bias of included studies. Risk of bias graph: review authors’ judgments about each risk of bias item presented as percentages across all included studies (a). Risk of bias summary: review authors’ judgments about each risk of bias item for each included study (b). [Color figure can be viewed at wileyonlinelibrary.com]

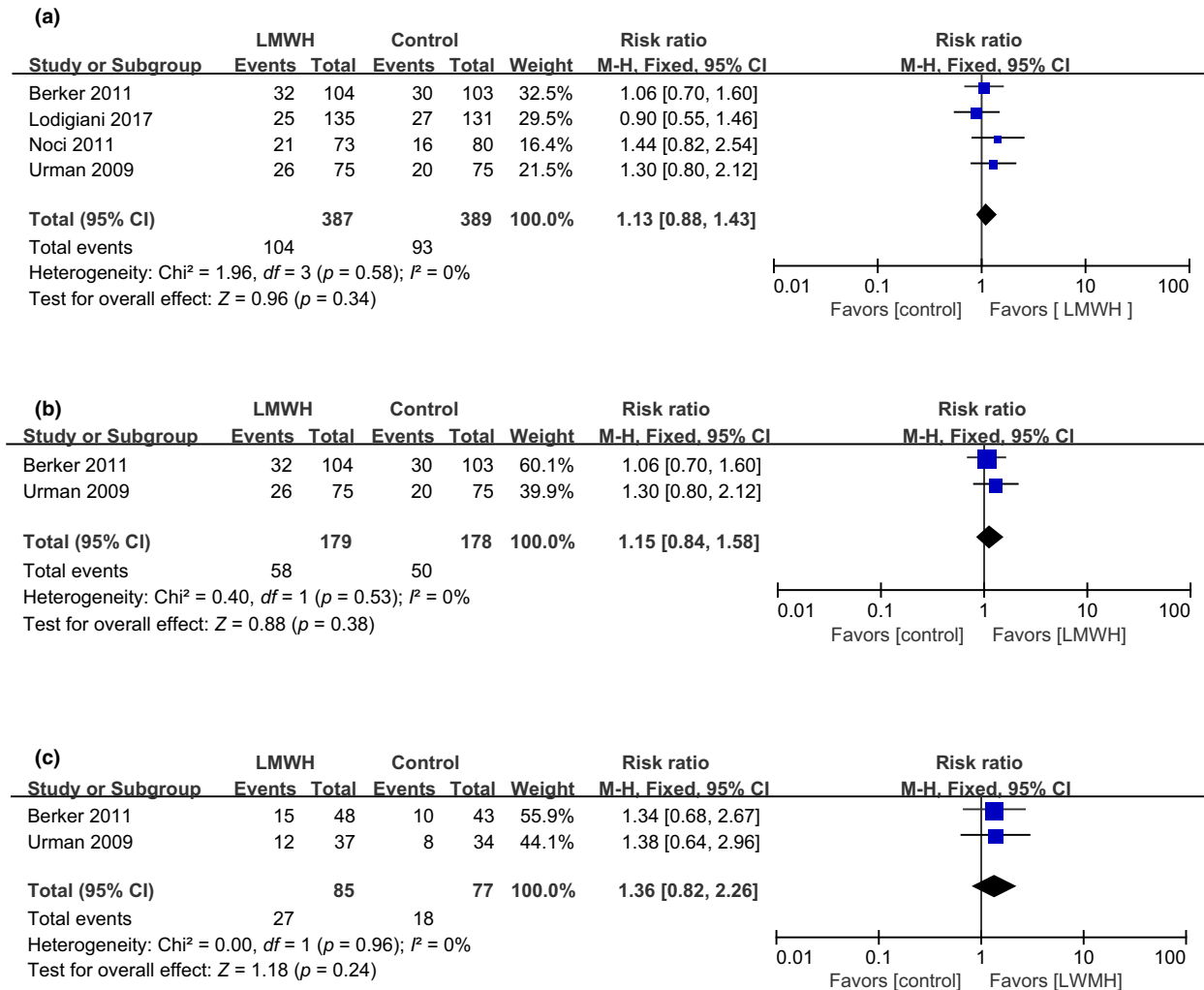


Figure 3. Forest plot of live birth rate (LBR) in women without thrombophilia treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) by fixed effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) of the association between low-molecular-weight heparin (LMWH) and control groups (a), in women with two or more failed IVF/ICSI cycles (b), and in women with three or more failed IVF/ICSI cycles (c). [Color figure can be viewed at wileyonlinelibrary.com]

into two subgroups according to age: ≤ 35 years, >35 years. Our result found no significant differences in the effect of LMWH for maternal age on the CPR. Three studies that reported LMWH were given for oocyte retrieval (11,16,17). We pooled the results, showing no significant differences between the groups (data not shown).

We also used a random-effects model for LBR, CPR and MR meta-analysis and got similar results (see Supporting Information, Figures S1–S3).

Side effects

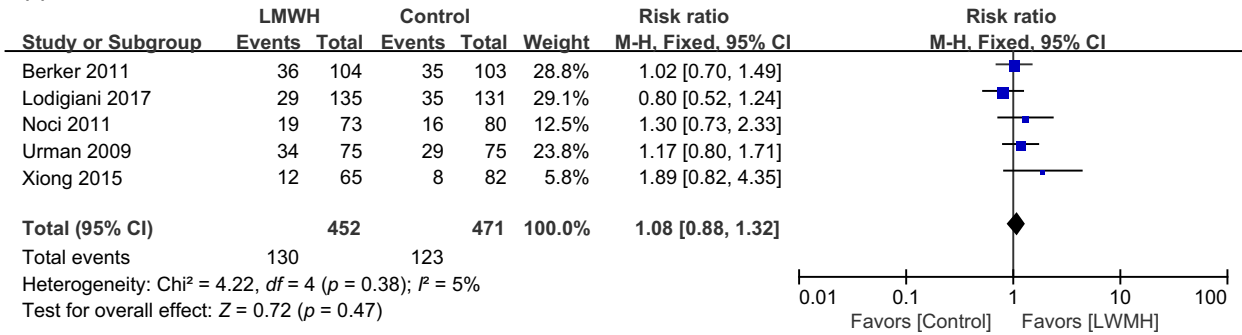
Three studies observed side effects of LMWH (8,10,11). In general, there were no serious side effects reported in

any study (8,10,11). One study reported five cases of vaginal bleeding or bloody discharge during the therapeutic procedure among the 62 women receiving LMWH treatment, but not serious enough to stop the use of LMWH (8). None of the studies reported developing preeclampsia during the gestational period. There were not enough data to pool the side effect data such as intrauterine growth restriction and preeclampsia outcomes.

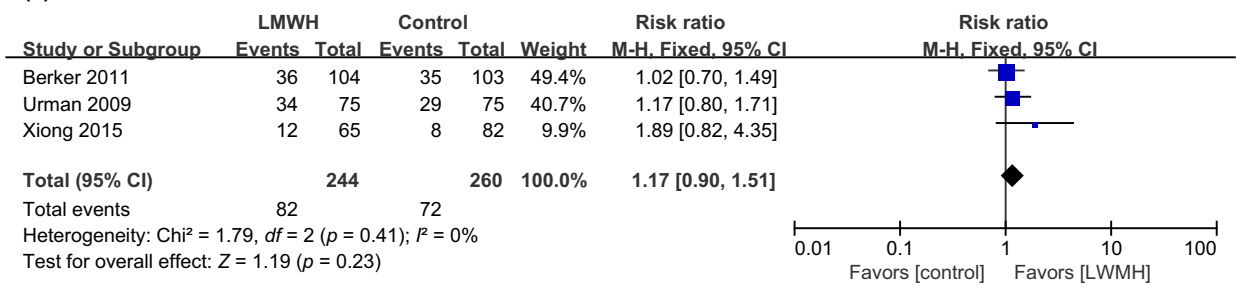
Discussion

The current systematic review summarizes the available evidence from RCTs or quasi-RCTs using LMWH as an adjuvant therapy in pregnant women undergoing IVF/

(a)



(b)



(c)

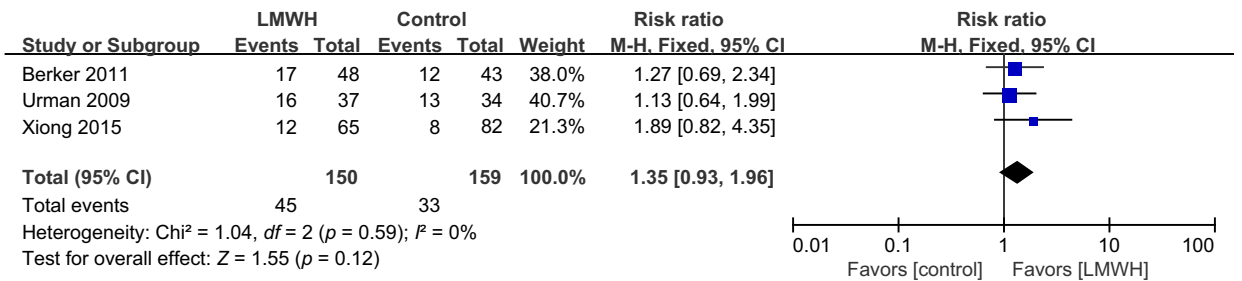


Figure 4. Forest plot of clinical pregnancy rate (CPR) in women treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) by fixed effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) of the association between low-molecular-weight heparin (LMWH) and control groups (a), in women with two or more failed IVF/ICSI cycles (b), and in women with three or more failed IVF/ICSI cycles (c). [Color figure can be viewed at wileyonlinelibrary.com]

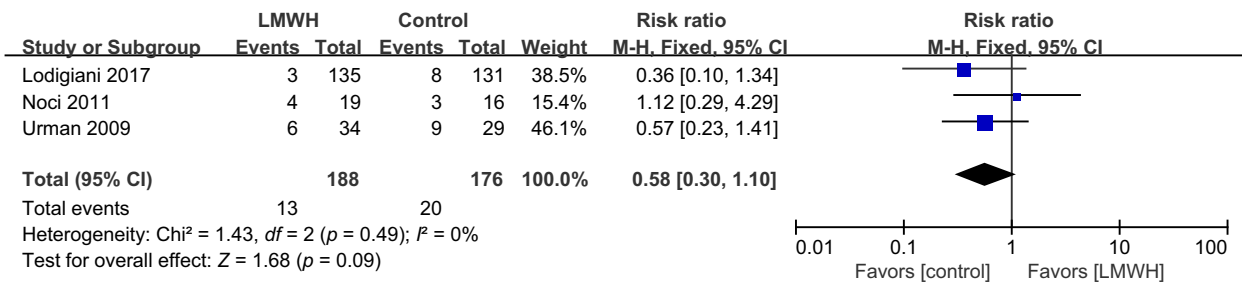


Figure 5. Forest plot of miscarriage rate (MR) of in women treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) women by fixed effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) of the association between low-molecular-weight heparin (LMWH) and control. [Color figure can be viewed at wileyonlinelibrary.com]

ICSI treatment, with the aim to evaluate pregnancy outcomes in non-thrombophilic women. This meta-analysis demonstrated no significant differences in LBR, CPR and MR between the LMWH-treated and control groups. In addition, no significant difference in the number of previous IVF cycles or different age groups was observed.

Compared with previous systematic reviews of LMWH on the outcomes of assisted reproduction, this meta-analysis had more updated data, with different inclusion and exclusion criteria. An extended and systematic search was conducted in the electronic databases and a broad syntax was used, which produced many papers in the search results.

The limitations of this study were that the evidence was assessed to be of moderate quality. Overall, the risk of bias was not sufficiently substantial affecting the results of these included studies. Another limitation of the included studies was lack of exact data on embryo transfer. As most of the RCTs focused on implantation rate, they only reported the percentage instead of the exact number of embryo transfers. This made the pooling and analysis of these data impossible.

The outcomes of IVF/ICSI pregnancies are associated with multifactorial pathophysiology including thrombophilia. Thrombophilia is a condition of the blood with a tendency to clot (20). Studies from animal models have shown that placental thrombosis was closely related to pregnancy loss (21). Reports suggested that it was also closely related to recurrent miscarriages (22–24). Hence, clinicians had begun using anticoagulant therapy, such as LMWH, to improve the pregnancy outcomes. Favorable results with LMWH led to a new question of whether this therapy could be applied to women undergoing IVF treatment for routine clinical use. Studies have shown that administration of aspirin has no positive effect on the implantation and pregnancy rates (25,26). Results of clinical trials suggested that neither aspirin alone nor aspirin combined with LMWH improved LBR compared with the placebo among women with unexplained recurrent miscarriages (27). In fact, evidence showed that this therapy may even increase the risk of prematurity (28). Studies also suggested that LMWH could not reduce the risk of recurrent placenta-mediated pregnancy complications (4). Hence, it is worth pooling the results of completed RCTs to explore the effect of LMWH in non-thrombophilic women on IVF/ICSI outcomes.

This paper was different from previous meta-analysis reports (3,22,29) that included thrombophilic women. Three previous meta-analyses (3,22,29) assessed the efficiency of LMWH in the outcomes of assisted reproduction. Seshadri *et al.* (3) included five RCT studies and five prospective comparative studies in their review. The treatment group was either heparin or heparin

plus aspirin, and thrombophilic participants were also included in the analysis. Seshadri *et al.* have concluded that the role of adjuvant heparin therapy showed no positive effect during IVF. In 2013, Potdar *et al.* (29) pooled two RCTs and one quasi-RCT (7,11,17), including women with thrombophilia (7). The pooled risk ratios in women with three or more failed IVF/ICSI cycles showed a significant improvement in the LBR (RR 1.79, $p = 0.02$) and a reduction in the MR (RR 0.22, $p = 0.02$) for the LMWH-treated group compared with the control group. This is because the participants pooled in this review had risk bias and with limited number, so the authors discouraged the routine use of LMWH as an adjunctive therapy in women with recurrent implantation failure. In the same year, Akhtar *et al.* (22) pooled the data of two RCT studies (11,16) and one study (7) with thrombophilic women receiving LMWH for assisted reproduction as a systematic review in the Cochrane database. In 2015, Akhtar *et al.* (22) summarized their previous work again but without a data update. These results showed no benefit of heparin on pregnancy outcomes. The authors concluded that the evidence does not justify the use of heparin in this context, except for the well-conducted research trials.

There are several reports on the side effects of LMWH administration (4,30). However, these reports also noted that the bleeding showed no differences between the treatment and control groups (4,30). One study (19) using intrauterine injection indicated that intrauterine injection of LMWH was considered a safe intervention without any beneficial effect.

Publication bias affects the results of systematic review. We used funnel plots to analyze for any potential bias. Funnel plots were symmetric, demonstrating no publication bias, with regard to live birth, pregnancy and miscarriage rates.

In conclusion, our results did not support the routine use of LMWH as an adjuvant therapy in non-thrombophilic IVF/ICSI-treated women in terms of pregnancy outcomes. However, there are many factors, such as differences at the time of administration, length of therapy and gestational age at discontinuation of LMWH *et c.*, that might affect the results. Moreover, the included studies were conducted in one center, potentially limiting their external validity. Therefore, these findings need to be further investigated with well-designed, adequately powered double-blinded, randomized, placebo-controlled, multicenter trials.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Forest plot of live birth rate (LBR) of women without thrombophilia treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) by random effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) of the association between low-molecular-weight heparin (LMWH) and control groups (a), in women with two or more failed IVF/ICSI cycles and (b), and in women with three or more failed IVF/ICSI cycles (c).

Figure S2. Forest plot of clinical pregnancy rate (CPR) of women treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) by random effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) of the association between low-molecular-weight heparin (LMWH) and control women (a), in women with two or more failed IVF/ICSI cycles (b), and in women with three or more failed IVF/ICSI cycles (c).

Figure S3. Forest plot of miscarriage rate (MR) of women treated with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) by random effects model analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIS) of the association between low-molecular-weight heparin (LMWH) and control.

Table S1. Supporting PRISMA checklist.

Table S2. Search strategy.