Heparin for assisted reproduction (Review)

Akhtar MA, Sur SD, Raine-Fenning N, Jayaprakasan K, Thornton JG, Quenby S

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[Intervention Review]

Heparin for assisted reproduction

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ABSTRACT

Background

Heparin as an adjunct in assisted reproduction (peri-implantation heparin) is given at or after egg collection or at embryo transfer. Heparin has been advocated to improve embryo implantation and clinical outcomes. It is proposed that heparin may enhance the intrauterine environment by improving decidualisation with an associated activation of growth factors and a cytokine expression profile in the endometrium that is favourable to pregnancy.

Objectives

To investigate whether the administration of heparin around the time of implantation (peri-implantation heparin) improves clinical outcomes in subfertile women undergoing assisted reproduction.

Search methods

A comprehensive and exhaustive search strategy was developed in consultation with the Trials Search Co-ordinator of the Cochrane Menstrual Disorders and Subfertility Group (MDSG). The strategy was used in an attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). Relevant trials were identified from both electronic databases and other resources (last search 6 May 2013).

Selection criteria

All randomised controlled trials (RCTs) were included where peri-implantation heparin was given during assisted reproduction. Live birth rate was the primary outcome.

Data collection and analysis

Two review authors independently assessed the eligibility and quality of trials and extracted relevant data. The quality of the evidence was evaluated using GRADE methods.

Main results

Three RCTs (involving 386 women) were included in the review. Peri-implantation low molecular weight heparin (LMWH) during IVF/ICSI was given at or after egg collection or at embryo transfer in these studies. The characteristics of the participants differed across studies. One included women having their first IVF cycle, with no blood clotting disorder; another included women with at least one blood clotting disorder and the third included women who had undergone at least two previous unsuccessful ART cycles.

Our findings differed according to choice of statistical model. When we used a fixed effect analysis, the evidence suggested that peri-implantation heparin was associated with an improvement in live birth rate compared with placebo or no heparin (odds ratio (OR) 1.77, 95% confidence interval (CI) 1.07 to 2.90, three studies, 386 women, I^2 = 51%, very low quality evidence) and also an improvement in the clinical pregnancy rate (OR 1.61, 95% CI 1.03 to 2.53, three studies, 386 women, I^2 = 29%, low quality evidence). However when a random effects model was used there was no longer a difference between the groups for either live birth (OR 1.85, 95% CI 0.80 to 4.24) or clinical pregnancy (OR 1.66, 95% CI 0.94 to 2.90). Moreover there was high heterogeneity (I^2 = 51%) for the analysis of live birth.

Adverse events were poorly reported in all the included studies. Events such as bleeding, and thrombocytopenia were reported in women receiving heparin and affected 5-7% of women in the heparin group in one study. However no studies reported data suitable for analysis and so no firm conclusions could be drawn regarding adverse effects.

The main limitations in the evidence were inconsistency, imprecision and inadequate reporting of adverse events.

Authors' conclusions

It is unclear whether peri-implantation heparin in assisted reproduction treatment (ART) cycles improves live birth and clinical pregnancy rates in subfertile women, as the evidence was sensitive to choice of statistical model and no benefit was apparent when a random effects model was used. Side effects have been reported with use of heparin and no firm conclusions can be drawn regarding its safety. Our results do not justify the use of heparin in this context, except in well-conducted research trials.

These findings need to be further investigated with well-designed, adequately powered, double-blind, randomised, placebo-controlled, multicentre trials. Further investigations could also focus on the effects of the local (uterine) and non-systemic application of heparin during ART.

PLAIN LANGUAGE SUMMARY

Heparin for assisted reproduction

Review Question

Researchers in the Cochrane Collaboration reviewed the evidence about the effect of heparin administered around the time of implantation on clinical outcomes in subfertile women undergoing assisted reproduction.

Background

Heparin is a class of blood thinning drug that is used in the prevention and treatment of blood clots. It has been suggested that heparin may improve the intrauterine environment in subfertile women, by increasing growth factors to improve attachment of the embryo to the lining of the womb. The result could be an improvement in live birth rates during assisted reproduction.

Study Characteristics

Three studies with 386 participants were included in the review. All participants were subfertile women undergoing assisted reproduction. Their characteristics differed across studies. One study included women having their first IVF cycle, with no blood clotting disorder. Another study included women with at least one blood clotting disorder. The third study included women with at least two previous unsuccessful assisted reproduction treatment cycles. In all cases a daily injection of low molecular weight heparin was given to women from the time of egg collection or embryo transfer during assisted reproduction. Control groups received placebo or no treatment. There were no issues with source of funding in any of the studies. The evidence is current to May 2013.

Key Results

It is unclear whether peri-implantation heparin in assisted reproduction treatment (ART) cycles improves live birth and clinical pregnancy rates in subfertile women. Although there was some suggestion of benefit, this disappeared when an alternative method

of analysis was used. Heparin had side effects such as bruising and bleeding, but no conclusions could be drawn regarding its safety because none of the studies reported comparative data on adverse effects. The evidence does not justify the use of heparin except in well-designed clinical research trials. Such trials are a priority.

Quality of Evidence

The evidence was of low or very low quality, mainly due to imprecision, inconsistency and inadequate reporting of advere events. Further well-designed randomised controlled trials with larger sample sizes are needed to clarify the possible role of heparin in assisted reproduction.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Heparin for assisted reproduction

Population: Subfertile women
Settings: Assisted reproduction treatment (ART)
Intervention: Heparin versus placebo or no heparin

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI) using fixed effect model	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Heparin				
Live birth rate per woman	**173 per 1000**	**271 per 1000** (183 to 378)	**OR 1.77** (1.07 to 2.9)	386 (3 studies)	⊕◯◯◯ **very low**[1,2]	Estimate using random effects model: OR 1.85, 95% CI 0.80 to 4.24
Clinical pregnancy rate per woman	**250 per 1000**	**349 per 1000** (256 to 458)	**OR 1.61** (1.03 to 2.53)	386 (3 studies)	⊕◯◯◯ **low**[2]	Estimate using random effects model: OR 1.66, 95% CI 0.94 to 2.90
Adverse effects	No comparative data available so no conclusions could be drawn. Adverse effects such as bleeding, and thrombocytopenia were reported in the heparin groups and affected 5-7% of women in one study					

*The basis for the **assumed risk** is the median control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Inconsistency (high heterogeneity: $I^2 = 51\%$)

[2] Imprecision: low overall event rate, confidence intervals compatible with substantial benefit or no appreciable benefit, findings sensitive to choice of statistical model. Random effects model gives non-significant findings

BACKGROUND

Description of the condition

Infertility is the failure of a couple of reproductive age to conceive after having regular unprotected sexual intercourse for a period of 12 months or more. Primary infertility refers to couples who have never conceived, and secondary infertility refers to couples who have previously conceived but are unable to do so again after a year of trying.

Infertility affects 15% of couples and is becoming increasingly common. Of these couples, 70% will have primary and 30% secondary infertility. Assisted reproduction techniques (ART) have been employed to help some of these couples achieve a pregnancy. Assisted reproduction has significant physical, social, psychological and financial implications. The success of ART can be defined as the live birth of a child. Live birth rates with ART vary from 30% to 50%; hence various adjuncts have been employed during assisted reproduction to increase the likelihood of pregnancy and live birth. The effectiveness of these adjuncts remains to be determined in many cases. Heparin, given as an adjunct to women with or without a known thrombophilia, is one such therapy and has been suggested as being efficacious in improving implantation (attachment of the fertilised egg to the wall of the uterus) and achieving pregnancy.

Description of the intervention

Heparan sulphates have an important role in conception and early pregnancy events. However the role of heparin (a structural analogue of heparan) in assisted conception is not clear. Heparin is a linear polydisperse polysaccharide consisting of 1-4 linked pyranosyluronic acid and 2-amino-deoxyglucopyranose (glucosamine) residues (Comper 1981). Owing to their highly anionic nature, heparin and heparan sulphate have high binding affinity to antithrombin, growth factors, growth factor receptors, viral envelope proteins and extracellular matrix molecules.

Heparan sulphate proteoglycans (HSPGs) are expressed throughout the reproductive tract and are involved in the regulation of endometrial cycling (Potter 1992; Kelly 1995, San Martin 2004; Germeyer 2007; Lai 2007; Xu 2007).

Low molecular weight heparins (LMWH) are derived from heparin by enzymatic (for example tinzaparin) or chemical (for example dalteparin, nadroparin and enoxaparin) depolymerisation of unfractionated heparin (UFH), which in itself cannot be synthesised in vitro.

Unfractionated heparin and LMWH facilitate the anticoagulant effect of antithrombin (Bick 2005) but, compared with unfractionated heparin, LMWH has reduced antifactor IIa activity leading to inefficient inhibition of thrombin by antithrombin. However, the smaller weight LMWH inactivates factor Xa with equal efficacy. Low molecular weight heparin has a longer half-life, a more predictable antithrombotic response, and a substantially lower risk of heparin-induced thrombocytopenia (HIT) (Warkentin 1995; Warkentin 2004) and osteoporosis (Murray 1995), thus having obvious clinical benefits. So in practice, LMWH is used routinely with daily self-administered subcutaneous injections, not requiring close monitoring and with lower risk of side effects.

Low molecular weight heparins have a mean molecular weight of 4300 to 5000 kDa (range 1000 to 10,000 kDa), compared to 15,000 kDa for unfractionated heparin (Nelson 2008).

How the intervention might work

Implantation is a complex, dynamic process which involves coordination of various interactions at an intra- and intercellular level. The interaction between the developing embryo and the endometrium is still not fully understood; however heparin can potentially modulate many of the known mechanisms that underlie the successful implantation of the developing embryo.

Traditionally the role of heparin in early pregnancy was believed to be in the prevention of blood clotting during implantation and placentation in women with inherited and acquired thrombophilia. However, more recent work suggests a possible therapeutic role for heparin in other mechanisms fundamental to implantation. Unfractionated heparin as well as LMWH are able to modulate the process of decidualisation, whereby the cells in the lining of the womb prepare for pregnancy. This positive effect on decidualisation is a potential mechanism by which heparin improves implantation in ART (Corvinus 2003, Poehlmann 2005, Fluhr H 2010).

Heparin also has the ability to bind with and modulate a wide variety of proteins, which can influence a number of physiological processes involved in implantation and trophoblastic development. These processes include adhesion of the blastocyst to the endometrial surface (Wang 2002) and trophoblastic differentiation and invasion (Arai 1994; Weigert 2001; Leach 2004; Quenby 2004; Erden 2006; Moller 2006; Di Simone 2007; d'Souza 2007; Nelson 2008).

Why it is important to do this review

Heparin is often offered to couples as an adjunct in an attempt to improve live birth rates, its presumed effect being to improve implantation. Clinicians may be using heparin as an adjunct based on biological plausibility rather than evidence of efficacy. A systematic review is required to determine the efficacy of heparin to increase pregnancy and live birth rates and reduce adverse perinatal outcomes for all women undergoing assisted reproduction.

When heparin is used as an adjunct treatment during assisted reproduction, there has been no consensus regarding the optimum type of heparin (unfractionated heparin or LMWH) timing or the dose. This is an area which we considered in the review.

This Cochrane review aims to provide evidence about the efficacy of heparin given in the peri-implantation period (around the time of conception) to reduce implantation failure in women who have a history of infertility and are undergoing assisted reproduction treatments. In this review we do not assess the efficacy of heparin as an anti-thrombophilic agent (preventing blood clots) later in pregnancy or in women with a history of recurrent miscarriage.

OBJECTIVES

To investigate whether the administration of heparin around the time of implantation improves clinical outcomes in subfertile women undergoing assisted reproduction.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs).

Types of participants

We included trials of women undergoing assisted reproduction treatment (ART) with a history of infertility. Trials of women with a previously known thrombophilia were included.
Trials involving women undergoing stimulated or unstimulated intrauterine insemination (IUI) were not included.

Types of interventions

1. Heparin versus no treatment.
2. Heparin versus placebo.
3. Heparin versus aspirin.
4. Heparin versus heparin and aspirin.
5. Unfractionated heparin (UFH) versus low molecular weight heparin (LMWH).

Studies were included if heparin was administered in the peri-implantation period (from the day of egg collection or embryo transfer (ET) to 14 days later).

Types of outcome measures

Primary outcomes

1. Live birth rate per woman. Number of live births divided by the number of randomised women (live birth is defined as delivery of one or more live infants).
2. Adverse effects of heparin e.g. any bleeding, bruising, heparin-induced thrombocytopenia (HIT), anaphylaxis and any other unexpected side effects.

Secondary outcomes

1. Clinical pregnancy rate per randomised woman. The presence of at least one gestational sac with fetal heart beat on ultrasound scan defines a clinical pregnancy.
2. Multiple pregnancy rate per randomised woman. The demonstration of more than one sac with a fetal pole on ultrasound scan defines multiple pregnancies.
3. Maternal pregnancy complications including first trimester miscarriage, second trimester miscarriage, preterm delivery, pre-eclampsia, pregnancy-induced hypertension, any maternal bleeding.
4. Fetal complications during pregnancy including intrauterine growth restriction, placenta previa, placental abruption.

Additional outcomes not appropriate for statistical pooling

Data per cycle, per pregnancy or per ET are not appropriate for pooling because of what statisticians refer to as 'unit of analysis errors'. Simple group comparison tests for categorical data require that observations are statistically independent. The use of multiple observations per woman leads to unpredictable bias in the estimate of treatment difference Vail 2003. However, due to the frequency with which this form of data are reported in subfertility research, we planned to report the following outcomes in narrative form:

- implantation rate, the number of fetal sacs divided by the number of embryos transferred;
- incidence of miscarriage per total number of pregnancies;
- incidence of multiple pregnancies per total number of pregnancies.

Search methods for identification of studies

A comprehensive search strategy was developed in consultation with the Trials Search Co-ordinator of the Cochrane Menstrual Disorders and Subfertility Group (MDSG). The strategy was used in an attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). Relevant trials were identified from both electronic databases and other resources.
This review will be updated every two years.

Electronic searches

We searched the following electronic databases, from inception to 6 May 2013 with the Cochrane highly sensitive search strategy for identifying randomised trials, which appears in the *Cochrane*

Handbook for Systematic Reviews of Interventions (version 5.1.0; chapter 6, 6.4.11) (Higgins 2011):

1. The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* latest issue) (see Appendix 1).
2. English language electronic databases: MEDLINE, EMBASE and PsycINFO (see Appendix 2, Appendix 3, Appendix 4).
3. *The Cochrane Library* (www.cochrane.org/index.htm) for DARE, the Database of Abstracts of Reviews of Effects (reference lists from non-Cochrane reviews on similar topics).
4. Current Controlled Trials (www.controlled-trials.com).
5. The World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx).

Searching other resources

We searched the references lists of all included studies and relevant reviews to identify further relevant articles and when required, we contacted authors and experts in the relevant field for potential studies.

We performed a search for grey literature.

Data collection and analysis

We performed statistical analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Review Manager 5.1 was used to input data.

Selection of studies

The title, abstract, and keywords of every record retrieved were scrutinised independently by two review authors (MA, SS) to determine which studies required further assessment. The full texts were retrieved when the information given in the titles, abstracts, and keywords suggested that the randomised controlled study intervention was heparin as an adjunct to assisted reproduction therapy.

If there were any doubts regarding these criteria from scanning the titles and abstracts, the full articles were retrieved for clarification. Disagreements were resolved by discussion with a third review author (Professor S Quenby), if necessary. We contacted the authors of trials to provide missing data, if required.

Data extraction and management

The following information was extracted from the studies included in the review. It is presented in the table 'Characteristics of included studies'.

Trial characteristics

This includes the following items.

1. Method of generating randomisation sequence.
2. Allocation concealment.
3. Trial design.
4. Number of women screened for eligibility then randomised, excluded, and finally analysed.
5. Duration, timing, and location of the trial.
6. Source of funding.

Baseline characteristics of the studied groups

1. Age of the women.
2. Duration of infertility.
3. Type of ART.
4. Previous fertility treatments.

Intervention

1. Type of intervention and control group.
2. Dose regimen and timing.

Outcomes

1. Outcomes.
2. How outcomes were defined.
3. How outcomes were measured.
4. Timing of outcome measurement.

All data were extracted independently by two review authors (MA, SS) using forms designed according to Cochrane guidelines. Additional information was sought from the authors on trial methodology and trial data for trials that appeared to meet the eligibility criteria but had aspects of methodology that were unclear or where data were in an unsuitable form for meta-analysis. We planned to settle any differences of opinion by discussion between the review authors, but there were no disagreements.

Assessment of risk of bias in included studies

Assessment of risk of bias in the included studies was independently performed by two review authors (MA, SS). Disagreements were noted and resolved by a third review author (SQ).

The 'Risk of bias' table was included in the Characteristics of included studies

The following 'Risk of bias' domains were assessed according to the criteria specified by the *Cochrane Handbook for Systematic Reviews of Interventions* 5.1.0.

1. Selection bias: Random sequence generation method (e.g. computer-generated, random number tables, or drawing lots) and allocation concealment: adequate(e.g. third party, sealed envelopes); inadequate (e.g. open list of allocation codes); not clear (e.g. not stated).
2. Performance bias: Blinding of participants and personnel.

3. Detection bias: Blinding of outcome assessments.
4. Attrition bias: Incomplete outcome data and intention-to-treat analysis if used.
5. Reporting bias: selective outcome reporting.
6. Other bias: Any other potential sources of bias not included in this protocol.

Measures of treatment effect

All outcomes were dichotomous. We used the numbers of events in the control and intervention groups of each study to calculate odds ratios (OR) with 95% confidence intervals (CI).

Unit of analysis issues

The primary analysis was per woman randomised. Reported data that did not allow valid analysis (for example, 'per cycle' rather than 'per woman', where women contribute more than one cycle) were briefly summarised in an additional table and were not used in meta-analysis. Multiple live births (for example, twins or triplets) were counted as one live birth event.

Dealing with missing data

The data were analysed on an intention-to-treat basis as far as possible and attempts were made to obtain missing data from the original trialists. Where these were unobtainable, only the available data were analysed.

Assessment of heterogeneity

The review authors (MA, SS) considered whether the participants, interventions, and outcomes in the included studies were similar enough to consider pooling in a meta-analysis.
Tests for statistical heterogeneity in pooled data were carried out using the Chi^2 test, with significance set at $P < 0.1$. The I^2 statistic was used to estimate the total variation across studies that was due to heterogeneity, where < 25% was considered as low-level, 25% to 50% as moderate-level, and > 50% as high-level heterogeneity. If high levels of heterogeneity ($I^2 > 50\%$) were seen for primary outcomes, we explored possible sources of heterogeneity using sensitivity and subgroup analyses.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases for primary outcomes, we performed a comprehensive search for eligible studies and were alert for duplication of data. We planned to use a funnel plot to explore the possibility of small study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies) if there were 10 or more studies in the primary analysis (Egger 1997).

Data synthesis

Meta-analyses were performed, as appropriate, where data were available from multiple studies investigating the same treatment and where the outcomes had been measured in a standard way. A fixed-effect model was used. We undertook this meta-analysis according to methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). An increase in the odds of a particular outcome, which may be beneficial (for example, live birth) or detrimental (for example, adverse effects), were displayed graphically in the meta-analyses to the right of the centre-line and a decrease in the odds of an outcome to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

If there were sufficient data, we planned to perform the following subgroup analyses.
1. Efficacy of heparin with different ART excluding IUI.
2. Efficacy of adjunct therapy of heparin with or without thrombophilia for women undergoing ART.
3. Duration, dose, timing and type of heparin therapy during ART.
4. Any other adjunct therapy used in addition with heparin during ART.
5. Efficacy of heparin during ART according to age.
6. Efficacy of heparin with fresh versus frozen ET.

Sensitivity analysis

We performed sensitivity analyses for the primary outcomes to determine whether the review conclusions would have differed if:
1. eligibility were restricted to studies without high risk of bias;
2. a random-effects model had been adopted;
3. the summary effect measure had been risk ratio rather than odds ratio.

Overall quality of the body of evidence: 'Summary of findings' table

A 'Summary of findings' table was generated using GRADEPRO software. This table evaluated the overall quality of the body of evidence for main review outcomes, using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness and publication bias). Judgements about evidence quality (high, moderate or low) were justified, documented, and incorporated into reporting of results for main outcomes.

RESULTS

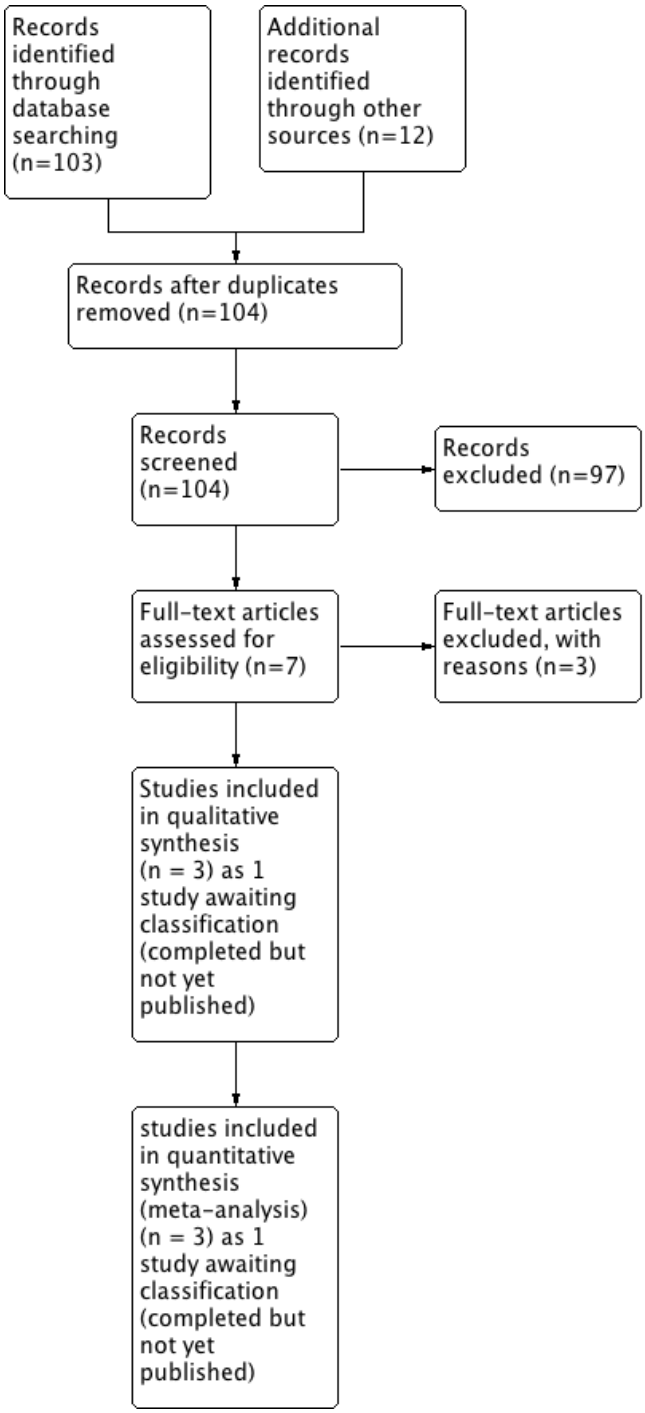
Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification.

Results of the search

Seven studies were identified that assessed the use of peri-implantation heparin in assisted reproduction. Of these only three studies were eligible for the review. They compared heparin alone versus either no heparin or placebo. The results of one study were not published yet, however, the characteristics of that study (Mashayekhy 2011) are available in 'Characteristics of studies awaiting classification (completed but not yet published)'. Full agreement existed between the two researchers, concerning inclusion or exclusion of trials. Figure 1

Figure 1. Study Review flow diagram.



Included studies

Three studies Qublan 2008; Urman 2009; Noci 2011 met the criteria for inclusion in this review. For details see Characteristics of included studies

Participants

The total number of trial participants was 386. The upper age limit was < 40 years in all participants in the included studies.

Interventions

All women were included for a single IVF/ICSI (in vitro fertilisation/intracytoplasmic sperm injection) cycle only. Low molecular weight heparin (LMWH) was administered from either oocyte retrieval or embryo transfer (ET), so the treatment protocol varied across studies.
In Qublan 2008, LMWH therapy treatment was started from the day of ET until results of Beta-hCG were available two weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed. In Noci 2011 LMWH treatment was started on the day of oocyte retrieval until nine weeks of pregnancy with positive pregnancy results. In Urman 2009 LMWH treatment was started a day after oocyte retrieval until 12 weeks of pregnancy with positive pregnancy test results. Control groups in these studies received placebo (Qublan 2008) or no heparin (Urman 2009; Noci 2011)

Outcomes

All three included studies reported live birth rate per woman as the primary outcome, adverse effects, clinical pregnancy rate per woman, multiple pregnancy rate per woman, implantation rate per woman and miscarriage rate per woman.

Additional outcomes not appropriate for statistical pooling

Data per cycle, per pregnancy or per ET were not appropriate for pooling. We have reported the following in additional tables:

- implantation rate, the number of fetal sacs divided by the number of embryos transferred; Table 1
- incidence of miscarriage per total number of pregnancies; Table 2
- incidence of multiple pregnancies per total number of pregnancies; Table 3

Excluded studies

Three studies failed to meet the inclusion criteria. Colicchia 2011 was excluded because LMWH was used in conjunction with prednisolone. Stern 2003 was excluded because unfractionated heparin (UFH) was used in conjunction with low-dose aspirin. Berker 2011 was excluded because it was a quasi-randomised study. Details are provided in Characteristics of excluded studies.

Risk of bias in included studies

The methodological quality of included studies was documented in the 'Risk of bias' table for each individual study. The 'Risk of bias' summary and 'Risk of bias' graph are presented as Figure 2 and Figure 3.

Figure 2. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

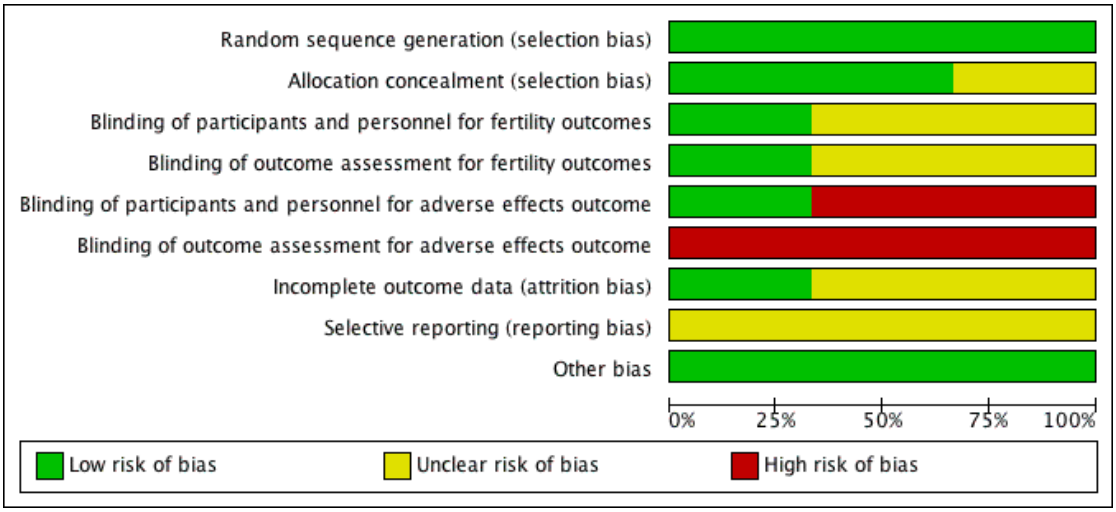
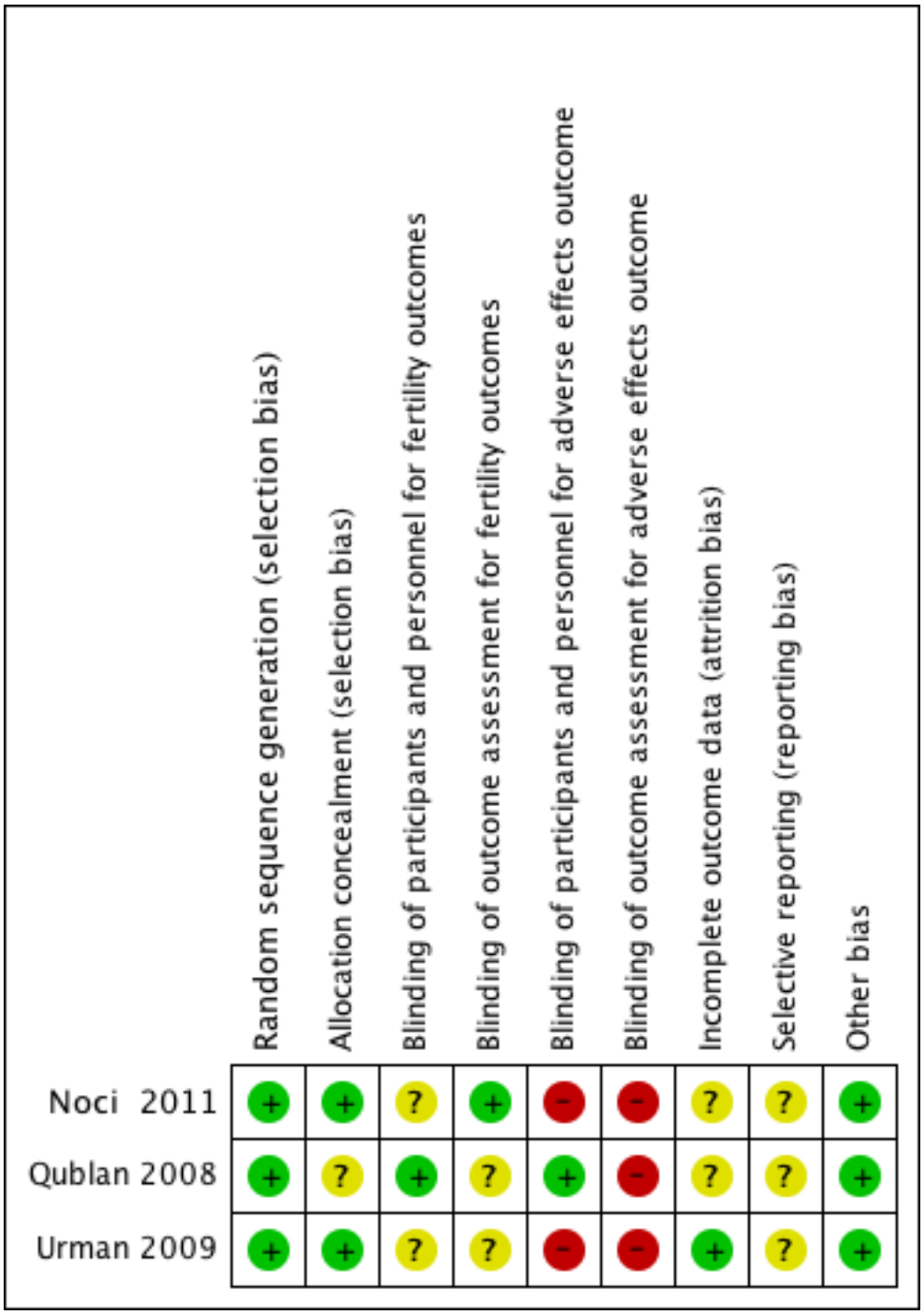


Figure 3. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence generation

All three studies were rated as at low risk of this bias.

Allocation concealment

Two studies were rated as at low risk of this bias (Noci 2011; Urman 2009). The third study was rated as at unclear risk, as concealment of allocation was not described Qublan 2008.

Blinding

Fertility outcomes

One of the studies described use of placebo (Qublan 2008) and was rated as at low risk of performance bias for fertility outcomes. Neither of the other studies described blinding of participants. However we considered that blinding was unlikely to influence fertility outcomes, so we rated these two studies as at unclear risk of performance bias for these outcomes. One study reported blinded assessment of fertility outcomes (Noci 2011) and we rated it as at low risk of detection bias. The other two studies were rated as at unclear risk of detection bias for fertility outcomes.

Adverse events

Lack of blinding may influence reporting of adverse events. The study using placebo (Qublan 2008) was rated as at low risk of performance bias for adverse events, but the other two studies were rated as at high risk. None of the studies reported blinded assessment of adverse events and we rated all studies as at high risk of detection bias for this outcome.

Incomplete outcome data

In Qublan 2008 the reporting in the trial publication was inconsistent. It was stated that 137 women were randomised but subsequently stated that 83 were randomised. All 83 were included in analysis. The study was rated as at unclear risk of bias in this domain.

In Urman 2009, 153 women were recruited to the trial. Three women in the treatment and control groups were lost to follow-up before completion of initial follow-up (completion of the 20th gestational week for the latest recruited participant who achieved an ongoing pregnancy), and another two women in the LMWH group were lost to follow-up after completion of the 20th gestational week but before delivery or expected completion of the 40th gestational week. Women lost to follow-up during the first period were considered not to have an ongoing pregnancy, and women lost to follow-up in the second period were considered not to have a live birth in the intention-to-treat analysis. The dropout rate was 5.22%. In the final analysis, 75 women in each group were considered. The study was rated as at low risk of attrition bias because trialists compensated for dropouts by imputing a negative outcome to losses to follow-up.

Noci 2011 enrolled 210 patients presenting all the necessary requirements and subjected to ovarian stimulation for IVF/ICSI. On the day of oocyte retrieval, 38 patients were excluded: 30 for the absence of retrieved oocytes or cancelled cycles and eight who decided to decline their participation. One hundred and seventy-two women were allocated to intervention and divided into two groups: 86 women in the control group and 86 women in the treatment group. The final series for analysis contained 153 women because 13 women belonging to the treatment group and six women belonging to the control group had no embryos to transfer, thus they were immediately excluded from the study. Thus in the final analysis, 73 women were in treatment group and 80 women were in the control group. The dropout rate was 8.72% after allocation to the intervention. The study was rated as at unclear risk of attrition bias.

Selective reporting

None of the studies reported comparative data on adverse events and so all were rated as at unclear risk of bias in this domain.

Other potential sources of bias

No other potential sources of bias were identified in any of the included studies, and all were rated as at low risk of bias in this domain.

Effects of interventions

See: **Summary of findings for the main comparison** Heparin for Assisted Reproduction (fixed effect); **Summary of findings 2** Heparin for Assisted Reproduction (random effects)

Primary Outcomes

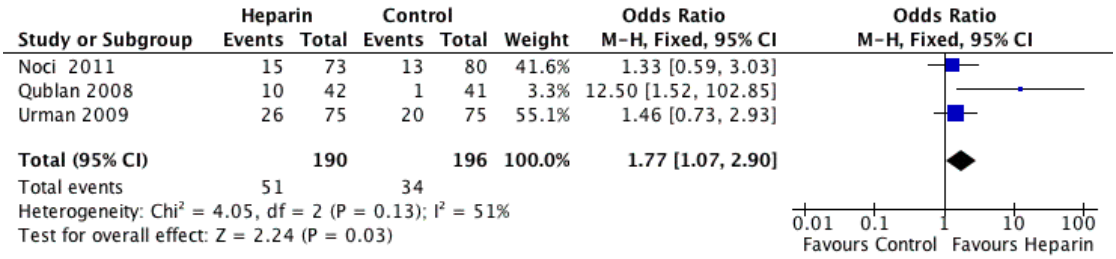
1. Live birth rate per woman

All three included studies assessed the primary outcome, namely 'live birth rate per woman'.

Results pooled in meta-analysis (fixed-effect model) showed that there was a significant improvement in live birth rate with the

use of LMWH (odds ratio (OR)1.77, 95% confidence interval (CI) 1.07, 2.90 P = 0.03, I^2 = 51%, three studies, 386 women) in comparison to placebo or no LMWH (Figure 4). Sensitivity analysis performed with a random-effects model showed that there was a non significant improvement in live birth rate with the use of LMWH compared to no LMWH (OR1.85, 95% CI 0.80, 4.24 P,=,0.15, $I^{2,}$ =,51%, three studies, 386 women)

Figure 4. Forest plot of comparison: 1 Heparin versus control, outcome: 1.1 Live Birth Rate per woman.



This finding should be viewed with extreme caution due to high heterogeneity and sensitivity to choice of statistical model.
The evidence was of very low quality as shown in Summary of findings for the main comparison.

2. Adverse effects

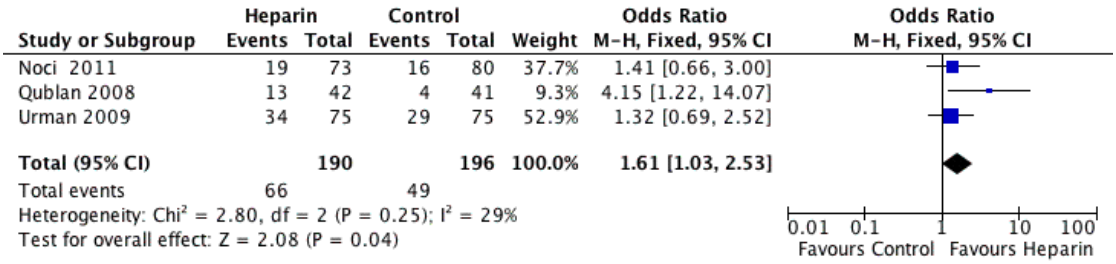
Direct adverse effects of heparin including bleeding, bruising, thrombocytopenia or any other side effects were described in all the included studies.
Qublan 2008 reported that the most frequent complications encountered in the heparin-treated group were bleeding (3/42, 7.1%) followed by thrombocytopenia (2/42, 4.8%) and allergic reactions (1/42, 2.4%).
Urman 2009 reported that platelet counts did not change significantly in the LMWH group during the study period and that none of the participants experienced any adverse effects other than small ecchymosis around the LMWH injection sites. None of the participants in the LMWH group discontinued treatment due to pain or ecchymosis around the injection site. It was unclear to what extent adverse effects in the control group were assessed.
Noci 2011 reported no other adverse effects in the study except minimal bruising at injection site of heparin.
It appeared from the studies that longer duration of heparin therapy increased the number of side effects; however this interpretation must be looked with caution as there was no available controlled comparative data for duration of therapy.
In Qublan 2008 LMWH therapy was started from the day of ET until results of Beta-hCG were available two weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed. In Noci 2011, LMWH treatment was started on the day of oocyte retrieval until nine weeks of pregnancy with positive pregnancy results. In Urman 2009 LMWH treatment was started a day after oocyte retrieval until 12 weeks of pregnancy with positive pregnancy test results.

Secondary Outcomes

1. Clinical pregnancy rates per woman

'Clinical pregnancy rate per woman' was described in all included studies.
Results pooled in meta-analysis (fixed-effect model) showed a significant improvement in clinical pregnancy rate with the use of LMWH compared with placebo or no LMWH (OR 1.61 95% CI 1.03, 2.53 P = 0.04, I^2 = 29%, three studies, 368 women) Figure 5. Sensitivity analysis performed with a random-effects model showed no significant improvement in clinical pregnancy rate with the use of LMWH compared to no LMWH (OR 1.66, 95% CI 0.94 to 2.90, I^2 = 29%, three studies, 368 women).

Figure 5. Forest plot of comparison: 1 Heparin versus control, outcome: 1.3 Clinical Pregnancy Rate per woman.



These results should be viewed with caution due to high heterogeneity and sensitivity to choice of statistical model.
The evidence is of very low quality, as shown in Summary of findings for the main comparison.

2. Multiple pregnancy rates per woman

'Multiple pregnancy rates per woman' were not reported in any of the included studies. "Multiple pregnancy rates per total number of pregnancies" was reported in all studies but cannot be pooled for meta-analysis due to unit of analysis errors. Please see Table 3

3. Maternal pregnancy complications

Qublan 2008 reported placental abruption (1/42, 2.4%) in LMWH group. Two (4.9%) women in the placebo group developed pre-eclampsia.
Urman 2009 reported that total numbers of preterm deliveries were nine (34.6%) in LMWH and six (30.0%) in control groups (P = 0.74). Three women delivered in the 32nd week (one set of quadruplets, one set of twins and a singleton, all in LMWH group), one woman (singleton in control group) delivered in the 33rd week, four women delivered in the 34th week (two sets of twins in LMWH group and two sets of twins in the control group), four women delivered in the 35th week (all twins, three and one in LMWH and control groups, respectively) and three women delivered in the 36th week (one singleton in LMWH group and two sets of twins in the control group).
Noci 2011 did not describe any maternal pregnancy complications.

4. Fetal complications during pregnancy

Qublan 2008 reported two intrauterine foetal deaths in the heparin-treated group compared to none in the control group. No further details were provided.
Urman 2009 reported that none of the infants delivered in the study had any congenital malformations. One boy (from the LMWH group) had a unilateral undescended testis, and another infant delivered at the 32nd week (from the LMWH group) underwent surgery due to necrotising enterocolitis.
Noci 2011 did not describe any fetal complications during pregnancy.

Other analyses

There were insufficient studies to conduct the planned subgroup analyses or to construct a funnel plot to assess publication bias.
We considered clinical and methodological differences between the studies that might account for the high heterogeneity in the analysis of live birth. Exclusion of the study that was clearly restricted to women with at least one thrombophilic defect (Qublan 2008) eliminated the heterogeneity (I^2 = 0%). However, with so few studies available for analysis it is unclear whether the effects of the intervention may differ in this population.

ADDITIONAL SUMMARY OF FINDINGS *[Explanation]*

Heparin for assisted reproduction

Population: Subfertile women
Settings: Assisted reproduction treatment (ART)
Intervention: Heparin versus placebo or no heparin

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI) using a random effects model	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Heparin				
Live birth rate per woman	**173 per 1000**	**280 per 1000** (144 to 471)	**OR 1.85** (0.8 to 4.24)	386 (3 studies)	⊕◯◯◯ **very low**[1,2]	Estimate using a fixed effect model: OR 1.77, 95% CI 1.07 to 2.9
Clinical pregnancy rate per woman	**250 per 1000**	**356 per 1000** (239 to 492)	**OR 1.66** (0.94 to 2.9)	386 (3 studies)	⊕◯◯◯ **low**[2]	Estimate using a fixed effect model: OR 1.61 95% CI 1.03 to 2.53
Adverse effects	No comparative data available so no conclusions could be drawn. Adverse effects such as bleeding and thrombocytopenia were reported in the heparin groups and affected 5-7% of women in one study					

*The basis for the **assumed risk** is the median control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **OR:** Odds ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Inconsistency (high heterogeneity: $I^2 = 51\%$)
[2] Imprecision: low overall event rate, confidence intervals compatible with substantial benefit or no appreciable benefit, findings sensitive to choice of statistical model.

DISCUSSION

Summary of main results

The aim of this review was to investigate whether the administration of heparin during the peri-implantation period improves clinical outcomes in subfertile women undergoing assisted reproduction. We found evidence suggesting that administration of peri-implantation low molecular weight heparin (LMWH) may improve live birth and pregnancy rates during assisted reproduction, however the studies were few and small (three studies, total 386 participating women) with high heterogeneity and sensitivity to choice of statistical model. Therefore all results must be interpreted with extreme caution.

Low molecular weight heparin was associated with adverse events, including bruising, ecchymosis, bleeding, thrombocytopenia and allergic reactions. There was a suggestion that adverse effects increased if heparin therapy was used over a longer duration. There were no reliable data on long-term side effects of heparin at this stage of pregnancy.

Overall, this evidence does not justify the present widespread use of LMWH in this population subgroup (previous failed IVF), outside well-conducted randomised trials. Such trials should be a priority.

Overall completeness and applicability of evidence

There were only three studies that could be included in the review and the total sample size was small (386 women) so the findings have to be viewed with caution. Moreover, study characteristics varied: one was a multicentre study Noci 2011 while the two others were conducted at a single centre (Qublan 2008; Urman 2009). There was no uniformity of dose, timing or duration of the intervention. Only one study Qublan 2008 used sodium chloride as placebo control, the other two included studies had no placebo, hence the patients were not blinded. Furthermore, none of the studies described blinding of clinicians.

We were unable to adequately assess the effect of heparin in women with or without thrombophilia undergoing assisted reproduction as only one study (Qublan 2008) included women with thrombophilia, Noci 2011 included women without thrombophilia, the other remaining study (Urman 2009), did not report about the presence or absence of thrombophilia in including participants.

The small numbers of underpowered trials means that there was insufficient evidence to change clinical practice until results of large high quality randomised controlled trials (RCTs) are available.

Quality of the evidence

The main limitations of individual studies were small sample size, failure to report blinded comparative data on adverse events and (in one case) failure to describe allocation concealment. When studies were combined there was high heterogeneity for the analysis of live birth, and findings for both live birth and clinical pregnancy were sensitive to choice of statistical model. The quality of the evidence for live birth and clinical pregnancy was rated as very low and low (respectively), using GRADE criteria (Summary of findings for the main comparison).

Potential biases in the review process

The findings were sensitive to methodological decisions made in the review process, and are therefore to be regarded very cautiously.

Agreements and disagreements with other studies or reviews

It has been suggested that heparin could potentially modulate many of the known mechanisms that underlie successful apposition, adhesion and penetration of the developing embryo. Heparin could improve the endometrial environment for implantation of embryo. Confirmation of the outlined potential of heparin to alter the molecular processes underpinning successful implantation was urgently required given the potential for clinical translation to increased pregnancy and live birth rate and a reduction in adverse perinatal outcomes for all women undergoing assisted reproduction (Nelson 2008).The following studies showed no efficacy of heparin in improving outcome.

- In one small non-randomised study, heparin with low-dose aspirin was given to women with antiphospholipid positive antibodies undergoing assisted reproduction. There were no statistically significant differences detected in implantation, pregnancy and ongoing pregnancy rates between both groups (Kutteh 1997).
- A double-blind, randomised cross-over trial was conducted to investigate whether heparin and low-dose aspirin increase the pregnancy rate in antiphospholipid antibody or antinuclear antibody-seropositive women with IVF implantation failure. Unfractionated heparin and low-dose aspirin were given from day of embryo transfer. It found that there was no significant difference in pregnancy rates or implantation rates between treated and placebo cycles. However, a cross-over design is not appropriate for a pregnancy trial (Stern 2003).
- Heparin was given to women with thrombophilia and repeated implantation failure undergoing assisted reproduction in this prospective cohort study. Authors suggested that it showed improvement in biochemical and clinical pregnancy rates. However, no precise data were published. This study also looked at other factors of implantation failure, therefore it cannot be inferred that this intervention of heparin only improved the success rate of assisted reproduction (Sharif 2010).

The American Society for Reproductive Medicine (Practice Committee of ASRM 2008) assessed available data in 2008 and suggested that assessment of antiphospholipid antibodies was not indicated among couples undergoing IVF, and heparin therapy was not justifiable on the basis of existing data to improve pregnancy and live birth rates.

In agreement with our review, Ricci 2010 suggested that heparin should not be used in women undergoing IVF until its efficacy is demonstrated in carefully designed RCTs.

Three published studies suggested that heparin did improve clinical outcome:

- One single centre non-randomised study found that heparin with low-dose aspirin given to women undergoing assisted reproduction with positive antiphospholipid antibodies showed improvement in live birth rate and clinical pregnancy rate Sher 1994.
- The same results were shown by a single centre case control study by the same author Sher 1998. However, these studies are non-randomised and significant bias was found.
- Lodigiani 2011 presented observational retrospective analysis of women with previous implantation failure and screened for thrombophilia undergoing assisted reproduction who were given LMWH showed significantly higher pregnancy rates. The results also showed that there was no relation between inherited thrombophilia and pregnancy rate in patients with previous IVF implantation failures. This was an observational retrospective study, which could be influenced by various other factors.

We found two reviews on this topic which also agree with our conclusions:

- Nardo 2009 suggested that clinicians should inform patients of factors including: our current lack of knowledge; potential adverse effects; and available weak evidence regarding adjuvant therapy during assisted reproduction. There was need for good clinical trials in many of the areas surrounding medical adjuncts in IVF to resolve the empirical/evidence divide.
- Bohlmann 2011 suggested that the available studies on heparin in assisted reproduction were characterised by heterogeneous inclusion criteria and a lack of proven effectiveness in special constellations. In conclusion, the application of heparin to improve assisted reproduction treatment (ART) outcome rates was not justified. A large RCT should be undertaken to answer this.

AUTHORS' CONCLUSIONS

Implications for practice

It is unclear whether peri-implantation heparin in assisted reproduction treatment (ART) cycles improves live birth and clinical pregnancy rates in subfertile women, as the evidence was sensitive to choice of statistical model and no benefit was apparent when a random effects model was used. Side effects have been reported with use of heparin and no firm conclusions can be drawn regarding its safety. Our results do not justify the use of heparin in this context, except in well-conducted research trials.

Implications for research

Well-designed RCTs with sufficient power are warranted to assess the efficacy of peri-implantation heparin in improving assisted reproduction outcomes. These should be large parallel-group RCTs with populations of subfertile women with unexplained infertility, recurrent failure of embryo implantation or a positive thrombophilia screen. No additional adjunct therapies should be used. Cross-over designs should always be avoided in trials where pregnancy is an intended outcome.

Studies should report data on adverse events in both study groups.

Studies should be done where local (uterine) rather than systemic heparin is used to see the effects of heparin on decidualisation, implantation and pregnancy rates in an attempt to avoid adverse effects.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Noci 2011

Methods	Multicentre Prospective randomised control pilot study
Participants	172 patients were allocated to intervention and divided into two groups: 86 women in the control group and 86 women in the treatment group. The final series for analysis contained 153 patients because 13 women belonging to treatment group and 6 women belonging to the control group had no embryos to transfer, thus they were immediately excluded from the study So in the final analysis 73 women were in treatment group (A) and 80 women were in the control group (B). Both groups were matched. Every woman was recruited for only one cycle. Cause infertility: variety of causes
Interventions	IVF or ICSI. The treatment group (A) received both luteal phase support with vaginal progesterone (Prometrium 200 mg twice per day) and a prophylactic dose of dalteparin sodium (Fragmin, 2500 IU s.c. daily; Pfizer Italia, Latina, Italy) from the afternoon of the day of oocyte retrieval until the day of pregnancy test. The control group (B) received luteal phase support with progesterone only until pregnancy test. Platelet count was performed on days7-8 of dalteparin treatment to evaluate possible adverse effects of the therapy. If platelet values dropped to below 50% of basal levels or <100,000/ μL, dalteparin administration was immediately stopped because of the risk of heparin induced thrombocytopenia COH: FSH, GNRH analogue. HCG 250 mcg. Luteal support: progesterone 200 mg pessaries vaginally twice daily until a pregnancy test was performed. If the test was positive, progesterone treatment was continued up to 12 gestational weeks
Outcomes	Live birth rate per woman: LMWH group (A): 21%, Control group (B): 16 % Adverse effect: Thrombocytopenia was not observed in any of the 73 patients treated with dalteparin and only a few patients reported the presence of minimal bruising at the injection point of the drug Clinical pregnancy rate per woman: LMWH group (A): 26%, Control group (B): 20% Multiple pregnancy rate per woman: LMWH group (A): 31.57%, Control group (B): 12.5% Implantation rate/ embryo transferred LMWH group (A): 15% Control group (B): 12% Spontaneous Miscarriage rate per woman: LMWH group (A): 21%, Control group (B) : 19%
Notes	Study population consisted of women aged < 40years, without congenital or acquired thrombophilia and undergoing their first IVF cycle

Risk of bias

Bias	Authors' judgement	Support for judgement

Noci 2011 (*Continued*)

Random sequence generation (selection bias)	Low risk	Computerised random sequence generation method was used
Allocation concealment (selection bias)	Low risk	Described clearly with sealed and numbered envelopes containing the allocation information
Blinding of participants and personnel for fertility outcomes	Unclear risk	Not described, but unclear whether lack of blinding could influence outcome
Blinding of outcome assessment for fertility outcomes	Low risk	The ultrasonography was performed by a gynaecologist unaware of the allocation of the patients
Blinding of participants and personnel for adverse effects outcome	High risk	Not described and lack of blinding could influence outcome
Blinding of outcome assessment for adverse effects outcome	High risk	Not described and lack of blinding could influence outcome
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	The study had a follow-up rate of 89% (153/172 women included in analysis)
Selective reporting (reporting bias)	Unclear risk	Describes selected adverse effects in intervention group, but no comparative data on adverse effects was reported
Other bias	Low risk	No other potential bias identified

Qublan 2008

Methods	Single centre Prospective randomised placebo controlled
Participants	States that of 137 women with a history of three or more previous IVF failures and who had at least one thrombophilic defect, adn who were randomised to heparin or placebo, 39 did not meet the inclusion criteria and 15 refused participation. The remaining 83 women were randomly allocated to each arm of the study. Randomisation was started on the day of ET
Interventions	The treatment group (A) (n = 42) had enoxaparin 40 mg/day subcutaneous injections. Control Group (B) (n = 41) received placebo (equivalent volume of NaCl 0.9% subcutaneous; Pharmaceutical Solutions Industry Ltd., Jeddah, SA). Treatment was started from the day of ET until results of Beta-hCG were available 2 weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed COH: HMG, GNRH antagonist. HCG 10,000 IU. Luteal support: Progesterone pessaries (Cyclogest: Alpharma, Barnstaple, UK) were used for luteal phase support in the

	two study groups
Outcomes	Live birth rate per woman: LMWH group (A): 23.8%, Control group (B): 2.4% Adverse effect: The frequency of complications did not differ between the two study groups. The most frequent complications encountered in the heparin-treated were bleeding (7.1%) followed by thrombocytopenia (4.8%), allergic reactions (2.4%) and placental abruption (2.4%) Pregnancy rate per woman: LMWH group (A): 31%, Control group (B): 9.6% Multiple pregnancy rate per woman: LMWH group (A): 23.1%, Control group (B): 25% Implantation rate/ embryo transferred LMWH group (A): 19.8% Control group (B): 6. 1% Spontaneous Miscarriage rate per woman: LMWH group (A): 7.7%, Control group (B) : 50% Intrauterine Fetal death rate: LMWH group (A) 15.4%, control group 0%
Notes	Study population consisted of women aged 19-35 years with a history of three or more previous IVF failures, and who had at least one thrombophilic defect

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Allocation was done by selection from table of random numbers
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding of participants and personnel for fertility outcomes	Low risk	Placebo used. States "only the subjects were blinded to the intervention" (Moreover it is unclear whether lack of blinding could influence this outcome)
Blinding of outcome assessment for fertility outcomes	Unclear risk	Not described, but unclear whether lack of blinding could influence outcome
Blinding of participants and personnel for adverse effects outcome	Low risk	Placebo used (equivalent volume of normal saline). States "only the subjects were blinded to the intervention"
Blinding of outcome assessment for adverse effects outcome	High risk	Not described and lack of blinding could influence outcome
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Reporting in trial publication is inconsistent. States that 137 women were randomised and subsequently states that 83 were randomised

Qublan 2008 (*Continued*)

Selective reporting (reporting bias)	Unclear risk	Describes "most frequent" complications in each group, but no comparative data on adverse effects was reported
Other bias	Low risk	No other potential bias identified

Urman 2009

Methods	Single centre Open labelled randomised controlled pilot trial
Participants	150 consecutive couples who met the inclusion criteria and gave informed consent were recruited to the trial. Each woman was included for one cycle only. 3 women in the LMWH and control group each were lost to follow-up before completion of the initially planned follow-up period (completion of the 20th gestational week for the latest recruited participant that achieved an ongoing pregnancy), and another 2 women in the LMWH group were lost to follow-up after completion of the 20th gestational week but before delivery or expected completion of the 40th gestational week. 75 women in each arm of the study
Interventions	ICSI. The study group was administered LMWH group (A) (Enoxaparin Sodium, Clexane, Aventis Pharma) at a dose of 1 mg/kg/day starting on the day after oocyte retrieval. Patients' weights were rounded to the closest multiple of 10 kg, and 0.1 mL/10 kg/day Clexane was self-administered subcutaneously by the participants. LMWH was discontinued if the pregnancy test 12 days after ET was negative, but continued up to the 12th week of pregnancy if the test was positive. The control group (B) received no medication besides progesterone gel. In the study group the platelet count was done on the day of oocyte retrieval and 1 week after commencement of LMWH treatment COH: FSH, GNRH agonist. HCG 10,000 IU. Luteal support: Progesterone pessaries 90 mg vaginal progesterone gel (Crinone 8%, Serono, Serono, Bedfordshire, UK) starting from the day of oocyte collection. LPS was continued until the pregnancy test performed 12 days after ET. Women with a positive pregnancy test continued the vaginal progesterone gel until the 12th week of gestation
Outcomes	Live birth rate per woman: LMWH group (A): 34.7%, Control group (B): 26.7% Adverse effect: Platelet counts did not change significantly in the LMWH group during the study period. Small ecchymoses around the LMWH injection sites were noted Clinical Pregnancy rate per woman: LMWH group (A): 45.3%, Control group (B): 38. 7% Ongoing Pregnancy rate per woman: LMWH group (A): 37.3%, Control group (B): 26.7% Multiple pregnancy rate per woman: LMWH group (A): 35.3%, Control group (B): 34. 5% Implantation rate/ embryo transferred LMWH group (A): 24.5% Control group (B): 19.8% Numbers of preterm deliveries were (34.6%) in LMWH and (30.0%) in control groups

Notes	Study population consisted of women aged < 38 years with a history of two or more previous IVF failures. Women lost to follow-up during the first period were considered not to have an ongoing pregnancy, and women lost to follow-up in the second period were considered not to have a live birth in the intention-to-treat analysis

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Women were randomised according to a computer-generated randomisation list. Study subjects were randomised in blocks of 10; i.e. of every 10 women randomised, five were allocated to the LMWH arm, and five were allocated to the control arm, in a random manner
Allocation concealment (selection bias)	Low risk	Opaque envelopes that were numbered and sealed containing the allocation information were given to the ART centre nurse coordinator who assigned patients to study arms following recruitment by attending physicians on the morning of oocyte retrieval procedure
Blinding of participants and personnel for fertility outcomes	Unclear risk	Open label but unclear whether lack of blinding could influence outcome
Blinding of outcome assessment for fertility outcomes	Unclear risk	Open label, but unclear whether lack of blinding could influence outcome
Blinding of participants and personnel for adverse effects outcome	High risk	Open label and lack of blinding could influence outcome
Blinding of outcome assessment for adverse effects outcome	High risk	Open label and lack of blinding could influence outcome
Incomplete outcome data (attrition bias) All outcomes	Low risk	This study compensated for dropouts by imputing a negative outcome to losses to follow-up
Selective reporting (reporting bias)	Unclear risk	Adverse effects in the intervention group were described but it was unclear to what extent adverse effects in the control group were assessed and no clear comparative data were reported
Other bias	Low risk	No other potential bias identified

COH: controlled ovarian hyperstimulation
ET: embryo transfer
FSH: follicle-stimulating hormone
GNRH: gonadotropin-releasing hormone
HCG: human chorionic gonadotropin
ICSI: intracytoplasmic sperm injection
IVF: in vitro fertilisation
IU: international units
LMWH: low molecular weight heparin
LPS: lipopolysaccharide,
NaCl: sodium chloride
s.c.: subcutaneous

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Berker 2011	Not a True RCT as quasi randomisation was performed for the purposes of this study
Colicchia 2011	Low molecular weight heparin (LMWH) was used in conjunction with prednisolone
Stern 2003	Unfractionated heparin (UFH) was used in conjunction with low-dose aspirin. Cross-over design study

RCT: randomised controlled trial

Characteristics of studies awaiting assessment *[ordered by study ID]*

Mashayekhy 2011

Methods	Single centre Prospective randomised controlled trial
Participants	86 patients with recurrent IVF-ET failure.
Interventions	Ovarian stimulation was performed with long protocol. The patients were randomly divided into two groups after embryo transfer, and one group received unfractionated heparin 5000 IU twice a day plus 100 mg progesterone and another group only received progesterone
Outcomes	There were no significant differences between individual characteristics of two groups. However, implantation rate and clinical pregnancy were significantly higher in patients who received unfractionated heparin. Thirty-six women had at least one thrombophilic mutation
Notes	Only the abstract has been published in The Iranian Journal of Reproductive Medicine spring 2011;9 (Suppl 2):30-30 The authors were contacted regarding the details of study results. The study is presently not able to be included in

	the review as it has been completed and submitted for publication. The authors were unable to provide me with the details of results till publication

ET: embryo transfer
IU: international units
IVF: iv vitro fertilisation

DATA AND ANALYSES

Comparison 1. Heparin versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live Birth Rate per woman	3	386	Odds Ratio (M-H, Fixed, 95% CI)	1.77 [1.07, 2.90]
2 Sens analysis Live Birth Rate (random effects)	3	386	Odds Ratio (M-H, Random, 95% CI)	1.85 [0.80, 4.24]
3 Clinical Pregnancy Rate per woman	3	386	Odds Ratio (M-H, Fixed, 95% CI)	1.61 [1.03, 2.53]
4 Sens analysis Clinical Pregnancy Rate (random effects)	3	386	Odds Ratio (M-H, Random, 95% CI)	1.66 [0.94, 2.90]

Analysis 1.1. Comparison 1 Heparin versus control, Outcome 1 Live Birth Rate per woman.

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 1 Live Birth Rate per woman

Study or subgroup	Heparin n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Noci 2011	15/73	13/80		41.6 %	1.33 [0.59, 3.03]
Qublan 2008	10/42	1/41		3.3 %	12.50 [1.52, 102.85]
Urman 2009	26/75	20/75		55.1 %	1.46 [0.73, 2.93]
Total (95% CI)	**190**	**196**		**100.0 %**	**1.77 [1.07, 2.90]**

Total events: 51 (Heparin), 34 (Control)

Heterogeneity: $Chi^2 = 4.05$, df = 2 (P = 0.13); $I^2 = 51\%$

Test for overall effect: Z = 2.24 (P = 0.025)

Test for subgroup differences: Not applicable

Analysis 1.2. Comparison 1 Heparin versus control, Outcome 2 Sens analysis Live Birth Rate (random effects).

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 2 Sens analysis Live Birth Rate (random effects)

Study or subgroup	Heparin n/N	Control n/N	Odds Ratio M-H,Random,95% CI	Weight	Odds Ratio M-H,Random,95% CI
Noci 2011	15/73	13/80		41.1 %	1.33 [0.59, 3.03]
Qublan 2008	10/42	1/41		12.7 %	12.50 [1.52, 102.85]
Urman 2009	26/75	20/75		46.2 %	1.46 [0.73, 2.93]
Total (95% CI)	**190**	**196**		**100.0 %**	**1.85 [0.80, 4.24]**

Total events: 51 (Heparin), 34 (Control)

Heterogeneity: $Tau^2 = 0.26$; $Chi^2 = 4.05$, df = 2 (P = 0.13); $I^2 = 51\%$

Test for overall effect: Z = 1.45 (P = 0.15)

Test for subgroup differences: Not applicable

Analysis 1.3. Comparison 1 Heparin versus control, Outcome 3 Clinical Pregnancy Rate per woman.

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 3 Clinical Pregnancy Rate per woman

Study or subgroup	Heparin n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Noci 2011	19/73	16/80		37.7 %	1.41 [0.66, 3.00]
Qublan 2008	13/42	4/41		9.3 %	4.15 [1.22, 14.07]
Urman 2009	34/75	29/75		52.9 %	1.32 [0.69, 2.52]
Total (95% CI)	**190**	**196**		**100.0 %**	**1.61 [1.03, 2.53]**

Total events: 66 (Heparin), 49 (Control)

Heterogeneity: Chi^2 = 2.80, df = 2 (P = 0.25); I^2 =29%

Test for overall effect: Z = 2.08 (P = 0.037)

Test for subgroup differences: Not applicable

Analysis 1.4. Comparison 1 Heparin versus control, Outcome 4 Sens analysis Clinical Pregnancy Rate (random effects).

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 4 Sens analysis Clinical Pregnancy Rate (random effects)

Study or subgroup	Heparin n/N	Control n/N	Odds Ratio M-H,Random,95% CI	Weight	Odds Ratio M-H,Random,95% CI
Noci 2011	19/73	16/80		37.1 %	1.41 [0.66, 3.00]
Qublan 2008	13/42	4/41		17.8 %	4.15 [1.22, 14.07]
Urman 2009	34/75	29/75		45.1 %	1.32 [0.69, 2.52]
Total (95% CI)	**190**	**196**		**100.0 %**	**1.66 [0.94, 2.90]**

Total events: 66 (Heparin), 49 (Control)

Heterogeneity: Tau^2 = 0.07; Chi^2 = 2.80, df = 2 (P = 0.25); I^2 =29%

Test for overall effect: Z = 1.76 (P = 0.079)

Test for subgroup differences: Not applicable

ADDITIONAL TABLES

Table 1. Table of Comparisons: Implantation rate per embryos transferred

Study ID	Heparin group	Control group
Noci 2011	15%	12%
Urman 2009	24.5%	19.8%
Qublan 2008	19.8%	6.1%

Table 2. Table of Comparisons: Incidence of miscarriage per total number of pregnancies and per woman

Study ID	Heparin group per pregnancy	Control group per pregnancy	Heparin group per woman	Control group per woman
Noci 2011	4/19	3/16	4/73	3/80
Urman 2009	n/a	n/a	n/a	n/a
Qublan 2008	1/13 *IUFD 2/13	2/4 *IUFD 0/4	1/42 *IUFD 2/42	2/41 *IUFD 0/41

IUFD: Intraunterine fetal death

Table 3. Table of Comparisons: Incidence of multiple pregnancies per total number of pregnancies

Study ID	Heparin group	Control group
Noci 2011	(6/19) 31.5%	(2/16) 12.5%
Urman 2009	(12/34) 35.3%	(10/29) 34.5%
Qublan 2008	(3/13) 23.1%	(1/4) 25%

APPENDICES

Appendix 1. CENTRAL search strategy

Menstrual Disorders and Subfertility Group Specialised Register (inception to 2 July 2012)
Ovid the Cochrane Central Register of Controlled Trials (CENTRAL) (inception to 2 July 2012)
There is no language restriction in these search.

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.
13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.
22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24 (8324)
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38

Appendix 2. MEDLINE search strategy

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1950 to 2 July 2012)

The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomized trials which appears in the *Cochrane Handbook of Systematic Reviews of Interventions (version 5.0.2; chapter 6, 6.4.11)*

There is no language restriction in this search

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.
13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.
22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38
40 randomized controlled trial.pt.
41 controlled clinical trial.pt.
42 randomized.ab.
43 placebo.tw.
44 clinical trials as topic.sh.
45 randomly.ab.
46 trial.ti.

47 (crossover or cross-over or cross over).tw.
48 or/40-47
49 exp animals/ not humans.sh.
50 48 not 49
51 39 and 50

Appendix 3. EMBASE search strategy

Ovid EMBASE (01.01.10 to 2 July 2012)
EMBASE is only searched one year back as the UKCC has hand searched EMBASE to this point and these trials are already in CENTRAL.
The EMBASE search is combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) http://www.sign.ac.uk/mehodology/filters.html#random
There is no language restriction in this search
1 exp embryo transfer/ or exp female infertility/ or exp fertilization in vitro/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp artificial insemination/
11 artificial$ inseminat$.tw.
12 reproductive technique$.tw.
13 reproduct$ technolog$.tw.
14 exp nidation/
15 (implant$ adj2 fail$).tw.
16 reproduct$ technique$.tw.
17 ((Female$ or women) adj2 infertil$).tw.
18 ((Female$ or women) adj2 subfertil$).tw.
19 exp recurrent abortion/
20 recurrent miscarriage.tw.
21 iui.tw.
22 intrauterine insemination.tw.
23 nidation.tw.
24 exp intracytoplasmic sperm injection/
25 or/1-24
26 exp HEPARIN/ or exp LOW MOLECULAR WEIGHT HEPARIN/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37

39 25 and 38
40 Clinical Trial/
41 Randomized Controlled Trial/
42 exp randomization/
43 Single Blind Procedure/
44 Double Blind Procedure/
45 Crossover Procedure/
46 Placebo/
47 Randomi?ed controlled trial$.tw.
48 Rct.tw.
49 random allocation.tw.
50 randomly allocated.tw.
51 allocated randomly.tw.
52 (allocated adj2 random).tw.
53 Single blind$.tw.
54 Double blind$.tw.
55 ((treble or triple) adj blind$).tw.
56 placebo$.tw.
57 prospective study/
58 or/40-57
59 case study/
60 case report.tw.
61 abstract report/ or letter/
62 or/59-61
63 58 not 62
64 39 and 63
65 (2010$ or 2011$).em.
66 64 and 65

Appendix 4. PsycINFO search strategy

Ovid PsycINFO (1806 to 2 July 2012)
There is no language restriction in this search

1 exp Reproductive Technology/
2 exp Infertility/
3 exp Embryo/
4 embryo transfer$.tw.
5 in vitro fertili?ation.tw.
6 ivf-et.tw.
7 (ivf or et).tw.
8 icsi.tw.
9 intracytoplasmic sperm injection$.tw.
10 (blastocyst adj2 transfer$).tw.
11 (assist$ adj2 reproducti$).tw.
12 artificial$ inseminat$.tw.
13 iui.tw.
14 intrauterine insemination.tw.
15 nidation.tw.
16 reproductive technique$.tw.
17 reproduct$ technolog$.tw.
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.

20 ((Female$ or women) adj2 infertil$).tw.
21 ((Female$ or women) adj2 subfertil$).tw.
22 exp Spontaneous Abortion/
23 recurrent miscarriage$.tw.
24 or/1-23
25 exp Heparin/
26 heparin$.tw.
27 LMWH$.tw.
28 liquemin.tw.
29 enoxaparin.tw.
30 heparinic acid.tw.
31 dalteparin.tw.
32 tinzaparin.tw.
33 clexane.tw.
34 lovenox.tw.
35 indenox.tw.
36 xaparin.tw.
37 or/25-36
38 24 and 37

WHAT'S NEW

Last assessed as up-to-date: 6 May 2013.

Date	Event	Description
4 September 2014	Amended	Second summary of findings table added, using random effects model. Abstract edited. No change to overall conclusions

CONTRIBUTIONS OF AUTHORS

Akhtar Muhammad A (Co-first author)

All correspondence with drafting of the protocol, develop a search strategy, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

Sur Shyamaly (Co-first author)

Drafting of the protocol, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

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Drafting of the protocol, select which trials to include, carry out the analysis, interpret the analysis, draft the final review and update the review.

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Quenby Siobhan

Drafting of the protocol, select which trials to include, help in carrying out the analysis, interpret the analysis, draft the final review and update the review.

DECLARATIONS OF INTEREST

The review authors have no commercial interest to disclose.

SOURCES OF SUPPORT

Internal sources

- Cochrane Menstrual Disorders and Subfertilty Group, New Zealand.
- Department of Obstetrics and Gynaecology, University of Auckland, New Zealand.
- Clinical Reproductive Medicine Unit, University Hospitals Coventry & Warwickshire NHS Trust, UK.
- Division of Obstetrics & Gynaecology, School of Clinical Sciences, University of Nottingham, UK.
- Clinical Sciences Research Institute, University of Warwick, UK.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Biological pregnancy rates and ongoing pregnancy rates per woman were included in the protocol but not in the review, as these outcome measures are not as important from a patient perspective as live birth rates and clinical pregnancy rates. We made these changes on the advice of the MDSG Co-ordinating Editor.

INDEX TERMS

Medical Subject Headings (MeSH)

*Birth Rate; *Embryo Implantation; *Live Birth; *Reproductive Techniques, Assisted; Anticoagulants [*administration & dosage; adverse effects]; Drug Administration Schedule; Embryo Transfer; Heparin, Low-Molecular-Weight [*administration & dosage; adverse effects]; Randomized Controlled Trials as Topic

MeSH check words

Female; Humans; Pregnancy