Table S1. Additional clinical data on the eight patients with heparin-induced thrombocytopenia treated with fondaparinux.

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Efficacy of low molecular weight heparin in patients undergoing *in vitro* fertilization or intracytoplasmic sperm injection

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In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have become widely used techniques. Despite their current popularity, the success rates of these techniques remain low [1]. The clinical pregnancy rate among women undergoing fresh embryo transfer (ET) after IVF or ICSI varies from 20% to 35% per transfer [1,2].

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Suboptimal uterine perfusion has been reported as a possible cause of infertility [3], suggesting the potential role for antithrombotic therapies in this setting. Previous studies failed to show a beneficial effect of aspirin [4]. Heparin may have a positive effect in conception and early pregnancy events, because of its ability to alter the hemostatic response to ovarian stimulation, modulate trophoblast differentiation and invasion, and decrease the risk of thrombosis [5]. Thus, we performed a systematic review and meta-analysis of the literature to investigate the effect of heparin on hard outcomes, including pregnancy rate and rate of live birth, in women undergoing IVF or ICSI.

We searched for studies using the MEDLINE and EMBASE (up to July 2011) electronic databases. The search strategy was developed without any language restriction, using

medical subjects headings and keywords. Research was supplemented by manually reviewing reference lists of all retrieved articles and abstract books from the Congress of the ISTH and the European Society of Human Reproduction and Embryology (ESHRE) Scientific Meetings. Only randomized controlled trials comparing unfractionated heparin or low molecular weight heparin (LMWH) with placebo or no therapy in women undergoing IVF or ICSI were included. Studies including patients with and without known thrombophilic abnormalities were considered eligible.

The following data were extracted: study (year of publication, design, and study center), patient characteristics (number of subjects enrolled, and mean or median age), and the aforementioned clinical outcomes. Study identification, study selection and data extraction were performed independently in duplicate by two reviewers. When there was a discrepancy between the reviewers, this was resolved by discussion or by the opinion of a third reviewer, as necessary. The agreement between the reviewers was calculated by use of the κ-statistic [6]. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated for each outcome under the assumption of fixed effects model with Review Manager 5 software. Analyses were repeated with a random effects model. Statistical heterogeneity was evaluated with the I^2-statistic [7]. Studies including patients with known thrombophilic abnormalities were excluded from the sensitivity analysis, as ET failure may have a different pathophysiologic mechanism [8]. Study quality was evaluated by considering the appropriateness of randomization, the presence of blinding, and the concealment of allocation.

A total of 535 (118 MEDLINE and 417 EMBASE) citations and 21 abstracts from the ISTH and ESHRE congresses were identified by our systematic search (search strategies and results are available upon request). After screening of the title and abstract with the predefined inclusion and exclusion criteria, seven studies were retrieved for more detailed evaluation [9–15]. Three studies were excluded because they assessed concomitant treatment with heparin and aspirin vs. placebo or no treatment [13–15], and one because it was not a randomized trial [9]; therefore, three studies with a total of 305 women were included in our systematic review [10–12]. The interobserver agreement for the study selection was good (κ = 0.79). Baseline characteristics of the included studies are summarized in Table 1. LMWH was the heparin used in all three studies. The method of randomization was adequate in all three studies [10–12]. One study was placebo-controlled [12], whereas the other two were open-label [10,11]. The allocation concealment was adequate in two studies [10,11] and unclear in one study [12]. Two studies evaluated the role of LMWH in patients without known thrombophilia [10,11], whereas only thrombophilic patients were included in one study [12]. Prophylactic LMWH dosage was used in two studies [10,12] and an intermediate dosage (i.e. half the therapeutic dose) in one study [11]. LMWH was continued up to 9–12 weeks of

Table 1 Baseline characteristics and treatment of patients included in the studies

Study, year	No. of patients included	Mean age (years)	Inclusion criteria	Primary outcome	Treatment and dose	Onset and duration of therapy
Noci, 2011 [10]	172	34.7 ± 3.6 vs. 35.1 ± 3.1	Patients undergoing IVF or ICSI Age < 40years Absence of thrombophilic abnormalities	Live birth ET	Dalteparin Sodium 2500 U vs. no treatment	On the day of oocyte retrieval and up to the day of the pregnancy test or up to the ninth week of pregnancy in the case of positive HCG
Urman, 2009 [11]	150	34.0 ± 5.0 vs. 34.8 ± 5.8	History of ≥ 2 previously failed fresh ET cycles, as demonstrated by negative serum β-HCG levels Age 38 years Fresh ejaculate sperm to be used for ICSI No hormonal, coagulation or immunologic disorders detected Normal uterine cavity, as assessed by hysteroscopy or saline infusion sonography Normal peripheral karyotype	Clinical pregnancy Live birth	Enoxaparin 1 mg kg^{-1} day^{-1} vs. no treatment	On the day of oocyte retrieval and up to the 12th gestational week in pregnant participants
Qublan, 2008 [12]	83	29.0 ± 6.3 vs. 29.2 ± 6.1	History of ≥ 3 previous IVF failures At least one thrombophilic abnormality	Implantation Clinical pregnancy Live birth	Enoxaparin 40 mg vs. placebo (NaCl 0.9%)	On the day of ET and up to delivery or fetal demise

ET, embryo transfer; HCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization.

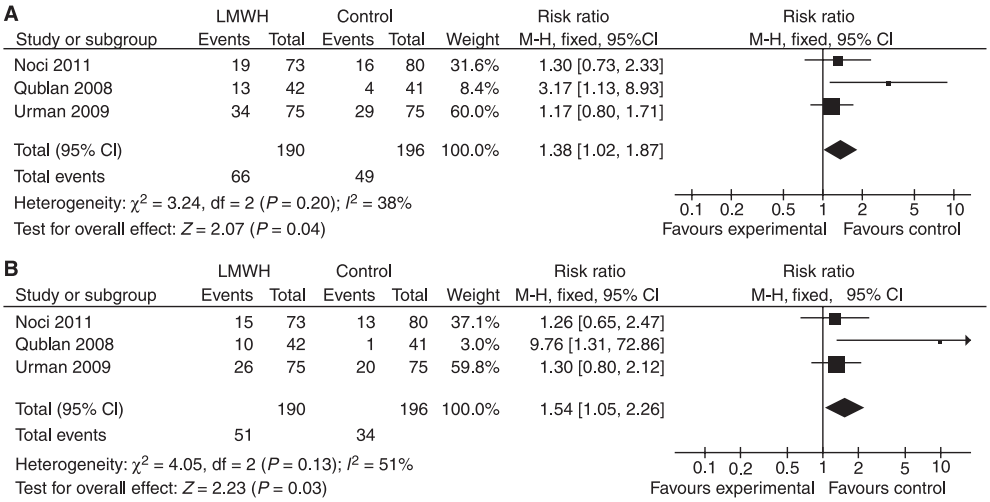

Fig. 1. Pooled relative risk and 95% confidence intervals (CIs) of the association between low molecular weight heparin (LMWH) vs. placebo or no treatment and pregnancy rate (A) and live birth (B). d.f., degrees of freedom; M-H.

gestation in two studies [10,11], and until delivery or fetal demise in one study [12]. Patients with previous failed reproduction treatment cycles were included in two studies [11,12], whereas the third study included patients undergoing a first IVF cycle [10].

The use of LMWH was associated with a higher rate of clinical pregnancies (RR 1.38, 95% CI 1.02–1.87; $P = 0.04$) and live births (RR 1.54, 95% CI 1.05–2.26) Fig. 1. Heterogeneity among the studies was moderate, but not significant ($I^2 = 38\%$ and $I^2 = 51\%$, respectively). When the analyses were repeated with the random effects model, the differences were no longer statistically significant ($P = 0.19$ and $P = 0.13$). Finally, sensitivity analyses excluding patients with known thrombophilic abnormalities showed similar but no longer statistically significant differences in the two outcomes ($P = 0.23$ and $P = 0.21$, respectively).

The results of this systematic review suggest that LMWH may be effective in increasing the rates of clinical pregnancies and live births in patients undergoing IVF or ICSI. However, these results should be interpreted with extreme caution, because only three, relatively small, trials explored this important issue, there is a fair degree of statistical and clinical heterogeneity in the included studies, and when the analyses were repeated with a random effects model, an approach that takes into account variance among the studies, differences were no longer statistically significant.

LMWH may act by preventing microthromboses that negatively impact on the implantation process and placental development [5]. Other potential mechanisms include modulation of differentiation and trophoblastic invasion, and the increase in the level of free insulin-like growth factor that is involved in the implantation process [16].

Non-randomized studies on this topic have led to inconsistent results [9,17]. The use of LMWH did not provide any beneficial effect on pregnancy outcomes in patients with two or more implantation failures [9]. On the other hand, in a more recent study, the use of LMWH was associated with a significantly higher pregnancy rate in patients with at least two IVF/ICSI cycles independently of the presence of thrombophilic abnormalities [17].

Although the evidence for the independent role of thrombophilic abnormalities in this setting is not compelling [18,19], whether LMWH is effective in patients without thrombophilic abnormalities remains to be established, as the differences between the pooled results of the two studies including non-thrombophilic patients were not statistically significant.

Furthermore, other factors, including maternal age at the time of conception and the number of previous failures, could influence the outcome of a pregnancy, and future studies should explore the role of LMWH in these different groups of patients.

In conclusion, our results suggest that the administration of LMWH may increase clinical pregnancy and live birth rates in women undergoing IVF or ICSI, although these results should be interpreted with caution, owing to the clinical and statistical heterogeneity among the studies. Thus, larger studies are warranted to evaluate the efficacy of LMWH in patients with and without thrombophilic abnormalities and in patients with and without previous implantation failure.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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In vivo von Willebrand factor size heterogeneity in spite of the clinical deficiency of ADAMTS-13

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von Willebrand factor (VWF) is synthesized by endothelial cells as ultra-large (UL) multimers. The activity of VWF is strongly correlated with multimer size and UL-VWF can spontaneously bind platelet GPIb. As the circulation of UL-VWF can lead to microvascular thrombosis, these hyperactive multimers are digested upon release into smaller, less active fragments by ADAMTS-13. It is generally assumed that VWF proteolysis generates a typical pattern consisting of a complex set of repeating multimers, characterized by a more prominent band surrounded by satellite bands ('triplets'), visible upon VWF analysis by SDS-agarose gel electrophoresis [1]. At least *in vitro*, there is clear evidence that ADAMTS-13 is responsible for the generation of these 'triplets', also often referred to as VWF size heterogeneity [2,3]. However, whether or not ADAMTS-13 is required for the generation of *in vivo* VWF size heterogeneity has never been systematically studied. To clarify the association between VWF size heterogeneity and ADAMTS-13 activity *in vivo*, we used a sensitive VWF multimer assay to analyze multimer patterns of VWF present in plasma of ADAMTS-13-deficient human and murine plasma.

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