Original Research Article

A prospective randomized controlled trial showing efficacy of luteal phase low molecular weight heparin in fresh non-donor IVF/ICSI cycles in women with previous implantation failures

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ABSTRACT

Background: Implantation failure is a major challenge in in-vitro fertilization (IVF) cycles. The present study was undertaken to determine the immunomodulatory effects of heparin in patients with previous implantation failures undergoing assisted reproductive techniques (ART).
Methods: This was a prospective randomized controlled trial with sample size of 100 patients who had history of at least one previously failed IVF/ICSI. Study group of 50 patients received heparin and 50 patients in control group received routine luteal phase support.
Results: Primary outcome of the study was implantation rate (IR) which was 11.03% in the study group was and 5.48% in the control group (p=0.08). Biochemical pregnancy rate and clinical pregnancy rate in the study group was 18% and 12% in the control group (p=0.401). Calculated live birth was 5.15% and 3.42% in the study and control groups respectively (p=0.562). 11 babies were taken home from the study group and 6 from the control group (p=0.18).
Conclusions: The result of this pilot study showed relative increase in implantation rates (IR) suggesting beneficial effects of heparin in patients with repeated implantation failures. Although these changes are not statistically significant, the presence of an increasing trend in all the outcome parameters signify the possible benefits of heparin proving for the present study hypothesis.

Keywords: Adjuvant therapy, Heparin, Implantation rate, Immunomodulator, Implantation failure, Luteal phase support, Recurrent

INTRODUCTION

Assisted reproductive technology (ART) has revolutionized the treatment of all forms of infertility and has made its application widespread. Since the introduction of this innovative approach for infertility, research is ongoing to improve all the crucial steps of ART, but 70% of apparently normal embryos transferred fail to implant.[1] Implantation requires orchestration of multiple events starting from development of embryo and expression of cytokines which play key role in cross talk of the embryo with the endometrium during implantation window.[2] Implantation is a harmonized event with a myriad of interactions between the blastocyst and uterus among which adhesion, basement membrane penetration and remodeling of the extracellular matrix are crucial. Implantation failure is related to either maternal or embryological factors.

To overcome repeated implantation failure, heparin is hypothesized to improve outcomes in previously failed IVF cases when used as adjunct. In the absence of any potential cause for failed implantation, heparin is given as empirical treatment in the hope of a successful pregnancy

outcome. Although the outcomes of both low molecular weight heparin (LMWH) and unfractionated heparin (UFH) are similar but the selection of either of these for the management would be individualized based on their pharmacodynamic, pharmacokinetic and various adverse effects caused by them.[3]

It is not very well established that systemically applied heparin might get into contact with the blastocyst or exert its effects exclusively on the decidual side of the embryo - maternal interphase. Interactions of heparin with enzymes responsible for degradation of the extracellular matrix is a subject of ongoing research. This was a prospective randomized controlled trial done in ART Centre of our institute to determine the effect of low molecular weight heparin in women with previous IVF-ET failure and to assess beneficial effects of low molecular weight heparin (LMWH) in females without hereditary or acquired thrombophilia.

The aim of this study was to evaluate the efficacy of heparin on implantation in women with previous implantation failures and looking for following outcomes;

- Implantation rate per embryo transfer (serum beta human chorionic gonadotropin of >100 IU) in women who were undergoing embryo transfer
- Clinical pregnancy rate (presence of fetal cardiac activity at 6 weeks period of gestation
- Ongoing pregnancy rate (20 weeks period of gestation)
- Live birth rate
- Take home baby rate.

METHODS

It was prospective randomized controlled trial.

Sample size calculation

Earlier study by Urman et al, showed that ongoing pregnancy rate in LMWH group and control group was 37% and 27% respectively.[4] Assuming the same level of outcome in the present study, the required sample size in each group at 5% level of significance and 80% power is 322. Therefore a total of 650 patients are required for the study. However considering time constraints and logistic problems, it is decided to go for pilot study with 50 samples in each group. Therefore a total of 100 patients are required. Flowchart of the study is presented in Figure 1.

The study was conducted in assisted reproductive technology centre of a tertiary care institute which was referral center for ART techniques. Presented study recruited 100 patients with fresh non donor oocyte who had history of at least two previous failed IVF/ICSI from December 2015 to August 2017, after taking informed written consent from the couple.

The study was registered under CTRI/2017/10/010176 and also approved by the ethics committee of the institute and initiated thereafter. Enrolled subjects who agreed to participate were randomized by using computer generated randomization table and divided into study group (Group 1) and control group (Group 2). This randomization was done on the day of oocyte retrieval.

Inclusion criteria

- History of at least two previously failed IVF/ICSI cycle
- Age ≤38 years
- No hormonal, coagulation or immunological disorders in woman
- Normal uterine cavity confirmed by hysteroscopy.

Exclusion criteria

- Women on anticoagulant therapy
- Causes impeding implantation failure like fibroids distorting uterine cavity, large hydrosalpinx, and non-availability of grade 1 or grade 2 embryos
- Congenital or acquired thrombophilia
- Severe male factor infertility requiring surgical sperm retrievals.

Statistical analysis

Subjects were randomized into two groups in the ratio 1:1 using computer generated randomization technique. Adhering to the inclusion and exclusion criteria, a total of 50 patients were included in the Group 1 who received injection low molecular weight heparin (LMWH) and Group 2 patients (n=50) received routine luteal phase support. Data was computerized using Excel spread sheet. All statistical analysis was performed using statistical package for social sciences (SPSS) IBM version 20.0. Normality assumption was tested using Kolmogorov-Smirnov test.

For normally distributed data, descriptive statistics such as mean, standard deviation (SD) and range values were calculated. For non- normal data, median and interquartile range (IQR) were calculated. Comparison of two groups' mean was done using Student's t-independent test. Comparison of two median values was done using non-parametric Mann Whitney U test. Categorical variables were expressed as frequency and percent values. Comparison of categorical variables was tested using x^2/Fisher exact test as appropriate. For all statistical tests, a two-sided probability value of p <0.05 was considered statistically significant.

RESULTS

100 patients were analyzed. Table 1 depicts the baseline characteristics of the study population (n=100). The baseline characteristics of participants were similar in

two groups. Primary and secondary outcomes are detailed in Table 2 and Figure 2. Two major parameters depicting success of IVF are implantation rate and live birth rate. The primary outcome was implantation rate and clinical pregnancy rate. Implantation rate was 11.03% in cases and 5.48% in controls (p=0.08) Clinical pregnancy rate achieved was 18% and 12% in cases and controls respectively (p=0.401). Live birth rate was 5.15% and 3.42% in cases and controls respectively (p=0.562). Take home baby rates were 22% in cases and 12% in controls respectively. Complications observed are depicted in Table 3.

Table 1: Baseline characteristics of the patients in both groups.

Characteristics	LMWH (Group 1)	Control (Group 2)	P value
Age in years (Mean±SD)	31.84±4.0	31.64±4.6	0.34
BMI (kg/m^2) (Mean±SD)	24.3±3.3	25.03±3.2	0.07
Duration of infertility (years)	6.7	6.1	0.206
Etiology of infertility			
Male factor n (%)	10 (20%)	8 (16%)	0.603
Female factor			
Ovulatory n (%)	6 (12%)	7 (14%)	0.766
Tubal n (%)	26 (52%)	25 (48%)	0.841
Endometriosis n (%)	5 (10%)	6 (12%)	0.995
Unexplained n (%)	13 (26%)	12 (24%)	0.817
Multiple factors n (%)	4 (8%)	6 (12%)	0.741
Tubal status			
Patent n (%)	21 (42%)	28 (56%)	0.161
Blocked n (%)	29 (58%)	22 (44%)	
Received ATT n (%)	23 (46%)	24 (48%)	0.841
Semen analysis (count)			
Mean±SD	60.28 ± 43.9	52.1±46.3	0.072
Median	60	41	
Interquartile range	38.5 - 85.75	21.25 - 76.0	
Semen analysis (motility)			
Mean±SD	46.0±20.79	39.5±20.53	0.149
Median	50	35	
Interquartile range	30 - 60	25 - 60	
Number of attempts			
2 n (%)	36 (72%)	43 (86%)	0.086
>2 n (%)	14 (28%)	7 (14%)	
Number of previous failed cycles (Mean±SD)	2.3±0.61	2.2±0.53	
Stimulation protocols			
Agonist n (%)	29 (58%)	27 (54%)	0.687
Antagonist n (%)	21 (42%)	23 (46%)	
Number of days of stimulation (Mean±SD)	11.11±1.65	11.64±2.08	0.1612
Number of follicles on the day of trigger (Mean±SD)	8.31±4.07	8.97±3.37	0.379
Hormonal profiles			
AMH (ng/mL)	4.14±3.5	3.64±2.12	0.389
FSH (ng/mL)	5.5±1.9	5.93±2.22	0.300
LH (ng/mL)	4.705±2.67	4.60±2.38	0.839
D2 Antral follicle count (n)	6.5±3.6	7.1±3.5	0.400
Endometrial biopsy on day 22			
Proliferative n (%)	16 (32%)	9 (18%)	0.269
Secretory n (%)	27 (54%)	33 (66%)	
Embryo transfer			
Easy n (%)	44 (80%)	43 (86%)	0.766
Difficult n (%)	6 (12%)	7 (14%)	
Endometrial thickness (mean ± SD) (mm)	8.9±1.67	8.8±1.2	0.724
Endometrial quality on day of trigger (n)	42 - trilaminar 8 - diffuse	39 - trilaminar 11 - diffuse	0.444
Mean number of oocytes (mean±SD)	7.86±4.08	8.48±4.05	0.282

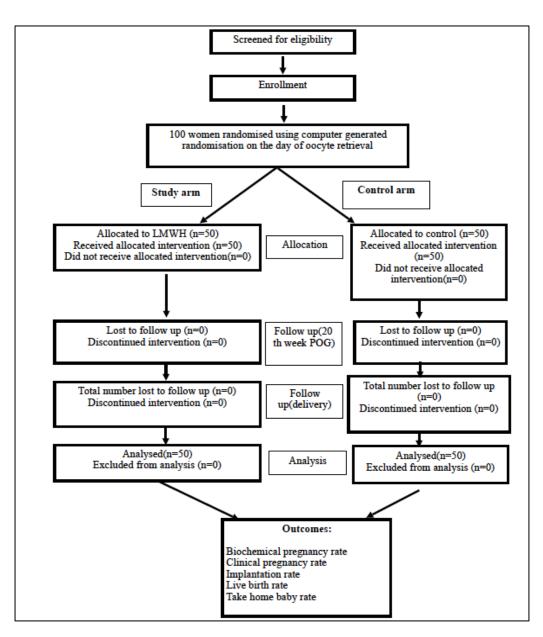| Characteristics	LMWH (Group 1)	Control (Group 2)	P value
Mean number of grade 1 and grade 2 oocytes (mean±SD)	2.56±2.4	3.02±2.63	0.365
Number of follicles on the day of trigger	8.31±4.07	8.97±3.37	0.375*
ET on the day of trigger	8.9±1.67	8.86±1.224	0.892
Median estradiol levels on the day of trigger (IQR)	3045.5 (2449-4730)	4305.5 (2616 - 5110)	0.148
Procedures			
IVF n (%)	40 (80%)	34 (68%)	0.171
ICSI n (%)	10 (20%)	16 (32%)	
Mean number of embryos transferred (Mean±S.D)	5.37±2.50	5.76±3.06	0.486
Mean number of grade 1 embryos transferred (Mean±SD)	3.4±1.9	3.5±2.3	0.593
Mean number of grade 2 embryos transferred (Mean±SD)	1.02±1.01	1.3±1.35	0.243

Figure 1: Flow chart of the study.

Table 2: Overall outcomes of the study.

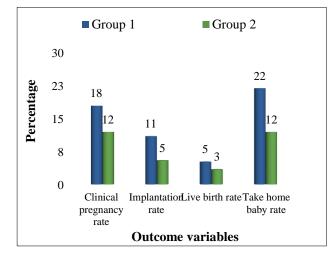| Overall outcomes	Group 1	Group 2	P value
Biochemical pregnancy rate	9/50 (18%)	6/50 (12%)	0.401
Clinical pregnancy rate	9/50 (18%)	6/50 (12%)	0.401
Clinical pregnancy rate per transfer	9/136 (6.61%)	6/146(4.10%)	0.348
Implantation rate	15/136 (11.03%)	8/146 (5.48%)	0.08
Live birth rate	7/136(5.15%)	5/146 (3.42%)	0.562
Take home baby rate	11/50 (22%)	6/50 (12%)	0.18
Delivery			
Normal vaginal delivery (n)	0	0	
Caesarean delivery n (%)	9/50 (18%)	6/50 (12%)	0.401
Newborns n (%)	11/50 (22%)	6/50 (12%)	0.18

Figure 2: Outcome variables depicted in bar graph.

Table 3: Complications of pregnancy.

Complications	Group 1	Group 2	P value
Multiple pregnancies (twins/triplets) n	3/2=5 5/9=55%	0/1=1 1/6=16%	0.287
Spontaneous miscarriages/IUD (n)	3/9=33%	0	0.229
Ectopic (n)	0	1/6=16%	0.40

DISCUSSION

Many factors are known to be involved in the complex hormonal process of implantation including many cytokines, growth factors, adhesion molecules, matrix metalloproteinases and the list goes on with ongoing research on the same. Implantation rate is the rate limiting step between embryo transfer and achievement of clinical pregnancy and implantation and subsequently live birth. Many confounding factors have been known to act in the process. Despite conquering all other failures of ART techniques this has been a challenging problem. Several hypothetic adjuvant therapies have been tried to improve the success rate like androgens, glucocorticoids, growth hormone to improve oocyte number and quality. To improve endometrial response sildenafil, granulocyte colony-stimulating factor, endometrial scratching, low dose aspirin, heparin, intrauterine injection of human chorionic gonadotropin and corticosteroids have been used. Antioxidants, Chinese herbal medicine, acupuncture, assisted hatching and preimplantation genetic screening to correct embryonic factors. Among these LMWH was used as a therapy in women with previous failed IVF/ICSI at our centre.

Hamdi et al, describes the role of heparin in complex cross talk during the implantation window even without the presence of thrombophilia.[5] Nelson S et al concluded that heparin modulates the production and interaction of several molecules like cytokines, integrins, growth factors and matrix - metalloproteinases.[6] Wilcox A et al showed that during implantation window endometrium prepares itself by regulating the synthesis of insulin-like growth factor (IGF-1), insulin like growth-factor-binding protein (IGFBP-1), inteleukin-1, leucocyte inhibitory factor-1, colony stimulating factor-1 and integrins.[7] Also heparin regulates heparin-binding epidermal growth factor (EGF), which is expressed maximally during implantation. Thus it enhances implantation, trophoblast invasion and promotes the early stages of embryo development. Heparin promotes trophoblastic invasion while reducing expression of e-cadherin thus promoting trophoblastic invasion and proliferation into endometrial tissue. Besides anti thrombotic effect, heparin restores trophoblast invasiveness and differentiation and blocks complement activation and modulates inflammatory responses in women with APA (antiphospholipid antibody).[8,9]

In unexplained RIF, with the absence of any anatomical, endocrine, immunological or genetic abnormality, suboptimal endometrial receptivity is known to be key limiting factor.[10] Heparin is known to improve endometrial receptivity in these patients. Heparin also acts at cellular level to improve endometrial receptivity and implantation. In a meta-analysis by Potdar et al, they

concluded that use of adjunct heparin improved live birth rate by 79%.[11] Urman et al observed relative increase in live birth rate by 30%.[4]

In this RCT, we observed notable increase in implantation rates. Although, there is sufficient evidence so far in the literature about the efficacy of heparin to improve implantation, it was not effectively tried in Indian population. The study group showed significant increase in implantation rates (IR) and multiple pregnancy rates suggesting beneficial effects of heparin in patients with repeated implantation failures. There was an observed marginal rise in BPR, CPR and LBR in the study group when compared to control group though statistical significance was precluded by lower sample size. Although these changes are not statistically significant, the presence of an increasing trend in all the outcome parameters signify the possible benefits of heparin proving for the present study hypothesis. Further multi centric double blinded randomized controlled trials with larger sample size need to be undertaken in order to achieve sufficient evidence and prove the study hypothesis statistically.

The limitations of this study are, it is a pilot study with a small sample size. The study has included patients with previous two implantation failures.

CONCLUSION

The result of this pilot study showed relative increase in implantation rates (IR) suggesting beneficial effects of heparin in patients with repeated implantation failures. Although these changes are not statistically significant, the presence of an increasing trend in all the outcome parameters signify the possible benefits of heparin proving for the present study hypothesis.

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