

Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial

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BACKGROUND: The pathology underlying recurrent implantation failures (RIF) is not clear and treatment options proposed are generally not evidence based. Although the effect of heparin on trophoblast biology has not been studied extensively, given the available data suggesting a possible beneficial effect of heparin on embryo implantation, we decided to undertake this pilot study.

METHODS: One hundred and fifty women with ≥ 2 failed assisted reproduction treatment cycles were included in this randomized open-label pilot trial. Participants underwent controlled ovarian stimulation with the long protocol and were randomly allocated to receive 1 mg/kg/day low molecular weight heparin (LMWH) or no treatment in addition to routine luteal phase support (LPS) on the day after oocyte retrieval. LPS and LMWH was continued up to the 12th gestational week in pregnant participants.

RESULTS: There were 26 (34.7%) live births in the LMWH group, and 20 (26.7%) in the control group (absolute difference 8.0%, 95% CI –4.2 to 24.9%, $P = 0.29$). There were 34 (45.3%) and 29 (38.7%) clinical pregnancies in the LMWH and control groups, respectively (absolute difference 6.6%, 95% CI –9.0 to 21.8%, $P = 0.41$). Implantation rates were 24.5 and 19.8% in the LMWH and control groups, respectively (absolute difference 4.7%, 95% CI –4.7 to 14.1%, $P = 0.33$).

CONCLUSION: Despite lack of statistical significance, observed relative increase by 30% in live birth rates with LMWH may be regarded as a clinically significant trend necessitating further research on the use of empirical LMWH in women with RIF and possibly in all women undergoing assisted reproduction treatment. Failure to demonstrate statistical significance of the observed treatment difference may be due to limited sample size of this pilot study.

Clinicaltrials.gov registration number: nCT00750451.

Key words: *in vitro* fertilization / assisted reproduction / recurrent implantation failure / heparin / randomized controlled trial

Introduction

The efficacy of assisted reproduction therapy (ART) in terms of live birth rates has remained relatively constant showing only gradual improvements over the years (Andersen *et al.*, 2007). The rate-limiting step appears to be implantation of the pre-embryo which is a complex process dependent upon many variables, most of which have not been adequately defined. Implantation requires orchestration of multiple events including trophoblast development and timely expression of numerous molecules playing roles in apposition, nidation and invasion

of the embryo into the endometrium. Failure of implantation in couples undergoing assisted reproduction is a relatively common occurrence despite the transfer of seemingly good quality embryos. Implantation failure occasionally may be a recurring phenomenon leading to despair in couples and frustration in their caregivers. Implantation failure has been attributed to many factors; however, for most, the causal relationship is not established. Although not all studies concur, congenital and acquired coagulation defects have been found to be more prevalent in women with recurrent implantation failures (RIF) (Sher *et al.*, 1998b; Martinelli *et al.*, 2003a, b; Stern *et al.*,

2003; Qublan *et al.*, 2006; Stern and Chamley, 2006). The notion that coagulation disorders may lead to implantation failure led to the use of anticoagulants mainly heparin during the course of ART treatment. Several studies have examined the effect of heparin on the outcome of ART in women with antiphospholipid antibodies (Sher *et al.*, 1994; Schenk *et al.*, 1996; Kutteh *et al.*, 1997; Sher *et al.*, 1998a; Stern *et al.*, 2003). Despite the heterogeneity of studies with regard to selection of participants and interventions, results suggest a potential for improvement in outcome with anticoagulant therapy.

More recently, heparin has been proposed to play a role in the complicated process of implantation beyond its anticoagulant effects (Fiedler and Wurfel, 2004; Nelson and Greer, 2008). Several lines of evidence from *in vivo* and *in vitro* studies suggest a beneficial effect of heparin on embryo implantation through interactions with several adhesion molecules, growth factors, cytokines and enzymes such as matrix metalloproteinases (Nelson and Greer, 2008).

Given the available data on heparin and embryo implantation we decided to undertake a pilot clinical trial with the aim to assess a possible beneficial effect of low molecular weight heparin (LMWH) on the outcome of ART in women with RIF but without hereditary or acquired thrombophilias. Couples with RIFs of unexplained etiology were randomly allocated to receive LMWH or no treatment in addition to routine luteal phase support (LPS) following oocyte retrieval (OR).

Materials and Methods

Study design and study population

This was an open-label, randomized, controlled pilot trial to assess whether administration of LMWH in the luteal phase after ICSI and embryo transfer cycles would increase implantation and pregnancy rates in women with RIF. The study was undertaken in the American Hospital of Istanbul Assisted Reproduction Unit between January 2006 and May 2008. The last participant was recruited in January 2008, and the follow-up was completed in January 2009. Although, the follow-up was initially planned to be terminated upon women's completion of the 20th gestational week, due to the fact that some of them had already delivered before the end of the planned follow-up period, it was continued until all participants delivered.

Institutional review board of the American Hospital approved the study and informed consents were obtained individually from all couples.

All couples were thoroughly re-evaluated prior to initiation of the new treatment cycle. Serum thyroid stimulating hormone and prolactin assays, screening for coagulation disorders (the presence of Factor V Leiden mutation, Prothrombin gene mutation, methylene tetrahydrofolate reductase (MTHFR) gene mutation, or abnormal levels of anticardiolipin IgG, IgM, and lupus anticoagulant, antithrombin, protein C and protein S) were done.

The inclusion criteria for the study were as follows:

- (i) History of at least two previously failed fresh embryo transfer cycles, as demonstrated with negative serum beta human chorionic gonadotrophin (hCG) levels.
- (ii) All previously failed cycles to be performed in the American Hospital of Istanbul.
- (iii) Female age ≤ 38 years.
- (iv) Fresh ejaculate sperm to be used for ICSI.
- (v) No hormonal, coagulation or immunological disorders detected.

- (vi) Normal uterine cavity, as assessed by hysteroscopy or saline infusion sonography.
- (vii) Normal female and male peripheral karyotype.

Women requiring anticoagulant therapy for other medical reasons, obvious causes of implantation failure (hydrosalpinx visible on transvaginal ultrasound, fibroids distorting the uterine cavity, the absence of Grade I or Grade II embryos available for transfer) or clinical or laboratory findings of congenital or acquired thrombophilia were excluded.

One hundred and fifty consecutive couples who met the inclusion criteria and gave informed consent were recruited to the trial. Each woman was included for one cycle only.

Laboratory tests

Mutations of the MTHFR, Factor V and Prothrombin genes were assessed with LightCycler[®] 2.0 Realtime PCR system (Roche Applied Science). Genotypes were identified by running melting curves with specific melting points. The activities of protein C and protein S were measured by a functional clotting assay (Dade Behring, Marburg, Germany). Plasma antithrombin activity was measured with the use of Berichrom (Dade Behring, Liederbach, Germany). Resistance to activated protein C was determined with a kit (Pro C Global[®], Dade Behring, Marburg, Germany). Simplified Dilute Russel Viper Venom test reagents (Dade Behring, Marburg, Germany) were used for detection of lupus anticoagulant with one stage clotting tests. Anticardiolipin antibody levels were measured with a commercial enzyme-linked immunosorbent assay kit in accordance with manufacturer's instructions (Euroimmun, Medizinische Labordiagnostika AG, Lübeck, Germany).

The criterion for the diagnosis of deficiencies of protein C, protein S and antithrombin was an activity level below the lower limit of normal (cutoff, 95th percentile), as previously determined by the laboratory. The lower limits of normal were 70% for protein C activity, 60% for protein S activity and 75% for antithrombin activity.

Randomization protocol

Women were randomized according to a computer-generated randomization list prepared by one of the investigators (BA). Study subjects were randomized in blocks of 10; i.e. of every 10 subjects randomized, five were allocated to the LMWH arm, and five were allocated to the control arm, in a random manner. Opaque envelopes that were numbered and sealed containing the allocation information were given to the ART center nurse coordinator who assigned patients to study arms following recruitment by attending physicians on the morning of OR procedure.

Interventions

Ovarian stimulation was undertaken with a long gonadotrophin releasing hormone agonist (GnRHa) protocol combined with recombinant human follicle stimulating hormone (rhFSH) (Gonal-F, Serono, Bari, Italy). Final maturation of the oocytes was induced with hCG (Pregnyl, Organon, Istanbul, Turkey). hCG 10 000 IU was administered intramuscularly when the leading follicle reached 20 mm in the mean diameter accompanied by ≥ 2 follicles of > 16 mm. Stimulation protocols and the indications for hCG injection or cycle cancellation did not change throughout the study period.

OR was performed 36 h after the administration of hCG. ICSI was used to fertilize the oocytes. Cleavage stage embryos were graded according to Hardarson *et al.* (2001). Embryos were transferred on the third day after ICSI. A maximum of four embryos were transferred.

The luteal phase was supported with 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 embryo transfer. Women with a positive pregnancy test continued the vaginal progesterone gel until the 12th week of gestation.

The study group was administered LMWH (Enoxaparin Sodium, Clexane, Aventis Pharma) at a dose of 1 mg/kg/day starting on the day after OR. Pre-filled syringes that contained 100 mg enoxaparin sodium per 1 ml were included in trial medication packages. 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 embryo transfer was negative, but continued up to the 12th week of pregnancy if the test was positive. The control group received no medication besides progesterone gel. In the study group platelet count was done on the day of OR and 1 week after commencement of LMWH treatment.

Women with high-order pregnancies at the end of the 12th gestational week were offered fetal reduction with transabdominal ultrasound guided intracardiac potassium chloride injection in the 13th gestational week.

Outcome measures

Pregnancy was confirmed by measuring serum beta-hCG levels 12 days after embryo transfer. Clinical pregnancy was defined as the presence of a fetus with a heartbeat at 6 weeks of gestation, a multiple pregnancy was defined as a gestation with more than one fetus, and ongoing pregnancy was defined as pregnancy proceeding beyond the 20th gestational week. Live birth was defined as delivery of one or more live infants. Preterm delivery was defined as delivery before completion of 37th week of gestation. Implantation rate was calculated separately for each participant as number of gestational sacs divided by number of transferred embryos multiplied by 100, and treated as a continuous variable to account for multiple implantations in a woman.

Statistical analysis

Sample size calculation

Ongoing pregnancy rate was the primary outcome measure. Assuming an ongoing pregnancy rate of 25% in the control group in accordance with previous figures achieved in women with ≥ 2 failed treatment cycles in our center, it was calculated that approximately 700 participants would be required to detect an absolute 10% increase with an alpha error level of 0.05 and beta error level of 0.2. Considering the difficulty of recruiting so many participants with RIF to a single center trial, the current trial aimed to be a pilot study with 150 participants, which could provide data for assessing the feasibility of future trials, and be included in future meta-analyses on the issue.

Statistical tests

Efficacy analysis was done according to the intention-to-treat principle. Differences between the study groups were assessed using the χ^2 test with or without Yates' correction for categorical variables, and independent samples *t*-test for continuous variables. Difference between repeated measures of platelet counts was assessed with paired samples *t*-test. Odds ratios and 95% confidence intervals were calculated for binary outcomes. All analyses were performed with the Statistical Package for Social Sciences 11.5 (SPSS for Windows 11.5.0, SPSS Inc., IL, USA).

Results

Demographic characteristics of participants and the features of assisted reproduction treatment cycles are presented in Table I. The average numbers of embryos transferred were similar between groups.

Table I Characteristics of participants and assisted reproduction treatment cycles

	LMWH	Control
Number of participants	75	75
Female age in years ^a	34.0 \pm 5.0	34.8 \pm 5.8
Etiology of infertility		
Ovulatory (%)	9 (12.0)	11 (14.7)
Tubal (%)	7 (9.3)	10 (13.3)
Endometriosis (%)	2 (2.7)	0 (0)
Male (%)	33 (44.0)	29 (38.7)
Unexplained (%)	13 (17.3)	16 (21.3)
More than one factor (%)	11 (14.7)	9 (12.0)
Median number of previous failed cycles (interquartile range, minimum and maximum values)	2 (2–4, 2–14)	2 (2–4, 2–7)
Mean number of oocytes (standard deviation)	8.7 (4.5)	7.6 (4.7)
Mean number of MII oocytes (standard deviation)	6.4 (3.2)	5.3 (3.6)
Mean number of embryos transferred (standard deviation) ^a	2.6 (0.7)	2.6 (0.8)
Mean number of grade I–II embryos transferred (standard deviation) ^b	2.2 (0.9)	2.3 (1.1)

LMWH: low molecular weight heparin; MII: metaphase two.

^aP-value = 0.99.

^bP-value = 0.54.

Three women in the LMWH group and three women in the control group 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 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 (Fig. 1).

Platelet counts did not change significantly in the LMWH group during the study period. None of the patients experienced any adverse effects other than small ecchymoses around the LMWH injection sites. None of the participants in the LMWH group discontinued treatment due to pain or ecchymoses around the injection site. Ecchymoses that would be suggestive of LMWH self-administration were not observed in control group women during the physical examination undertaken on the day of the pregnancy test and again at the 6th week of gestation.

There were 34 (45.3%) and 29 (38.7%) clinical pregnancies in the LMWH and control groups, respectively ($P = 0.41$). Implantation rates were 24.5 and 19.8% in the LMWH and control groups, respectively ($P = 0.33$).

Four of five women with high-order multiple pregnancies opted for fetal reduction and their pregnancies were successfully reduced to

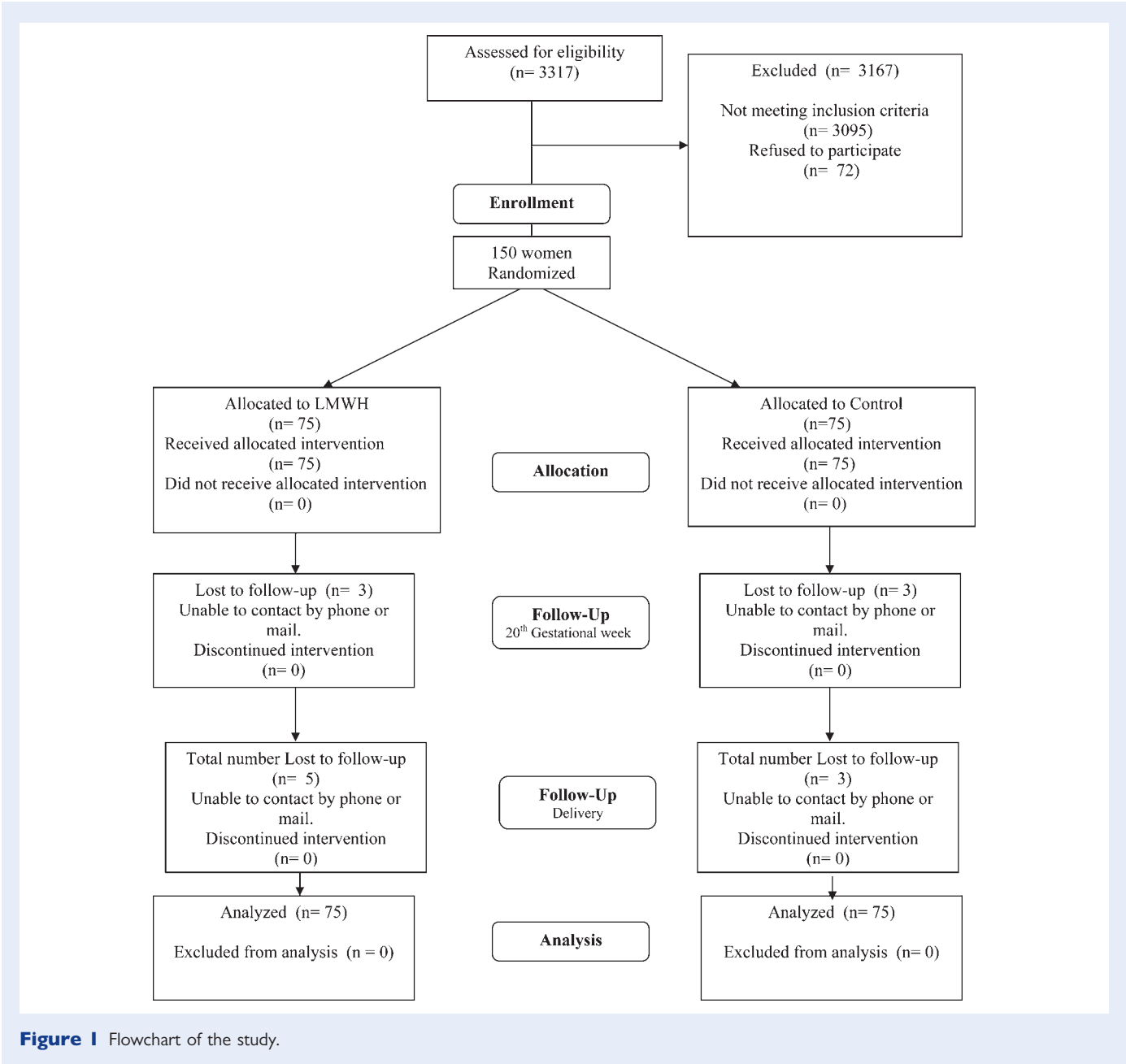


Figure 1 Flowchart of the study.

twins in the 13th gestational week. While one of these women was lost to follow-up after the 20th gestational week, other three delivered live twins at 34th, 35th and 38th weeks. One participant in the LMWH group, with a quadruplet pregnancy refused fetal reduction and delivered quadruplets at 32nd week of gestation.

Number of live births were 26 (34.7%) and 20 (26.7%) in LMWH and control groups, respectively ($P = 0.29$). There were 28 (37.3%) ongoing pregnancies beyond 20th gestational week (initially defined primary end-point) in the LMWH group, and 20 (26.7%) in the control group ($P = 0.16$) (Table II, Fig. 2).

Numbers of preterm deliveries were 9 (34.6%) in LMWH and 6 (30.0%) in control groups ($P = 0.74$). Three women delivered in 32nd week (one set of quadruplets, one set of twins and a singleton, all in LMWH group), one women (singleton in control group)

delivered in 33rd week, four women delivered in 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), three women delivered in the 36th week (one singleton in LMWH group and two sets of twins in the control group).

None of the infants 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 necrotizing enterocolitis.

The administration of LMWH did not statistically significantly improve outcome parameters as compared with controls. Similar results were observed in cycles with high-order (≥ 3) previous implantation failures (Table III).

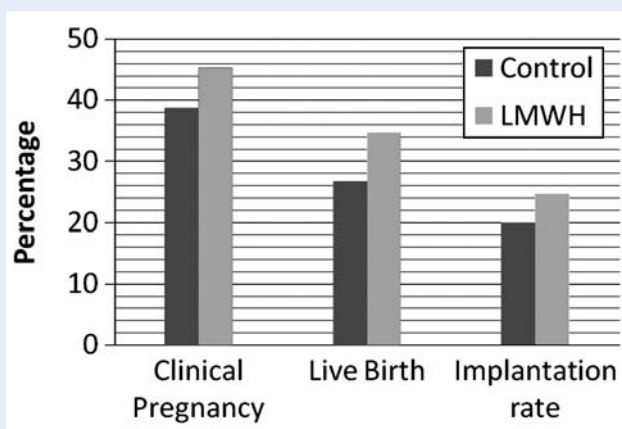


Figure 2 Overall outcomes.

LMWH, low molecular weight heparin.

None of the women reported developing pre-eclampsia during the gestational period.

Discussion

To the best of our knowledge this is the first study assessing the efficacy of empirical LMWH administration throughout the luteal phase of ART cycle in women with RIF but without any evidence of thrombophilia. The results of this pilot study demonstrate a trend towards

increased embryo implantation and live birth rates with empirical administration of LMWH in couples with unexplained RIF. Despite a lack of statistical significance, the observed relative increase of 30% in live birth rates with LMWH may be regarded as a clinically significant trend necessitating further research on the use of empirical LMWH in ART. Lack of statistical significance might be due to low statistical power associated with the small sample size of the current trial. A sample size of 150 would enable demonstration of an absolute difference of 24% up from a baseline event rate of 25% in the control group as statistically significant with the same alpha and beta error levels of 0.05 and 0.2. A difference of such magnitude corresponds to a relative increase by 100%, which is obviously not a realistic expectation. The present trial was defined as a pilot trial, in order to note limitations of its size. On the other hand, it should be considered that the observed differences might have been reduced had more participants been recruited.

Couples with laboratory or clinical findings of thrombophilias were excluded in order to ascribe any observed beneficial effect of LMWH on the outcome to its non-anticoagulant effects. The rationale behind selective inclusion of women with previously failed treatment cycles was 2-fold; firstly couples with previously failed treatment cycles generally demand a modification in their treatment protocols, and they were anticipated to be willing to participate in this pilot trial in the light of previous data suggesting a beneficial effect of heparin in RIF. The second reason was to evaluate the effect of LMWH on the outcome in this difficult to manage subset of patients with the aim to provide them another treatment option if found to be effective. Enoxaparin was chosen due to its established safety profile in

Table II Overall outcomes

	LMWH	Control	Absolute difference, 95% CI	P-value
Clinical pregnancy (%)	34/75 (45.3)	29/75 (38.7)	6.6%, -9.0% to +21.8%	0.41
Multiple pregnancy (%) (twins, triplets, quadruplets)	12/34 (35.3) (9,1,2)	10/29 (34.5) (8,2,0)	0.8%, -22.8% to +24.4%	0.94
Implantation	24.5%	19.8%	4.7%, -4.7% to +14.1%	0.33
Ongoing pregnancy ^a (%) (>20 weeks)	28/75 (37.3)	20/75 (26.7)	10.6%, -4.2% to +24.9%	0.16
Live birth ^b (%)	26/75 (34.7)	20/75 (26.7)	8.0%, -6.7% to +22.7%	0.29

LMWH: low molecular weight heparin; CI: confidence interval.

^aThree patients in each group were lost to follow up at the 20th gestational week.

^bFive patients in the LMWH group and three patients in the control group were lost to follow up prior to delivery.

Table III Outcome in high-order (≥ 3) RIF

	LMWH	Control	Absolute difference, 95% CI	P-value
Clinical pregnancy	16/37 (43.2)	13/34 (38.2)	5.0%, -19.0% to +28.1%	0.67
Multiple pregnancy (%) (twins, triplets, quadruplets)	6/16 (37.5) (5,1,0)	4/13 (30.8) (3,1,0)	6.7%, -30.3% to +40.3%	0.99
Implantation	21.6%	16.0%	5.6%, -7.9% to +18.9%	0.41
Ongoing pregnancy ^a (>20 weeks)	14/37 (37.8)	8/34 (23.5)	14.3%, -9.1% to +35.5%	0.19
Live birth ^b	12/37 (32.4)	8/34 (23.5)	8.9%, -11.9% to +29.7%	0.40

LMWH: low molecular weight heparin; CI: confidence interval.

^aOne patient in each group were lost to follow up at the 20th gestational week.

^bThree patients in the LMWH group and one patient in the control group were lost to follow up prior to delivery.

pregnancy (Greer and Nelson-Piercy, 2005). Platelet counts were not routinely repeated after the first week of treatment, because of a relatively low risk of heparin induced thrombocytopenia with the administration of LMWH. None of the participants had clinical or laboratory findings suggesting a decrease in renal function that would prolong the half-life of the medication and increase the risk for thrombocytopenia.

The overall lost to follow-up rate is acceptable for a prospective trial (8/150, 5.3%). Although the sample size of the trial is limited, the outcomes in these patients are not expected to significantly alter the current results. Moreover, the present study is not a trial that was aimed at providing definitive results on the subject, but rather it was designed as a pilot trial inquiring into the feasibility of further research on the issue. Recruitment of women with two rather than three previous failed cycles may be regarded as a limitation of the study. Although the primary goal of the present study was to assess the effect of LMWH on the outcome of ART in women with RIF, considering the proposed mechanisms of action discussed below, LMWH can be expected to affect all women undergoing ART in a similar manner. Thus, it was decided to include women with two previous failed cycles, rather than sticking to the traditional definition of RIF in order to facilitate timely collection of data in this single center pilot trial. Non-blinded design may be regarded as another limitation of the current trial; however, the outcome parameters are binary variables that do not allow for subjective evaluation. Hence the foreknowledge of treatment allocation is not expected to alter the outcome assessment. Moreover, physicians were not aware of treatment allocation at the time of embryo transfer. Participants were randomized to treatment arms after OR by the nurse coordinator, and previous treatment procedures were administered uniformly for all participants. Therefore, treatment bias was eliminated as well.

Regulation of trophoblast invasion is integral to successful implantation and maintenance of pregnancy. Women who are carriers of congenital or acquired thrombophilia are at increased risk of implantation failure and pregnancy complications such as spontaneous miscarriages, fetal growth restriction, stillbirth and pre-eclampsia. Heparin with its anticoagulant effects has been used successfully in women who are carriers of these thrombophilic mutations. Effects of heparin beyond anticoagulation have led to its unsupported use in the absence of thrombophilias in women undergoing ART cycles, as well as in women with a bad obstetric history (Tzafettas *et al.*, 2002; Nelson and Greer, 2008).

Different mechanisms of action besides anticoagulation can be suggested for the beneficial effects of heparin on implantation (Nelson and Greer, 2008). These include the following.

E-cadherin is an intercellular adhesion molecule that has been showed to be involved in trophoblast differentiation and invasiveness (Xue *et al.*, 2003). Expression of E-cadherin has been shown to be modulated by unfractionated heparin as well as enoxaparin in rat pregnancies (Erden *et al.*, 2006) and by membrane-bound heparin-binding epidermal growth factor (HB-EGF) in human pancreatic cell lines (Wang *et al.*, 2007). Shih *et al.* (2002) reported decreased trophoblast motility and invasiveness in the presence of E-cadherin expression in implantation-site intermediate trophoblastic cell lines. These findings suggest that LMWH may facilitate implantation process by down-regulating E-cadherin expression in the decidua. However, there are other *in vitro* studies with conflicting results with

regard to effect of heparin and LMWH on trophoblast motility and invasiveness (Quenby *et al.*, 2004; Bose *et al.*, 2005; Di Simone *et al.*, 2007; Ganapathy *et al.*, 2007).

HB-EGF is considered to play a role in the implantation process. In mouse studies, HB-EGF was found to improve blastocyst formation and promote trophoblast outgrowth (Das *et al.*, 1994). HB-EGF expression in luminal and glandular epithelium from human endometrial biopsy specimens was shown to be maximized during the peri-implantation period, and presence of HB-EGF on the surface of pinopodes was demonstrated (Stavreus-Evers *et al.*, 2002). Tightly regulated expression of HB-EGF in luminal epithelium and of EGF-receptor in the blastocyst around the time of implantation suggests an important function for this ligand-receptor signaling in embryo-maternal cross-talk for implantation (Paria *et al.*, 1993; Hamatani *et al.*, 2004). Heparin, via heparan sulfate proteoglycans or HB-EGF may be involved in blastocyst adhesion and invasion (Fiedler and Wurfel, 2004; Nelson and Greer, 2008). Furthermore, HB-EGF was shown to enhance *in vitro* extravillous trophoblast differentiation (Leach *et al.*, 2004). The authors showed that human EGF receptor-mediated autocrine and paracrine signaling by HB-EGF or other EGF family members induced cytotrophoblast differentiation to an invasive phenotype. Other studies have reported similar effects with LMWH (Quenby *et al.*, 2004; Di Simone *et al.*, 2007).

Enhanced trophoblast migration and invasiveness due to a LMWH induced increase in free insulin-like growth factor I is another proposed mechanism for a beneficial effect of LMWH on the implantation process (Lacey *et al.*, 2002; Moller *et al.*, 2006; Nelson and Greer, 2008).

Possible interactions between heparin/LMWH and cytokines and matrix metalloproteinases that can modulate the potential for successful implantation have been assessed by Nelson and Greer in a review of the role of heparin in assisted reproduction (Nelson and Greer, 2008).

In conclusion, heparin and its derivatives seem to affect at least some of the many variables that are involved in the complex process of implantation. The results of this pilot study suggest a potential beneficial effect of LMWH on the clinical outcome of ART in women with RIF. This finding, however, has yet to be corroborated in larger trials. Despite *in vivo* and *in vitro* evidence in favor of the alleged beneficial effect, wholesale adoption of this strategy into routine practice should be avoided until further research confirms the presence of such an effect and reveals its exact mechanism of action. Even if the proposed beneficial effect is confirmed, the subsets of patients in whom LMWH would be most effective and the appropriate dosing and duration of LMWH administration needs to be determined before unselectively exposing women and their embryos to the potential side effects of the medication.

Author's Role

The author's contributions are as follows; B.U.: design and institution of the study protocol, drafting, review and final preparation of the article, approval of the final version; B.A.: design and institution of the study protocol, collection and analysis of data, drafting, review and final preparation of the article and approval of the final version; K.Y., C.A., S.A., R.M. and B.B.: institution of the study protocol, review and approval of the final version.

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