Thrombosis Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

The effect of parnaparin sodium on in vitro fertilization outcome: A prospective randomized controlled trial

Corrado Lodigiani[a,*], Francesco Dentali[b], Elena Banfi[a], Paola Ferrazzi[a], Luca Librè[a], Ilaria Quaglia[a], Luca Cafaro[c], Emanuela Morenghi[d], Veronica Pacetti[a], Elena Zannoni[c], Anna Maria Baggiani[c], Paolo Emanuele Levi-Setti[c]

[a] Humanitas Research Hospital, Cardiovascular Department, Thrombosis and Haemorragic Diseases Center, Rozzano, Milan, Italy
[b] Department of Clinical and Experimental Medicine, Insubria University, Varese, Italy
[c] Humanitas Research Hospital, Department of Gynecology, Division of Gynecology and Reproductive Medicine, Humanitas Fertility Center, Rozzano, Milan, Italy.
[d] Humanitas Research Hospital, Biostatistics Unit, Rozzano, Milan, Italy

ARTICLE INFO

Keywords:
In vitro fertilization
Live-birth pregnancy rate
Low-molecular weight heparin
Reproductive sterility
Thrombophilia

ABSTRACT

Introduction: In-vitro and in-vivo models suggest the influence of low-molecular weight heparin on conception in infertile women undergoing in vitro fertilization procedures (IVF). In this randomized controlled trial we assessed whether a low-molecular weight heparin (parnaparin) could affect IVF outcomes.

Materials and methods: 271 cycles were analyzed in 247 women having a first or subsequent IVF cycle at Fertility Center of Humanitas Research Hospital. Patients, without severe thrombophilia and hormonal or active untreated autoimmune disorders, were randomly allocated (1:1) to receive for the whole cycle parnaparin, or routine hormonal therapy only. The primary endpoint was the clinical pregnancy rate and the secondary endpoints included implantation rate and live birth rate.

Results: The clinical pregnancy and the live birth rate were similar in treated and controls (21.5% vs. 26.7%, $p = 0.389$; 18.5% vs. 20.6%, $p = 0.757$). The abortion rate was 10.3% vs 22.9%, $p = 0.319$, respectively. The subgroups analysis, ≤ 35, 36–38, 39–40 years, showed the following: comparable clinical pregnancy rate (22.5% vs 38.8%, $p = 0.124$; 21.8% vs 17.3%, $p = 0.631$; 19.4% vs 23.3%, $p = 0.762$ respectively) and live birth rate (16.3% vs 32.7%, $p = 0.099$; 20.0% vs 13.5%, $p = 0.443$; 19.4% vs 13.3%, $p = 0.731$ respectively) in treated vs controls. Sensitivity analyses on women with ≥ 3 previous attempts and first enrolment only, and subgroup analyses according to trial conclusion conditioning a small sample size with low statistical power.

Conclusions: Our study excludes positive effect of parnaparin, once a day for the whole cycle, on clinical pregnancy rate in infertile women undergoing in vitro fertilization techniques.

1. Introduction

Since the introduction into clinical practice of in vitro fertilization procedures (IVF) [1], improvements have been made to ovarian stimulation protocols, preparation of follicles and gamete culture medium. However, the implantation rates of the pre-embryo are still low [2], which is the actual limiting factor for the success of IVF in terms of live birth rate [3]. Since embryo implantation depends on several factors, the causes of its failure, even if the embryo is transferred at blastocyst stage after full chromosomal analysis [4], remain unknown. Unfortunately, this may occasionally be a recurring phenomenon leading to despair in couples and frustration in their caregivers. Recently, some experts have advocated the use of antithrombotic drugs (heparins and aspirin) in order to improve the implantation rate [5]. Heparin can have a positive effect in conception and early pregnancy events by altering the hemostatic response to ovarian stimulation, modulating trophoblast differentiation and invasion, and decreasing the risk of thrombosis [6]. In-vitro and in-vivo models suggest the influence of low-molecular weight heparin (LMWH) on different aspects of trophoblast adhesion and invasiveness by acting on matrix metalloproteinases and tissue inhibitors [7], cadherin-E [8,9], heparin-binding epidermal growth factor [10,11], and free insulin-like growth factor [6,12]. Clinical studies suggest an influence of heparin on IVF outcome under specific clinical conditions such as women affected by antiphospholipid antibodies (aPL) [13,14,15]. The role of heparin in patients with recurrent IVF failure without an aPL syndrome is more conflicting [16,17]. In general, original studies gave negative results. A comprehensive systematic review and meta-analysis of published literature on

* Corresponding author at: Humanitas Research Hospital, Cardiovascular Department, Thrombosis and Haemorragic Diseases Center, via Manzoni 56, 20089 Rozzano, Milan, Italy.
E-mail address: corrado.lodigiani@humanitas.it (C. Lodigiani).

http://dx.doi.org/10.1016/j.thromres.2017.08.006
Received 16 June 2017; Received in revised form 7 August 2017; Accepted 13 August 2017

the effect of heparin on the outcome of IVF was conducted by Seshadri et al. in 2012 which included both randomized and observational studies. The randomized studies included only small numbers of women, and had high methodological heterogeneity, and so had significant limitations. These showed no significant difference in implantation rate, clinical pregnancy, clinical miscarriage and live birth rate. Meta-analysis of the observational studies showed a significant increase in the clinical pregnancy rate and live birth rate, however the authors concluded that the potential role of heparin during IVF treatment required further evaluation in adequately powered randomized studies as they noted that the observational studies could have exaggerated the value of heparin in IVF due to selection bias. In a more recent meta-analysis, the use of LMWH significantly increased the clinical pregnancy rate and live birth rate [18,19,20]. Despite the absence of robust clinical evidence, maternal LMWH administration is used in IVF as an intervention that may improve implantation and reduce miscarriage, although clinical practice varies widely between individual clinicians and clinics. Thus, we conducted a randomized controlled trial (RCT) to assess whether LMWH administration in infertile women having a first or subsequent IVF cycle could contribute to improving the clinical outcome, with or without the presence of non-severe thrombophilia.

2. Materials and methods

2.1. Study design

This randomized, prospective, controlled, stratified, open label and phase III study was carried out and reported according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines for randomized controlled studies [21]. The study was approved by the institutional review board of our Institute and written informed consent was obtained for all women and is registered on ClinicalTrials.gov NCT02991950.

2.2. Aim of the study

To assess whether a low molecular weight heparin, parnaparin, administration could contribute to improving the outcome of IVF in terms of clinical pregnancy rate in infertile women undergoing a cycle of IVF, aged ≤ 40 years and ≥ 18 years, without severe thrombophilia, and hormonal or active untreated autoimmune disorders.

2.3. Patients and methods

The study was undertaken at the Fertility Center and Thrombosis and Haemorragic Diseases Center of Humanitas Research Hospital, Milan, Italy, between November 2011 to December 2015. The last participant was recruited in April 2015, and the follow-up was completed in December 2015. Originally we planned the conduct the study as a multicenter study. Subsequently it was amended as single center study due to the high management of costs. Infertile women satisfying all the inclusion and exclusion criteria were potentially eligible for the study. Women between 18 and 40 years of age who underwent a fresh IVF cycle with routine ovulation induction protocol were candidates for the study. Cycles with frozen ejaculated or testicular sperm and cycles with frozen embryos were excluded from the study, in order to include a quite homogeneous population with exclusion of all possible bias (e.g. severe male factor leading to the use of frozen or testicular sperm). Women with chronic disease (liver, renal, thyroid) or hormonal disorder not compensated with a specific therapy, with a immunological disease (e.g., autoimmune thyroiditis, connectivitis, rheumatoid arthritis, systemic lupus erythematosus), with abnormal platelet count ($< 100{,}000/mm^3$), with antiphospholipid autoantibodies or other severe thrombophilia (antithrombin, protein S, protein C deficiency or homozygous FV Leiden or FIIG20210A, or double heterozygous FV Leiden and FIIG20210A), and patients with previous thrombosis or with a contraindication for heparin therapy were excluded. Patients with a family history (even strong) of thrombosis were not excluded from the study. According to the study protocol, patients could be enrolled in the study more than once. Participating women were submitted to a block computer-generated randomization and allocated to receive or not to receive parnaparin for the whole cycle at a daily dose of 4250 anti-Xa IU/0.4 mL and 6400 anti-Xa IU/0.6 mL, if the body weight was respectively under or over 60 kg. The randomization list was prepared by a biostatistician not involved in patient recruitment. Opaque numbered and sealed envelopes containing the allocation were managed by the study data manager, who informed the attending physicians. Data were collected on BMI, smoking habits, infertility causes, previous pregnancies, miscarriages, and IVF procedures, retrieved oocytes, transferred embryos and implantation rate, IVF outcome and parameters used for randomization.

2.4. Laboratory tests

Factor VG1691A and FIIG20210A were assessed with HTRBio-to-Bit-PCR Platform, Fleming Research. The activities of anticoagulant proteins were measured in human citrated plasma on IL Coagulation System® (Instrumentation Laboratory, Bedford, USA): protein C and antithrombin with an automated chromogenic assay; free protein S with automated latex ligand immunoassay; resistance to activated protein C with COATEST™ APC RESISTENCE™. Lupus anticoagulant was determined with one-stage clotting test using Simplified Dilute Russel Viper Venom (IL). Anti-Cardiolipin IgG/IgM antibodies were quantified in human serum in accordance with manufacturer's instructions (ORGENTEC, Diagnostika GmbH, Germany).

2.5. Interventions

During the study period, patients underwent a controlled ovarian hyperstimulation (COH) protocol. The ovarian stimulation protocol was determined according to ovarian reserve parameters, evaluating anti-Müllerian hormone (AMH), antral follicle count (AFC), and body mass index (BMI) before the treatment [22,23] and considering previous treatment cycles [24,25].

COH was performed with a GnRH agonist (Enantone die, Takeda, Italy or Triptorelin Depot 3.75 mg IM, Decapeptyl®, Ipsen, Milan, Italy) or GnRH antagonist protocol (Cetrotide®, Merck Serono, Rome, Italy; Orgalutran®, Organon, MSD-Italy) [26].

In the GnRH, agonist protocol induction was started after evidence of pituitary desensitization (absence of ovarian follicles > 10 mm and endometrial thickness < 5.4 mm on transvaginal ultrasound examination). In the GnRH antagonist protocol, women received a low dose oral contraceptive and gonadotrophins were started the first day of withdrawal bleeding [27].

The starting dose of rFSH (Puregon®, MSD-Italy; Gonal-F, Serono, Rome, Italy) or hMG (Meropur®, Ferring, Milan, Italy) was decided according to ovarian reserve parameters and or previous induction cycle for the first four days and thereafter, on the basis of transvaginal follicular parameters (number and diameter) and results of estradiol and progesterone determination, a variable dose of gonadotropin was administered until the day of ovulation trigger with a 250 mcg rhCG (Ovitrelle®, Serono, Rome, Italy Merck Serono) injection.

Parnaparin sodium (Fluxum 4250 anti-Xa IU/0.4 mL or 6400 anti-Xa IU/0.6 mL; Alfa Wassermann S.p.A., Bologna, Italy) was administered for the whole cycle, from the day before the beginning of the stimulation phase of the cycle until the result of the procedure and, in the case of evolutive pregnancy, until delivery or the end of pregnancy. According to the study protocol, LMWH was discontinued in the case of patient refusal, toxicity or other complications precluding further therapy, or in the case of a decision by the physician. During LMWH administration, concomitant therapy with acetylsalicylic acid or steroids was not allowed, so patients on chronic treatment with these drugs