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ARTICLE

Effect of dalteparin sodium administration on IVF outcome in non-thrombophilic young women: a pilot study

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Abstract This study evaluated whether heparin administration could affect IVF outcome. A total of 172 women, aged <40 years, without laboratory findings of thrombophilia and undergoing their first IVF cycle, were randomly allocated to treatment ($n = 86$) and control ($n = 86$) groups. Patients allocated to the treatment group received low-molecular-weight heparin dalteparin sodium 2500 IU s.c. daily, in addition to routine luteal phase support, from oocyte retrieval up to the day of the pregnancy test or up to the ninth week of pregnancy in the cases of positive human chorionic gonadotrophin. From the day after the oocyte retrieval, all patients began standard supplementation with vaginal progesterone 200 mg twice a day. At the sixth week of pregnancy, patients underwent an ultrasound scan to assess the number/viability of gestational sacs. Implantation rates were 15% and 12% in the dalteparin and control groups, respectively. The clinical pregnancy rates/embryo transfers were 26% (19/73) and 20% (16/80), in the dalteparin and control groups, respectively, with live birth rates/embryo transfer of 21% (15/73) and 16% (13/80). Despite the lack of statistical significance, the increase in pregnancies observed in the treatment group may be considered as an important clinical point in the optimization of IVF clinical outcome.

KEYWORDS: assisted reproduction, heparin, implantation rate, IVF

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Introduction

Since the introduction into clinical practice of oocyte IVF techniques and the transfer of obtained embryos to the uterus (Steptoe and Edwards, 1978), several improvements have been made to ovarian stimulation protocols, preparation of follicles and gamete culture medium. However, the implantation rates are still low (Donaghay and Lessey, 2007), which is the actual limiting factor for the success of assisted reproduction treatment in terms of live birth rate (LBR) (Anderson et al., 2008).

Embryo implantation succeeds only if several factors coexist, including perfect timing and efficiency of biological events involving the embryos, the endometrium and an adequate dialogue between the endometrium and the embryos. Since these events depend on several factors, the causes of failed embryo implantation, even if the embryo is morphologically normal, remain unknown.

In the literature, different clinical studies suggest an influence of heparin treatment on assisted reproduction outcome under specific clinical conditions. Actually, the beneficial effect of heparin treatment seems to be relevant in thrombophilic patients, in particular women affected by antiphospholipid antibodies (Kutteh et al., 1997; Sher et al., 1998; Stern and Chamley, 2006) and in patients with recurrent IVF/embryo transfer failure (Qublan et al., 2008). Moreover, heparin administration in non-thrombophilic women with failed embryo transfer cycles has been demonstrated as an effective treatment. Despite the lack of statistical significance, a clinically relevant positive trend in implantation rate, clinical pregnancy rate and LBR has been observed in these patients treated with enoxaparin sodium (Urman et al., 2009).

Heparin can have a positive effect in conception and early pregnancy events, thanks to its ability to alter the haemostatic response to ovarian stimulation, modulate trophoblast differentiation and invasion and decrease the risk of thrombosis (Nelson and Greer, 2008).

Moreover, in-vitro and in-vivo models suggest the influence of low-molecular-weight heparin (LMWH) therapy on different aspects of trophoblast adhesion and invasiveness by acting on matrix metalloproteinases and tissue inhibitors (Di Simone et al., 2007), cadherin-E (Erden et al., 2008; Shih Ie et al., 2002), heparin-binding epidermal growth factor (Das et al., 1994; Leach et al., 2004) and free insulin-like growth factor (Lacey et al., 2002; Nelson and Greer, 2008).

Considering previous clinical and experimental data, this pilot clinical trial aimed to assess whether LMWH administration in non-thrombophilic women having a first IVF/embryo transfer cycle could contribute to improve the clinical outcome.

Materials and methods

Study design and population

The study enrolled all consecutive patients undergoing their first assisted reproduction cycle (IVF or intracytoplasmic sperm injection (ICSI)) between May 2008 and December 2008 at the Department of Sciences for Women's and Child's Health, Division of Obstetrics and Gynecology of the University of Florence and at the Department of Reproductive Medicine and Child Development, Division of Obstetrics and Gynecology of the University of Pisa.

The enrolment included all patients who met the following inclusion criteria: age $<$40 years without congenital or acquired thrombophilic state. In particular, all patients presented normal-range results of antithrombin, protein C, protein S, activated protein C resistance, homocysteine, prothrombin gene G20210A polymorphism, lupus anti-coagulant, anticardiolipin antibody S and anti-2GPI antibody S. Laboratory determinations have been performed as previously described by Marcucci et al. (2007). Factor V Leiden mutation was not evaluated because activated protein C resistance values were in the normal range in all patients. Moreover, all women had normal homocysteine concentrations, thus MTHFR C677T polymorphism was not investigated. None of the patients received treatment with LMWH in the previous 3 months in order to exclude heparin-induced thrombocytopenia syndrome risk, typically $<$0.1% (Warkentin et al., 2008). Moreover, enrolled women were characterized by the absence of endocrine/haematological abnormalities, chronic diseases, relevant tubal or uterine pathology interfering with embryo implantation (e.g. fibroids $>$5 cm and/or distorting the uterine cavity, hydrosalpinx visible on transvaginal ultrasonography).

Selected patients who agreed to participate in the study were informed that the day of oocyte retrieval would have been randomized by a computer-generated randomization list into two groups: the treatment group (low doses of LMWH plus vaginal progesterone) or the control group (only vaginal progesterone). This study was approved by the Local Ethics Committee of both hospital units and a written informed consent was read and signed by each patient.

Interventions

All patients underwent ovarian stimulation with a long gonadotrophin-releasing hormone agonist (Enantone, 0.1 mg/day during the last 5 days of oral contraception; Takeda Italia Farmaceutici, Rome, Italy; and Enantone, 0.05 mg/day; Takeda Italia Farmaceutici, Rome, Italy; from the first day of FSH) combined with recombinant FSH (Gonal-F; Merck-Serono, Rome, Italy; or Puregon; Organon-Schering-Plough, Milan, Italy). When at least three follicles $>$17 mm were observed by ultrasonography, recombinant human chorionic gonadotrophin (HCG) (Ovitrelle, 250 μg s.c., Merck-Serono) was administrated to induce oocyte maturation. After 34–36 h, oocyte retrieval was arranged and standard IVF or ICSI techniques were performed. Cleavage-stage embryos were graded according to the literature (Hardarson et al., 2001) and embryos were transferred on the second day after oocyte insemination. In accordance with the Italian law, a maximum of three oocytes were inseminated and all obtained embryos were transferred.

On the day of oocyte retrieval, women were randomized into two groups (treatment and control group) according to a computer-generated randomization list prepared by one of the authors (IN). Sealed and numbered envelopes, containing the allocation information, were given to the

treatment centre's nurse co-ordinator, who assigned patients to the study arms following recruitment by the physician on the morning of oocyte retrieval.

The control-group patients started luteal phase support with vaginal progesterone (Prometrium, 200 mg twice per day; Rottapharm, Milan, Italy) from the day after the oocyte retrieval until the day of pregnancy test (12 days after embryo transfer).

The treatment-group patients 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. Platelet count was performed on days 7–8 of dalteparin treatment to evaluate possible adverse effects of the therapy. Platelet levels were compared with the previously measured basal levels: 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.

All patients belonging to both groups were tested for pregnancy 12 days after embryo transfer. Treatments in both groups were stopped if the βHCG concentrations were $<$50 IU/ml; conversely, therapies were continued until the sixth week of pregnancy, when a transvaginal ultrasound scan was performed to assess the number and viability of gestational sacs. These ultrasound controls were performed by a gynaecologist unaware of the allocation of the patients. If a clinical pregnancy was confirmed, the treatment to patients belonging to both groups was continued until the ninth week of pregnancy. A complete follow-up of these patients was performed until delivery.

Outcome measures

The primary outcome was the LBR/embryo transfer. The secondary outcomes included the implantation rate and the clinical pregnancy rate/embryo transfer. The implantation rate was calculated separately for each participant and then compared using Mann Whitney *U* non-parametric test. The clinical pregnancy rate/embryo transfer was defined as the ratio between the cases with a positive heartbeat embryo and the number of transferred embryos. Single or multiple clinical pregnancies were finally registered.

Statistical analysis

The statistical analysis used the *t*-test for independent variables and the chi-squared test for categorical variables. The Mann Whitney *U* non-parametric test was also used. All analyses were performed with the Statistical Package for Social Sciences 15.0 (SPSS; IL, USA).

Results

The study 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 oocyte or cancelled cycles and eight who decided to decline their -participation. Consequently, 172 patients were allocated to intervention and divided into two groups: 86 women were included in the treatment group and 86 women in the control group. The final series for analysis contained 153 patients 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. A flow chart presenting patient participation in the study is shown in **Figure 1**.

No statistically significant differences between the treatment group and the control group were demonstrated in relation to demographic characteristics or ovarian stimulation. Moreover, the two groups were similar for laboratory biological parameters (**Table 1**). There were 1.9 embryos/transfer in the control group and 2.3 embryos/transfer in the treatment group (not statistically significant; **Table 1**).

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.

Among the 73 patients in the treatment group, 53 cases presented a negative pregnancy test and in one case an ectopic pregnancy was observed. Concerning the control group, consisting of 80 patients, 64 women had a negative pregnancy test.

Nineteen clinical pregnancies were obtained in the treatment group, while 16 were obtained in the control group (**Table 2**), and the number of gestational sacs with a positive heartbeat was 25 in the treatment group and 18 in the control group.

The treated patients obtained a 15% implantation rate versus 12% of the control patients (**Table 2**). However, the implantation rates were not statistically significantly different (two-tailed). Similarly, the treated patients obtained a 26% clinical pregnancy rate per transfer (19/73) versus 20% (16/80) of the control patients (**Table 2**). Once again, this difference was not statistically significant (odds ratio (OR) 1.30; 95% confidence intervals (CI) 0.58–2.89).

Concerning the incidence of multiple pregnancies, there was approximately one multiple pregnancy out of three (6/19: five twin cases, one triplet case) in the treatment group. In contrast, only 2/16 patients in the control group had a multiple pregnancy (twins).

Regarding the follow-up of these patients until the delivery, the spontaneous miscarriage rates were similar in the treatment (4/19; 21%) and the control (3/16; 19%) groups (**Table 2**). Fifteen women in the treatment group delivered (nine singletons, six twins) and 21 children were born. Thirteen women in the control group delivered (11 singletons, two twins) and 15 children were born. Therefore, the LBR/embryo transfer was 21% (15/73) in the treatment group and 16% (13/80) in the control group. This difference, however, was not statistically significant (OR 1.33; 95% CI 0.54–3.27) (**Table 2**).

Discussion

The administration of LMWH has been shown to increase the efficiency of assisted reproduction treatment in congenital (Qublan et al., 2008) or acquired (Kutteh et al., 1997; Sher et al., 1998; Stern and Chamley, 2006) thrombophilia, or in

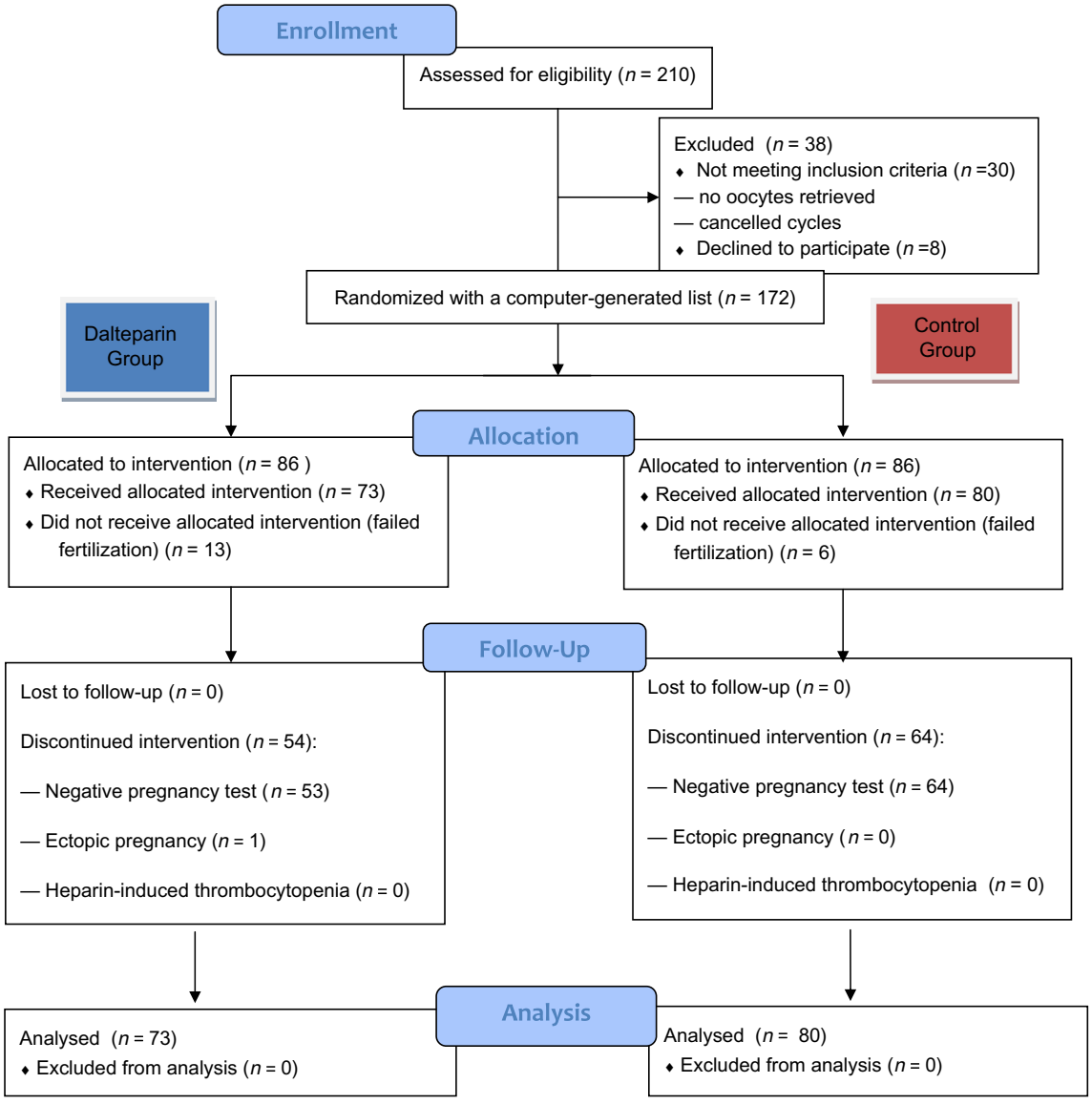

Figure 1 Patient participation flowchart.

case of repeated failures of IVF/ICSI-embryo transfer cycles (Urman et al., 2009).

This study observed that the administration of a prophylactic dose of LMWH (2500 IU/day) in non-thrombophilic women undergoing their first IVF cycle showed a non-significant trend towards increased LBR/embryo transfer and clinical pregnancy rate.

This study stopped luteal supplementation (dalteparin and/or progesterone) at the ninth week of pregnancy. It was not continued until the 12th week because patients were not in a thrombophilic state and different studies have suggested that after the ninth week the critical phases of embryo development are concluded and the natural pregnancy loss significantly decreases (Tong et al., 2008).

As far as is known, this is the first report in the literature that analyses the role of LMWH in non-thrombophilic young patients. Fiedler and Wurfel (2004) performed a similar study on the efficiency of heparin administration in assisted reproduction treatment. They observed that only a prolonged heparin treatment (14 days) resulted in an increased pregnancy rate in the patient population, while a shorter treatment with heparin was not effective. However, the authors did not indicate the number of cases or the dose used.

The mechanism of positive interference by heparin on embryo implantation is not clear; but various hypotheses have been given.

Heparin is a polydispersed linear polysaccharide that presents structural similarities with heparan sulphate proteoglycans (HSPG) expressed in the reproductive tract, which are involved in the regulation of folliculogenesis, sperm viability and capacitation and the endometrial cycle (Nelson and Greer, 2008). LMWHs are derived from heparin by depolymerization of unfractionated heparin. Like heparin, LMWH facilitates the anticoagulant effect of antithrombin, but it has many advantages, such as a longer half-life that allows once-daily dosing, a more predictive antithrombotic response and even a reduction of adverse effects. In

Table 1 Laboratory and clinical data.

	Treatment (n = 73)	*Control (n = 80)*
Age (years)	34.7 ± 3.6	35.1 ± 3.1
Type of infertility		
Primary infertility	42	48
Secondary infertility	31	32
Duration of infertility (years)	2.8 ± 1.4	3.3 ± 2.1
Standard protocol		
IVF	39	38
ICSI	34	42
Cause of infertility		
Tubal factor	14	16
Male factor	30	38
Endometriosis	9	5
Unknown	16	15
Mixed factors	4	6
Antral follicle count	10.4 ± 4.6	9.5 ± 4.8
Stimulation days	9.9 ± 9.6	10.3 ± 9.9
FSH total dose (IU)	2304 ± 727.9	2427 ± 882.1
Follicles ≥16 mm on day of HCG	3.5 ± 2.3	3.3 ± 2.4
Endometrial thickness on day of HCG (mm)	9.3 ± 1.9	9.5 ± 1.7
Oestradiol peak (pg/ml)	1036 ± 598.3	1181 ± 670.3
Inseminated oocytes	244	243
Fertilization rate (%)	79	72
Cleavage rate (%)	94	93
Transferred embryos	168	154
Transfers	73	80
Embryos/transfer	2.3 ± 0.7	1.9 ± 0.7
Embryo grade (%)		
A	60	57
B	27	31
C	11	8
D	2	4

Values are mean ± SD or *n*, unless otherwise stated. There were no statistically significant differences between the two groups. HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection.

Table 2 Outcomes.

	Treatment (n = 73)	*Control (n = 80)*
Clinical pregnancy (*n*)	19	16
Clinical pregnancy rate per transfer (%)	26	20
Implantation rate (%)	15	12
Spontaneous miscarriages (%)	21	19
Delivery (*n*)	15	13
Newborns (*n*)	21	15
Live birth rate/embryo transfer (%)	21	16

There were no statistically significant differences between the two groups.

experimental animal models, the importance of the role played by HSPG in reproduction is highlighted by the observation that mice deficient in enzyme hs-3-O-sulphotransferase-1, which is essential for the synthesis of heparan sulphate, show an abortion rate of 50%. Moreover, the surviving embryos also present some phenotypic aspects compatible with intrauterine growth restriction (Shworak et al., 2002).

In human reproduction, HSPG seems to be important in congenital or acquired thrombophilia, which is frequently characterized by placenta-mediated complications. The most important acquired thrombophilic condition is the antiphospholipid syndrome, associated to an increased risk of thrombosis, a high probability of pregnancy loss and an increased risk of recurrent miscarriage. In this group of patients, antithrombotic therapy (low-dose aspirin or heparin) is effective in preventing recurrent pregnancy loss (Miyakis et al., 2006; Nelson and Greer, 2008). Moreover, in women with either antiphospholipid syndrome or repeated implantation failure and antiphospholipid seropositivity, LMWH and aspirin should be started in association with ovarian stimulation and continued throughout pregnancy (Kutteh et al., 1997; Sher et al., 1998; Stern and Chamley, 2006). A recent report showed the efficiency of LMWH therapy for repeated IVF cycle failures (Urman et al., 2009).

The possible beneficial effects of antithrombotic therapy consist in the prevention of microthromboses that negatively impact the implantation process and placental development. However, recent data suggest an alternative mechanism of action, consisting in a modulation of differentiation and trophoblastic invasion. The implantation is a complex process involving endocrine, paracrine, autocrine and juxtacrine modulators that span cell—cell and cell—matrix interactions. Heparin can potentially modulate many of the known mechanisms that underlie successful apposition, adhesion and penetration of the developing embryo (Nelson and Greer, 2008). In-vitro experiments suggest an important role of a direct promotion of trophoblast invasion, rather than placental thrombosis during embryo implantation; LMWH seems to up-regulate several specific proteins and so is able to promote trophoblast invasion (Di Simone et al., 2007). Moreover, other molecular systems could be implied in the beneficial effects of LMWH on implantation like the cadherin-E system that seems to be down-regulated in the decidua during heparin therapy (Shih Ie et al., 2002; Erden et al., 2008). In mouse models, heparin-binding epidermal growth factor improves blastocyst adhesion and invasion, suggesting an important role in the uterus—embryo cross-talk (Das et al., 1994; Leach et al., 2004). Finally, LMWH induces an increase of free insulin-like growth factor that could be implied in the implantation process (Lacey et al., 2002; Nelson and Greer, 2008).

The complex mechanism of action of LMWH on molecular trophoblast invasion could justify the positive effect of this therapy observed also in the young non-thrombophilic women enrolled in the present study. Moreover, the beneficial effect of LMWH on embryo implantation could be highlighted by the increased number of multiple pregnancies: the treatment group presented six twin pregnancies out of 19 cases, while the control group presented only two twin pregnancies out of 16 cases.

In conclusion, this study observed that the administration of prophylactic-dose LMWH in non-thrombophilic women undergoing their first IVF cycle could increase the LBR/embryo transfer, the implantation rate and the clinical pregnancy rate. Although these changes are not statistically significant, the presence of an increasing trend certainly encourages the undertaking of a larger and multicentre study in order to achieve sufficient statistical power.

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