

Adjunct low-molecular-weight heparin to improve live birth rate after recurrent implantation failure: a systematic review and meta-analysis

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BACKGROUND: Poor fertility outcomes in women with recurrent implantation failure (\geq RIF) present significant challenges in assisted reproduction, and various adjuncts, including heparin, are used for potential improvement in pregnancy rates. We performed this systematic review and meta-analysis to evaluate the effect of low-molecular-weight heparin (LMWH) on live birth rates (LBRs) and implantation rates (IRs) in women with RIF and undergoing IVF.

METHODS: Studies comparing LMWH versus control/placebo in women with RIF were searched for on MEDLINE, EMBASE, Cochrane Library, conference proceedings and databases for registered and ongoing trials (1980–2012). Statistical analysis was performed using Review Manager 5.1. The main outcome measure was LBR per woman.

RESULTS: Two randomized controlled trials (RCTs) and one quasi-randomized trial met the inclusion criteria. One study included women with at least one thrombophilia (Qublan *et al.*, 2008) and two studies included women with unexplained RIF (Urman *et al.*, 2009; Berker *et al.*, 2011). Pooled risk ratios in women with ≥ 3 RIF ($N = 245$) showed a significant improvement in the LBR (risk ratio (RR) = 1.79, 95% confidence interval (CI) = 1.10–2.90, $P = 0.02$) and a reduction in the miscarriage rate (RR = 0.22, 95% CI = 0.06–0.78, $P = 0.02$) with LMWH compared with controls. The IR for ≥ 3 RIF ($N = 674$) showed a non-significant trend toward improvement (RR = 1.73, 95% CI 0.98–3.03, $P = 0.06$) with LMWH. However, the beneficial effect of LMWH was not significant when only studies with unexplained RIF were pooled.

The summary analysis for the numbers needed to be treated with LMWH showed that approximately eight women would require treatment to achieve one extra live birth.

CONCLUSIONS: In women with ≥ 3 RIF, the use of adjunct LMWH significantly improves LBR by 79% compared with the control group; however, this is to be considered with caution, since the overall number of participants in the studies was small. Further evidence from adequately powered multi-centered RCTs is required prior to recommending LMWH for routine clinical use. This review highlights the need for future basic science and clinical research in this important field.

Key words: heparin / low-molecular-weight heparin / recurrent implantation failure / IVF / thrombophilia

Introduction

Implantation is a complex signaling process between the embryo and the endometrium, which involves adhesion, nidation and invasion of the trophoblast within the endometrial tissues. The term recurrent implantation failure (RIF) has been used since 1983 to describe the failure of embryos to implant following IVF. There is no unanimous definition for RIF in terms of the number of failed cycles or the total number of transferred embryos that have not successfully implanted (Rinehart, 2007; Simon and Laufer, 2012). The ESHRE PGD consortium document (Thornhill *et al.*, 2005) mentioned that RIF can be considered after more than three high-quality embryo transfers (ETs) or implantation failure with transfer of ≥ 10 embryos in multiple transfers with exact numbers to be determined by each center. In order to improve pregnancy outcomes in women with RIF, various investigations and treatment adjuncts including heparin have been studied (Margalioth *et al.*, 2006; Nardo *et al.*, 2009).

Heparin is a polysulphated glycosaminoglycan that interacts with proteins containing positively charged amino acids. Low-molecular-weight heparin (LMWH) is derived from unfractionated heparin by depolymerization and has an activity similar to heparin but with increased bio-availability and half-life. The primary biological activity of heparin is anticoagulation or the antithrombin effect, where heparin catalyzes the inhibition of factor Xa and thrombin (Linhardt *et al.*, 1992). Heparin, alone or in conjunction with aspirin, has been used for treating women with recurrent miscarriage, diagnosed with antiphospholipid syndrome (APLS) (Cowchock *et al.*, 1992; Kutteh, 1996; Rai *et al.*, 1997). It has been postulated that the anticoagulation effect of heparin prevents placental thrombosis and infarction and promotes establishment and continuation of pregnancy in these women (Nelson and Greer, 2008). However, in recent years studies have shown that there is no evidence regarding the efficacy of heparin and low-dose aspirin in women with two or more consecutive previous pregnancy losses (Clark *et al.*, 2010), in those with unexplained recurrent miscarriage (Kaandorp *et al.*, 2010) or in women with inherited thrombophilic disorders (Tan *et al.*, 2012).

Similarly, there is controversial evidence to suggest an association between RIF and thrombophilia, both inherited and acquired (Sher *et al.*, 1998b; Grandone *et al.*, 2001; Stern *et al.*, 2003; Coulam *et al.*, 2006; Qublan *et al.*, 2006). Inherited disorders include factor V Leiden mutation, methylene tetrahydrofolate reductase (MTHFR) polymorphisms, prothrombin gene mutation, protein C deficiency, protein S deficiency and antithrombin deficiency; acquired defects include antiphospholipid antibodies (APA), which are lupus anticoagulant, anticardiolipin and anti- β_2 glycoprotein I. Kutteh (1998) showed that APA

are found more frequently in women undergoing IVF treatment (18.8%) compared with normal controls (5.5%). Similarly, other researchers reported positive APA in $\sim 60\%$ of women with infertility and endometriosis and undergoing IVF (Sher *et al.*, 1994). Furthermore, various investigators have shown that thrombophilia are more common in women with RIF compared with healthy fertile controls (Coulam *et al.*, 2006; Qublan *et al.*, 2006; Bellver *et al.*, 2008). This association is explained by thrombophilia causing microthrombosis at the implantation site and thereby impairing the initial invasion of maternal vessels by the syncytiotrophoblast, leading to implantation failure (Geva *et al.*, 1995; Grandone *et al.*, 2001; Azem *et al.*, 2004). In these women, heparin treatment can potentially enhance the implantation process. However, observational and randomized controlled trials (RCTs) using heparin as an adjunct to IVF treatment have shown conflicting evidence for improved fertility outcomes in women with thrombophilia with or without RIF. Six studies for acquired thrombophilia have been conducted (Sher *et al.*, 1994, 1996, 1998a; Kutteh *et al.*, 1997; Stern *et al.*, 2003; Qublan *et al.*, 2008). Three studies showed that unfractionated heparin and low-dose aspirin do not improve pregnancy rates (Schenk *et al.*, 1996; Kutteh *et al.*, 1997; Stern *et al.*, 2003), whereas three other studies showed a significant difference in pregnancy rates in women with thrombophilia and having heparin treatment with or without low-dose aspirin compared with untreated controls (Sher *et al.*, 1994, 1998a; Qublan *et al.*, 2008).

Recently, heparin has been shown to be effective in improving implantation rates (IRs) without the presence of thrombophilia (Urman *et al.*, 2009). There is emerging evidence that heparin modulates endometrial receptivity and decidualization of endometrial stromal cells and improves implantation. Fluhr *et al.* (2010) have shown that heparin increases the production of prolactin and insulin-like growth factor (IGF-1) and inhibits the production of insulin-like growth-factor-binding protein (IGFBP-1). The expression of these proteins plays an important role in endometrial development and receptivity during the 'implantation window' (Wilcox *et al.*, 1999; Fluhr *et al.*, 2007, 2008). Additionally, heparin regulates heparin-binding epidermal growth factor (EGF), which is expressed maximally at the time of implantation, thus enhancing implantation, trophoblast invasion and promoting the early stages of embryo development (Tamada *et al.*, 1999; Constancia *et al.*, 2002; Stevenson *et al.*, 2005). In animal models, heparin has been demonstrated to act on adhesion molecules like the E-cadherin system to regulate implantation (Erden *et al.*, 2006). It reduces the expression of E-cadherin and promotes trophoblast invasion and proliferation into the endometrial cells. Moreover, in the presence of APLS, apart from its antithrombin effect, LMWH prevents APA binding to the trophoblast cells and restores trophoblast

invasiveness and differentiation (Di Simone et al., 1999). Heparin has also been shown to block complement activation and modulates inflammatory responses in women with APA (Girardi et al., 2004).

The aforementioned evidence suggests that there could be a potential role of heparin in improving implantation by enhancing endometrial receptivity and decidualization with or without the antithrombin effect. Moreover, in women with unexplained RIF, where in the absence of any anatomical, endocrine, immunological or genetic abnormality there is recurrent failed implantation, suboptimal endometrial receptivity is known to be the key factor that adversely affects implantation (Altmann et al., 2010; Garrido-Gomez et al., 2013). Again, heparin might have a role in improving or enhancing endometrial receptivity in this cohort of patients.

In light of the challenges faced in overcoming RIF, heparin is being used as an adjunct to IVF treatment in women with RIF. In the absence of any potential cause for RIF (anatomical, endocrine, immunological or genetic abnormality), empirical treatment becomes an anchor of hope for a successful pregnancy outcome. This systematic review and meta-analysis aims to evaluate the effect of LMWH on live birth rates (LBRs) and IRs in women with RIF and undergoing IVF.

Materials and Methods

Literature search

Online searches of databases were performed in MEDLINE (January 1980–December 2012), EMBASE (January 1980–December 2012) and the Cochrane Library. The searches also included Conference Proceedings Citation Index and databases for registered and ongoing trials. A combination of Medical Subject Headings and words were used to generate a subset of citations for heparin ('heparin', 'low molecular weight heparin', 'unfractionated heparin', 'heparin' and 'thrombo*'); citations including thrombophilia ('thrombophilia', 'antiphospholipid syndrome', 'heparin' and 'antiphospholipid*'); citations including RIF ('recurrent implantation failure', 'implantation failure', and 'failed cycle'); and citations including outcomes after IVF and ICSI ('outcome', 'IVF', 'in vitro fertilisation', 'intracytoplasmic sperm injection', 'ICSI', and 'assisted reproductive techniques'). These subsets were combined using 'AND' to generate final citations addressing the research question. The reference list of all published articles including review articles were examined to identify articles not noted by the electronic search of the databases. No language restrictions were placed on the searches, for all non-English articles of the relevant studies. The authors were contacted to obtain further information, as appropriate.

Study eligibility criteria

We included randomized controlled, quasi-randomized and prospective studies that compared the use of LMWH (intervention) with placebo or no adjuvant treatment in women with RIF undergoing IVF/ICSI. RIF was defined as ≥ 3 failed ET cycles. In the included studies, the intervention (LMWH) was commenced after oocyte retrieval (OR) or from the day of ET and continued until the day of the pregnancy test. In the presence of a positive pregnancy test, the intervention continued up to 12 weeks of pregnancy or beyond. Reasons for excluding studies were retrospective study design, use of intervention in non-RIF study population, RIF considered as more than one failure or use of a different intervention such as unfractionated heparin with or without aspirin and immunoglobulins (Table II). Two authors (N.P. and T.A.G.) independently performed the study selection and data extraction; all articles including abstracts from the electronic searches were assessed and citations that met the initial pre-defined selection

criteria were obtained. Trial quality assessment and final inclusion–exclusion decisions were made after examination of full manuscripts. After independent assessment of the manuscripts, any disagreement between the two reviewers was resolved by consultation with the third reviewer (L.G.N.).

Data extraction

The selected studies were assessed for the methodological quality using the domain-based risk for bias assessment tool recommended by the Cochrane Collaboration (Higgins et al., 2011). Information was sought on the method of randomization, blinding, allocation concealment, blinding of outcome assessment, data collection and selective reporting. For each study, information was obtained on the participants (number of previous failed IVF/ICSI cycles, ovarian response in the previous failed cycle and investigations for RIF), intervention used (LMWH) and timing of intervention in relation to treatment cycle. Where there was doubt or lack of information, authors were contacted for further details.

Outcome measures

The primary outcome measure was LBR per woman. Secondary outcome measures were IR, clinical pregnancy rate (CPR), miscarriage rate and multiple pregnancy rate (MPR). Other reported observations were drug (LMWH) related side effects (bruising and thrombocytopenia) and congenital abnormalities at birth. IR was defined as the number of sacs seen divided by the number of embryos transferred; CPR was defined as gestational sac and fetal heart activity seen per woman on transvaginal ultrasound scan after 6 weeks of gestation and MPR was defined as the number of multiple pregnancies divided by the total number of clinical pregnancies. Miscarriage was defined as the loss of pregnancy after identification of clinical pregnancy per woman.

Search results

The studies were selected and reported according to the PRISMA guidelines (Moher et al., 2009). Of 602 citations identified, 24 were selected for detailed evaluation and finally just two RCTs and one quasi-randomized study were included in the meta-analysis (Fig. 1).

Although, three studies met the pre-defined criteria, there were differences in defining RIF; Urman et al. (2009) and Berker et al. (2011) defined RIF with two consecutive failed IVF/ICSI cycles and performed further subgroup analysis for those with ≥ 3 failed cycles, whereas Qublan et al. (2008) defined RIF as three consecutive failed IVF/ICSI (Table I). Similarly, there was difference in the study population, i.e. those with at least one thrombophilia (Qublan et al., 2008) and those with unexplained RIF (Urman et al., 2009; Berker et al., 2011). In view of the heterogeneity in study populations (defining RIF and inclusion of women with or without thrombophilia), analysis was performed for ≥ 3 RIF and sensitivity analyses was done for studies with unexplained RIF only, after excluding the quasi-randomized study and in women with ≥ 2 RIF.

Statistical analysis

Study features and outcomes were assembled in a tabular form, and formal meta-analysis was performed using Review Manager 5.1 Software (Review Manager, 2011). A fixed effect model (using a Mantel–Haenszel method) was used and where the I^2 statistic showed heterogeneity of $>50\%$, a random effect model was applied. The effect estimate was expressed as a pooled risk ratio (RR) with 95% confidence interval (CI) and was represented graphically by forest plots. Statistical heterogeneity was examined using the χ^2 test and a P value of <0.05 was suggestive of heterogeneity. Clinical heterogeneity was examined by assessing the participants, intervention used, study quality and outcome measures. Further sensitivity analysis was performed to investigate the clinical and methodological variations in the

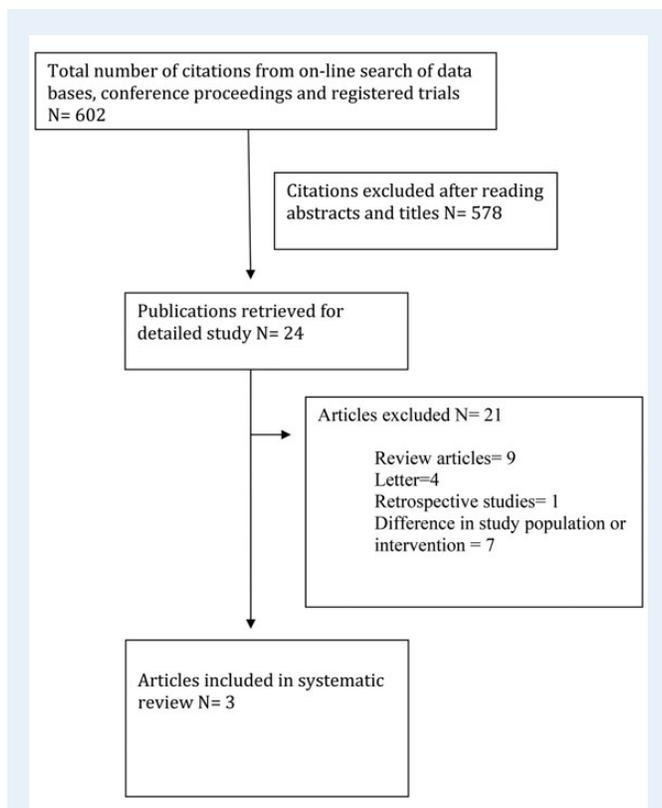


Figure 1 PRISMA flow chart of selected studies for the systematic review.

studies. Publication bias was assessed visually using funnel plot and by formal testing.

Results

The process of the literature searches and selection of studies for the quantitative meta-analysis is shown in Fig. 1. After the initial screening 21 publications were excluded, of which 8 were original studies as shown in Table II. There were no prospective studies identified that met the inclusion criteria. Two randomized studies and 1 quasi-randomized study were selected for the meta-analysis and included 243 women with RIF who underwent IVF/ICSI, 127 in the intervention group and 116 in the control/placebo group (Table I).

Study quality assessment and publication bias

Studies included in the meta-analysis were 2003 onwards since the earlier studies did not meet the selection criteria. The two RCTs (Qublan *et al.*, 2008; Urman *et al.*, 2009) were at low risk of bias for method of randomization, allocation concealment, attrition bias and selective reporting (Fig. 2). In the study by Qublan *et al.*, patients were blinded to the treatment arm, whereas in the study by Urman *et al.*, the control group did not receive placebo; therefore, were not blinded to the treatment received. In contrast, the quasi-randomized study (Berker *et al.*, 2011) had high risk of bias for method of randomization and blinding, unclear risk for allocation concealment and low risk of bias for attrition and selective reporting. All three studies were unclear for detection bias and none of the studies explicitly stated whether the

individuals assessing the outcome were blinded to the trial or not. However, assessment for pregnancy outcome is unlikely to be subjective since implantation, clinical pregnancy, multiple pregnancies and miscarriage are all objectively assessed on ultrasound scan. In view of the high risk of bias for the study by Berker *et al.* (2011) and its potential impact on the results, we performed a sensitivity analyses after excluding the quasi-randomized study. Publication bias for the outcome of LBR for the three included studies showed a symmetrical funnel plot (Supplementary data, Fig. S1).

Included studies

Baseline characteristics of the study population and controlled ovarian hyperstimulation (COH) regime in the three studies were almost similar and progesterone for luteal phase support was used in the intervention and control group in all three studies.

Berker *et al.* (2011) performed a quasi-randomized study in women with RIF, defined by failure of two consecutive ICSI ET cycles, from June 2007 to October 2009. They provided subgroup analysis for women with ≥ 3 RIF. Patients were assigned consecutively to one of the three clinicians in the clinic to plan their treatment protocol. The study group consisted of the 110 consecutive RIF patients seen by clinician A who used LMWH empirically, whereas the control group consisted of 109 consecutive patients seen by clinicians B and C during the same time period who did not use LMWH empirically (quasi-randomization). For women with ≥ 3 RIF, the intervention and control groups were $n = 48$ and $n = 43$, respectively. The mean age of patients was 31 ± 5 years. All patients were screened for normal uterine cavity by hysteroscopy or hysterosalpingography and for coagulation disorders including mutations of factor V Leiden, prothrombin gene, MTHFR gene and abnormal levels of anticardiolipin immunoglobulin G, immunoglobulin M, lupus anticoagulant, antithrombin, protein C and protein S deficiency. The exclusion criteria were any of the above coagulation disorders, hydrosalpinx, polyps, myoma, lack of Grade I and II embryo for transfer, or no available sperm or oocyte.

Each woman was recruited for one cycle, sperm were fresh from ejaculation or testicular extraction. COH was done using recombinant FSH (r-FSH) with GnRH agonist or antagonist. Day three ET was performed with a maximum of three embryos in all but four patients, where four embryos were transferred. Luteal phase support was provided by vaginal micronized progesterone 200 mg three times a day until 12 weeks of gestation. Cases had LMWH (enoxaparin sodium, Clexane; Aventis Intercontinental, France) administered subcutaneously in a standard dose of 40 mg/0.4 ml per day from the day of OR. In contrast, the control arm did not receive any treatment. For higher order pregnancies, fetal reduction was offered between 11 and 13th weeks of gestation.

Qublan *et al.* (2008) conducted a prospective placebo controlled randomized trial in women with RIF and thrombophilia and who underwent ICSI, from October 2004 to March 2006. Randomization was done on the day of ET and the inclusion criteria were ≥ 3 previous IVF failures with at least one thrombophilic defect, age 19–35 years, basal FSH of ≤ 10 IU/L, body mass index (BMI) between 19 and 29 kg/m², presence of both ovaries, good quality embryos for transfer. Patients with polycystic ovary syndrome, endometriosis, hydrosalpinx, chronic medical condition, abnormal uterine cavity and those with any contraindication for use of heparin were excluded. All patients were screened for both inherited and acquired thrombophilia and had at least one defect. These

Table 1 Characteristics of included studies in the review.

Included studies					
Study	Design	Participants	Intervention	Control	Outcomes
Berker et al. (2011)	Quasi-RCT	2 consecutive failed cycles of ICSI-ET (defined as RIF) without coagulation defect. Mean age of 31 ± 5 years. Screened negative for anatomical, endocrine, coagulation and immunological causes. 110 cases and 109 control group, ICSI, Day 3 transfer and three embryos transferred. Subgroup analysis for women with ≥3 RIF, 48 cases and 43 controls	LMWH 40 mg/day SC from the day of OR, if positive pregnancy test continued until 12 weeks of gestation	No treatment	LBR, CPR, IR, MPR, miscarriage
Qublan et al. (2008)	RCT	3 consecutive failed IVF/ICSI with at least one coagulation defect. Age 19–35 years, basal FSH ≤ 10, BMI 19–29 kg/m ² . Screened negative for anatomical, endocrine and immunological causes. 42 cases and 41 control group, ICSI, Day 3 transfer and best one to three embryos transferred	LMWH 40 mg/day SC from the day of ET until delivery or fetal demise	Placebo (0.9% NaCl SC)	LBR, CPR, IR, MPR, miscarriage, drug side effects, fetal complications
Urman et al. (2009)	RCT	2 consecutive failed fresh cycles of ICSI-ET (defined as RIF) without coagulation defects. Age ≤38 years, screened negative for anatomical, endocrine, coagulation, immunological and genetic causes. 75 patients in each arm, ICSI, Day 3 transfer, maximum of four embryos transferred. Subgroup analysis for women with ≥3 RIF, 37 cases and 34 controls	LMWH 1 mg/kg/day SC from the day after OR, if positive pregnancy test continued until 12 weeks of gestation	No treatment	LBR, CPR, IR, MPR, miscarriage, drug side effects, congenital anomalies

CPR, clinical pregnancy rate; ICSI-ET, intracytoplasmic sperm injection-embryo transfer; IR, implantation rate; LBR, live birth rate; MPR, multiple pregnancy rate; RCT, randomised control trial.

included MTHFR C677T gene mutation, factor V Leiden mutation, G20210A mutation, Protein S, Protein C and antithrombin deficiency, homocysteine, anticardiolipin antibodies and lupus anticoagulant. Overall, the study population had a third of patients with an inherited disorder, a third with an acquired disorder and the remainder had combination of inherited and acquired thrombophilia. Amongst the patients with inherited thrombophilia, ~30% had MTHFR C677T mutation. There was no significant difference in the occurrence of various thrombophilia in the two study arms. There were 42 women in the intervention and 41 in the control arm.

COH was achieved using long down-regulation protocol with GnRH agonist and subsequent ovarian stimulation with human menopausal gonadotrophin. One to three best quality embryos were transferred on Day 3. Patients were randomized into treatment (enoxaparin 40 mg/day SC) and placebo group (equivalent volume of sodium chloride 0.9% SC). Treatment was started from the day of ET and if β-hCG was >25 IU/ml, medications were continued either until delivery or fetal demise was diagnosed. Progesterone pessaries were used for luteal phase support in both groups. In the LMWH group, 7% of subjects reported bleeding, 4.8% thrombocytopenia, 2.4% allergic reactions and 2.4% placental abruption. No fetal complications were noted.

Urman et al. (2009) performed an open-labeled RCT for women with RIF (January 2006–May 2008). The inclusion criteria were history of at least two previously failed fresh ET cycles, age ≤38 years, fresh ejaculate sperm for ICSI, no hormonal, coagulation (inherited or acquired thrombophilia) or immunological problems, normal uterine cavity by hysteroscopy or saline infusion sonography and normal peripheral karyotype for

both partners. Exclusion criteria included the presence of hydrosalpinx, fibroids distorting the uterine cavity, absence of Grade I or II embryos for transfer and requirement of anticoagulant therapy for other medical reasons. Male factor infertility was present in 44% and endometriosis was present in 3% of study group. Subgroup analysis for women with ≥3 RIF was clearly presented, with 37 women in the intervention and 34 in the control group.

Computer-generated randomization was done after OR and the physicians performing the ET were blinded to the treatment arm allocation. COH was undertaken with long GnRH antagonist protocol and r-FSH. Day three ET were done with a maximum of four embryos. Luteal phase support included 90 mg vaginal progesterone gel from the day of OR, and continued until 12 weeks of gestation if there was a positive pregnancy test.

The study group received LMWH (enoxaparin sodium) at a dose of 1 mg/kg/day SC starting from the day after OR and no treatment was given to the control group. Platelet counts were done on the day of OR and a week after starting LMWH. There were no significant changes in the platelet count and only small ecchymosis around the site of LMWH injection were reported and none of the patients discontinued treatment. Women with high-order pregnancies were offered fetal reduction in the 13th gestational week. None of the babies had congenital malformations noted at birth.

Meta-analysis

Primary outcome measures

LBR: the fixed effect forest plot for all three RCTs in women with ≥3 RIF (N = 245) showed significant improvement in LBR with LMWH

Table II Characteristics of excluded studies in the review.

Excluded studies					
Study	Design	Participants	Intervention	Control	Exclusion criteria
Kutteh <i>et al.</i> (1997)	Non-randomized	36 APA-positive women undergoing first cycle IVF	Unfractionated heparin and aspirin from OR to 13 weeks of gestation	No treatment	Not RIF
Lodigiani <i>et al.</i> (2011)	Retrospective	At least two failed IVF/ICSI, age ≤ 40 years, screened for thrombophilia	LMWH (enoxaparin) 40 mg daily or nadropin 80/100 IU/kg or dalteparin 80/100 IU/kg once daily. From the day before OR until pregnancy test		Retrospective study
Noci <i>et al.</i> (2011)	RCT	First cycle IVF/ICSI, 172 women, aged < 40 years, thrombophilia excluded. Luteal phase progesterone support given	LMWH (dalteparin sodium) 2500 IU SC daily from the day of OR to 9 weeks of gestation	No treatment	Not RIF
Perminova <i>et al.</i> (2010)	Non-randomized (abstract)	1–5 previous IVF failures, at least one acquired or inherited thrombophilia	LMWH 2850 IU, from Day 1 of COH to 12 weeks of gestation	No treatment	1–5 failures
Schenk <i>et al.</i> (1996)	Non-randomized (abstract only)	APA-positive women	Unfractionated heparin 5000 IU BD and low-dose aspirin from the day of OR to 12 weeks of gestation	No treatment in the APA seronegative women	Not RIF
Sher <i>et al.</i> (1994)	Non-randomized	Women seropositive or negative for APA	Unfractionated heparin 5000 IU BD and aspirin from Day 2 of COH to 34 weeks of gestation	No treatment	Not RIF
Sher <i>et al.</i> (1998a)	Parallel non-RCT	Women seropositive for APA, with fewer than two IVF attempts and those with RIF	Unfractionated heparin 5000 IU BD, aspirin \pm immunoglobulin from Day 2 of COH until 10 weeks of gestation	No treatment	Primarily for different types of APA, for those with two failed IVF, immunoglobulin was added with heparin and aspirin as intervention
Stern <i>et al.</i> (2003)	RCT	≥ 10 failed ETs with at least one APA- or ANA-positive antibody	Unfractionated heparin and aspirin from the day of ET until 14 weeks of gestation if β -hCG was ≥ 100 IU on Day 17 after ET	Placebo (0.9% NaCl SC and sucrose p.o.)	The use of unfractionated heparin and aspirin as intervention, crossover design after randomization

*APA, antiphospholipid antibodies; COH, controlled ovarian hyperstimulation; ICSI, intracytoplasmic sperm injection; RIF, recurrent implantation failure; RCT, randomised control trials.

[$n/N = 37/127$ (29%) intervention group versus $n/N = 19/118$ (16%) control group; RR = 1.79, 95% CI = 1.10–2.90, $P = 0.02$, $I^2 = 48\%$] (Fig. 3). Sensitivity analyses for women with ≥ 3 unexplained RIF (Urman *et al.*, 2009; Berker *et al.*, 2011) (Fig. 4), a trend toward improvement in LBR with LMWH but it was not significant [$n/N = 27/85$ (32%) intervention group versus $n/N = 18/77$ (23%) control group; RR = 1.36, 95% CI = 0.82–2.26, $P = 0.24$, $I^2 = 0\%$]. Supplementary data, Fig. S1 shows the funnel plot for publication bias for the LBR outcome.

Secondary outcome measures

IR: all three studies reported the IR (Qublan *et al.*, 2008; Urman *et al.*, 2009; Berker *et al.*, 2011). The random effects model for women with ≥ 3 RIF ($N = 674$) showed a non-significant trend toward improved IR in the intervention group with LMWH [$n/N = 75/344$ (22%) intervention group versus $n/N = 42/330$ (13%) control group; RR = 1.73, 95% CI 0.98–3.03, $P = 0.06$, $I^2 = 60\%$] (Qublan *et al.*, 2008; Urman *et al.*, 2009; Berker *et al.*, 2011) (Fig. 5). Subgroup analysis showed no statistically significant difference in the IR for women with unexplained RIF.

CPR: fixed effects for the three included studies ($N = 245$) showed no difference in CPR in the LMWH and control groups [$n/N = 46/127$ (36%) intervention group versus $n/N = 29/118$ (25%) control group;

RR = 1.46, 95% CI 0.99–2.15, $P = 0.05$, $I^2 = 37\%$]. Sensitivity analysis for those with ≥ 2 RIF and those with ≥ 3 unexplained RIF showed similar results (RR = 1.09, 95% CI 0.83–1.42; RR = 1.20, 95% CI 0.79–1.82, respectively).

Miscarriage rate: all three studies reported miscarriage rates and for women with ≥ 3 RIF ($N = 75$) (Qublan *et al.*, 2008; Urman *et al.*, 2009; Berker *et al.*, 2011), there was statistically significant reduction in the miscarriage rate for the intervention group [$n/N = 3/46$ (7%) intervention group versus $n/N = 8/29$ (28%) control group; RR = 0.22, 95% CI 0.06–0.78, $P = 0.02$, $I^2 = 0\%$] (Fig. 6). Subgroup analysis for women with unexplained RIF showed non-significant trend in the reduction of miscarriage rate (Supplementary data, Fig. S2).

MPR: all three RCTs reported the MPR (multiple births = 21, clinical pregnancies = 75) (Qublan *et al.*, 2008; Urman *et al.*, 2009; Berker *et al.*, 2011). Meta-analysis showed no significant difference in the MPR for the intervention and the control groups [$n/N = 13/46$ (28%) intervention group versus $n/N = 8/29$ (28%) control group; RR = 1.07, 95% CI 0.51–2.27, $P = 0.85$, $I^2 = 0\%$]. Subgroup analysis for women with ≥ 2 RIF and those with ≥ 3 unexplained RIF showed no significant difference (RR = 0.99, 95% CI 0.65–1.52; RR = 1.10, 95% CI 0.49–2.48, respectively) (Supplementary data, Fig. S3).

Other reported findings

Side effects of LMWH use: small ecchymoses were noted in one study (Urman et al., 2009); bleeding (7.1%), thrombocytopenia (4.8%) and allergic reactions (2.4%) were noted in another study (Qublan

et al., 2008). These side effects were comparable in both intervention and control groups.

Congenital anomalies: no congenital anomalies were reported at birth for babies in either the intervention or control group (Urman et al., 2009).

Discussion

The above meta-analysis shows that in women with ≥ 3 RIF ($N = 245$), the use of LMWH adjunct to IVF/ICSI treatment resulted in 79% improvement in the LBR; however, there was only a non-significant trend toward improved pooled RRs for the IR. For the miscarriage rates, there was a significant reduction in the intervention group compared with the controls. Furthermore, using the pooled results for LBR, the numbers needed to treat with LMWH would be 7.7 to achieve one live birth.

In this review, two RCTs and one quasi-randomized trial were included. The RCT by Qublan et al. (2008) was well designed and aimed at studying the effect of LMWH in women with RIF and thrombophilia. They included women with ≥ 3 failed IVF/ICSI, checked for both inherited and acquired thrombophilia, ensured there were no other associated factors for RIF, and used only LMWH as an intervention. Nevertheless, there are certain shortcomings in the study which have been noted by other authors too (Ricci et al., 2010). Qublan et al. included women with both inherited and acquired thrombophilia. Acquired thrombophilia like APA are associated with recurrent miscarriage and possibly RIF compared with certain heterozygous inherited thrombophilia. In this study, a third of the patients were carriers of MTHFR C677T polymorphism, and there is no conclusive evidence in the literature regarding the adverse effect of this heterogeneous mutation on IVF or pregnancy outcomes (Rey et al., 2003; Dobson et al.,

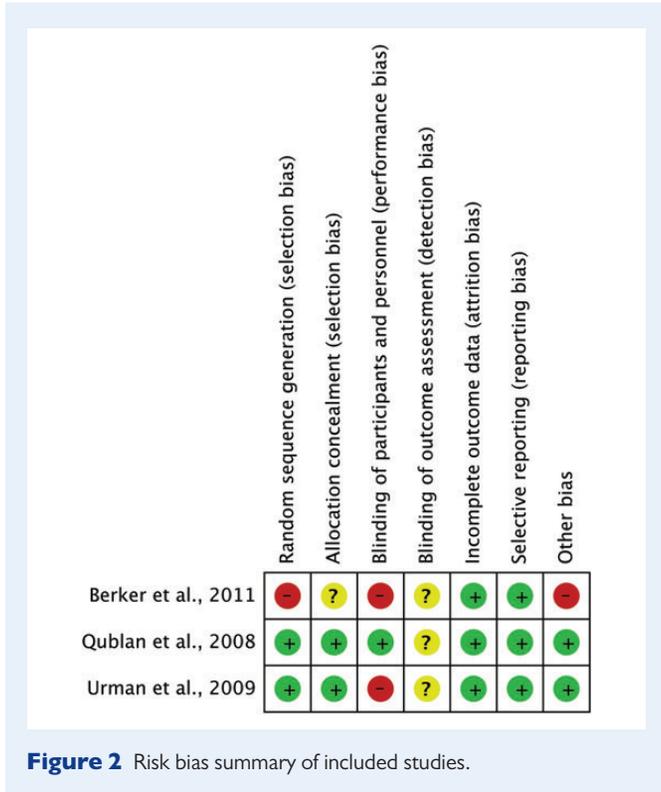


Figure 2 Risk bias summary of included studies.

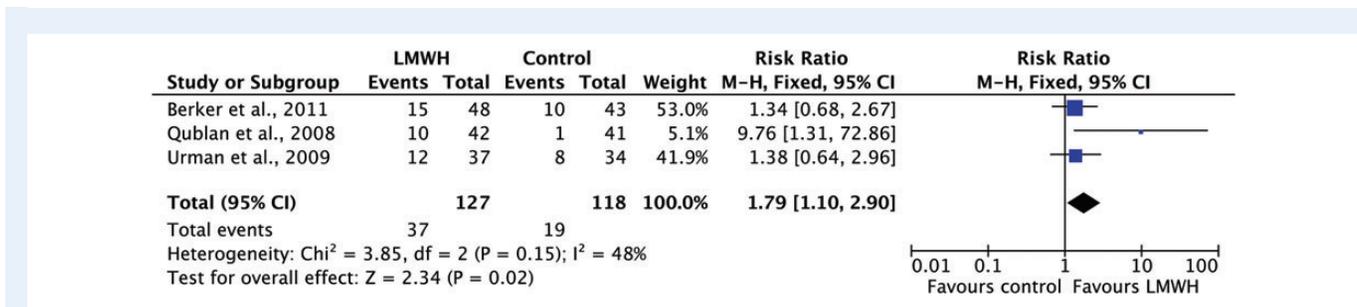


Figure 3 Live birth rate (LBR) in women with ≥ 3 recurrent implantation failure and LMWH as treatment adjunct.

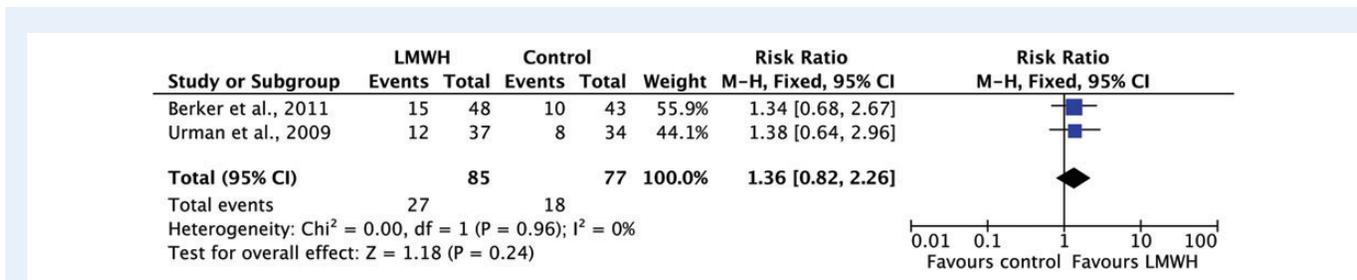


Figure 4 Live birth rate(LBR) in women with ≥ 3 unexplained recurrent implantation failure and LMWH as treatment adjunct.

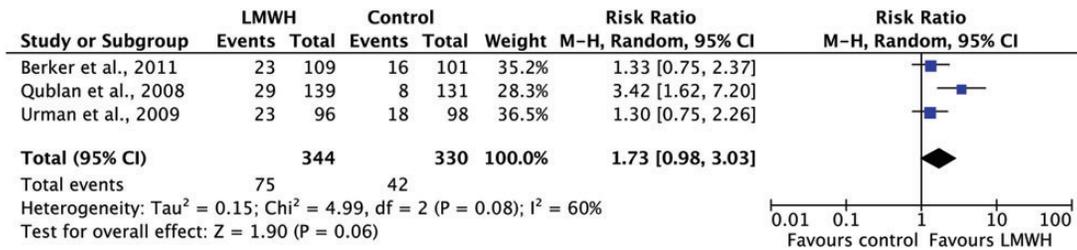


Figure 5 Implantation rate (IR) in women with ≥ 3 recurrent implantation failure and LMWH as treatment adjunct.

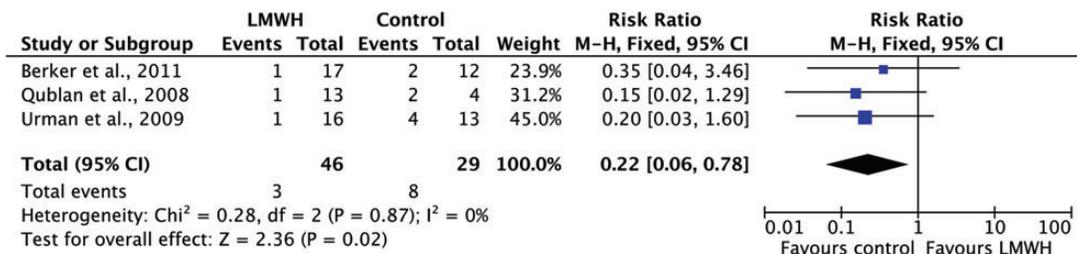


Figure 6 Miscarriage rate in women with ≥ 3 recurrent implantation failure and LMWH as treatment adjunct.

2007). Recent studies have shown that the C677T mutation does not have significant association with recurrent miscarriage, compared with the G1793A and A1298C mutations which are significantly associated with recurrent miscarriage (Seremak-Mrozikiewicz *et al.*, 2010; Klai *et al.*, 2011; Nair *et al.*, 2013). A single test with low positivity cut-off was used in this study to check for lupus anticoagulant and anticardiolipin antibodies, yet for diagnosis of acquired thrombophilia these antibodies should be confirmed by a repeat test 12 weeks apart (Miyakis *et al.*, 2006). These observations indicate that the study population was heterogeneous and that the women did not necessarily have thrombophilia which is associated with adverse IVF or pregnancy outcomes. The observed potential beneficial effect of LMWH could be due to its antithrombin effect or perhaps by other mechanisms of improving endometrial receptivity and decidualization (as mentioned in the Introduction section). However for the purpose of our meta-analysis, this study was a well-designed RCT of women with RIF that was useful for pooling results with other studies.

The second RCT by Urman *et al.* (2009) was well designed, although blinding of patients was not possible since the control group did not receive any treatment, but as the outcomes are unlikely to be subjectively influenced, a lack of blinding is unlikely to affect the results. A clear subgroup analysis of those with ≥ 3 RIF was presented and although the sample size was small, again the study provided valuable information for pooling results. It was observed that the dose of LMWH used in this study was 1.5 to 2 times higher compared with the other two studies (enoxaparin 1 mg/kg/day, compared with the standard dose of 40 mg/day). To explore if the difference in the dose had impact on the pooled estimates, a sensitivity analysis was performed after exclusion of this study. Estimates for the LBR did not change remarkably, and on the

contrary, there was a slight increase in the RR (RR = 2.08, 95% CI = 1.11–3.91, $P = 0.02$).

The third quasi-randomized study (Berker *et al.*, 2011) did not score well on the domain-based bias assessment since the method of randomization was based on clinical practice. Furthermore there was no blinding, and although the patients were consecutively allocated to physicians, allocation concealment was unclear. Nevertheless, trial conductance and reporting was comparable to the other two studies. One other limitation of the study was that tests for ovarian reserve (basal follicular phase FSH or antral follicular count or anti-Müllerian hormone) were not recorded and there is a possibility that women with reduced ovarian reserve were included. These limitations could potentially have an impact on the results of our meta-analysis; therefore a sensitivity analysis was performed after exclusion of this study. The results of this sensitivity analysis showed a non-significant trend in improvement of LBR (random effects model, RR = 3.01, 95% CI 0.40–22.72, $P = 0.29$, $I^2 = 73\%$), IR (random effects model, RR = 2.04, 95% CI 0.79–5.30, $P = 0.14$, $I^2 = 77\%$) and a significant reduction in miscarriage rate (fixed effects model, RR = 0.18, 95% CI 0.04–0.83, $P = 0.03$, $I^2 = 0\%$) (Supplementary data, Figs S4–S6). The non-significant trend toward improvement in the intervention group could be due to reduced power in this subgroup (small sample size despite pooling of results).

Although miscarriage is a secondary outcome measure, a significant reduction in the intervention group cannot be ignored and this highlights the potential role of LMWH in facilitating implantation. These findings are in contrast to the RCTs performed in women with recurrent miscarriage, where the use of intervention did not improve the pregnancy outcome (Clark *et al.*, 2010; Kaandorp *et al.*, 2010). However, recurrent miscarriage and RIF, although considered within the same spectrum, are two

different entities. In RIF, there is no spontaneous conception, and after exclusion of all potential causes, suboptimal endometrial receptivity is a likely factor. It might be that in this cohort, LMWH up- or down-regulates certain endometrial receptors or genes and the same would not be applicable to the miscarriage cohort. Furthermore, the recurrent miscarriage studies were performed in women with two or more miscarriages and there is a possibility that the combination of aspirin and LMWH could be different compared with using LMWH on its own.

We believe that in the light of emerging evidence for role of LMWH on adhesion molecules and heparin-binding EGFs, it has a potential to facilitate implantation and decidualization along with its anticoagulant effect. It has been shown that LMWH can enhance invasiveness of extravillous trophoblast cells by inducing activity of specific metalloproteases (MMP) (Di Simone et al., 2007). In the family of MMPs, MMP-2 and MMP-9 have been shown to be the most involved in trophoblast invasion into endometrial tissues (Isaka et al., 2003). Interestingly, in *in vitro* models, heparin has been shown to reduce aberrant apoptosis in the trophoblasts and enhance cell survival (Hills et al., 2006), which in turn would improve embryo attachment and trophoblast invasion and differentiation.

This systematic review and meta-analysis has been performed with the aim of studying outcomes in women with ≥ 3 RIF who have had only LMWH as intervention. We considered studies with ≥ 3 RIF since there is no unanimous definition of RIF and informal discussion with fertility specialists working at different clinics in the UK have informed us that they use the same cut-off as our clinic, of ≥ 3 failed ET cycles. Evidence suggests that LMWH has a greater antiXa activity, more consistent bio-availability, a longer plasma half-life and is less strongly bound to proteins compared with unfractionated heparin (Fareed and Walenga, 2007). Similarly, heparin and low-dose aspirin have different mechanisms of action; therefore, we excluded studies with interventions other than LMWH. Only three studies had LMWH as an intervention and had RIF defined clearly. The strength of this systematic review is that all three studies had almost similar baseline characteristics of the subjects, used similar definitions for RIF, used similar intervention and considered the same outcome measures. As stated above, although one study was quasi-randomized, it was well conducted and the outcomes have been demonstrated with and without inclusion of this study. However, there are certain limitations of this review; clinical heterogeneity between the studies was noted (with one study including women with thrombophilia) and the overall sample size of the pooled studies was small. This means that the results have to be interpreted with caution. Power calculations have shown that in order to detect an absolute difference of 10% in ongoing pregnancy rates in women with ≥ 2 RIF, with an 80% power and an alpha of 0.05, 700 participants would be required (Urman et al., 2009). Similarly, Berker et al. (2011) calculated that for 9% difference in LBR per cycle in women with ≥ 3 RIF, 920 participants would be required.

Of note, we excluded an RCT on women with RIF and thrombophilia (APA and antinuclear antibodies) since unfractionated heparin with aspirin were used as intervention (Stern et al., 2003). This was an RCT with a crossover design, thus the same women were present in both treatment arms at different time points. Since this was the only other study identified for women with ≥ 3 RIF and thrombophilia, even though the intervention was different, we performed a sensitivity analysis, but no statistically significant improvement was observed for LBR

in the intervention group (RR = 2.43, 95% CI 0.19–31.52, $P = 0.5$, $I^2 = 81\%$) (Stern et al., 2003; Qublan et al., 2008).

There is only one other systematic review and meta-analysis published recently about the use of heparin in IVF treatment (Seshadri et al., 2012). It differs significantly from the current review in terms of the primary objective, methodology and the results. The review by Seshadri et al. includes studies using heparin for IVF treatment irrespective of first cycle or recurrent failures and includes observational studies as well as RCTs. Thus the results have been pooled for a markedly heterogeneous population. Additionally, the quality assessment for the RCTs was performed using a quality assessment scale which is no longer considered appropriate to appraise clinical trials (Higgins et al., 2011). One other limitation is the pooling of studies with different interventions of LMWH, unfractionated heparin and or aspirin. In contrast, this review has evaluated primarily the use of LMWH in improving outcomes for women with ≥ 3 RIF, and thereby has a more consistent study population and the results of our meta-analysis can be used in the clinical setting to provide evidence-based information to women with RIF. The results of the meta-analysis by Seshadri et al. showed no difference in LBR, IR or miscarriage rate when RCTs were pooled together but significant improvements in LBR and CPR for meta-analysis of observational studies.

The results of this meta-analysis show that in women with ≥ 3 RIF, the use of LMWH as an adjunct to IVF treatment significantly improved LBR. Similarly, there was a 78% reduction in the miscarriage rate in the intervention group. However, IR was not significantly improved. The difference in outcomes for LBR and IR may be due to the inadequate power of the pooled studies ($N = 245$). It is important to note that these beneficial effects of LMWH were not significant when only the two studies with unexplained RIF were pooled. This could again be due to small numbers and quasi-randomized design of one study. The results suggest that there could be a potential role of LMWH in improving pregnancy outcomes for women with RIF. As mentioned previously, these effects could be mediated by enhancing endometrial receptivity and trophoblast invasion due to the expression of different proteins (IGF-I and IGFBP-1), regulation of heparin-binding EGF and adhesion molecules or inhibition of complement activation.

The results of this review do not advocate routine use of LMWH as an adjunct in women with RIF. However, they do indicate a strong need to evaluate the role of LMWH in enhancing endometrial receptivity in both clinical and basic science research. This can be accomplished by designing and performing adequately powered RCTs, possibly multi-centered, using standardized criteria for defining unexplained RIF and using only LMWH as an intervention.

Supplementary data

Supplementary data are available at <http://humupd.oxfordjournals.org/>.

Authors' roles

N.P. conceived the idea, executed the data extraction and analysis and prepared the draft; T.A.G. participated in the study design and analysis and reviewed the manuscript; J.C.K. analyzed and critically reviewed the manuscript and L.N.G. contributed to study design and data extraction and critically reviewed the manuscript.

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