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Research Article

Optimization of Assisted Reproduction Technologies in Patients with Hereditary Thrombophilia and Recurrent IVF Failures

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Abstract

Study Background: Poor fertility outcomes in women with recurrent implantation failure and hereditary thrombophilia present significant challenges in assisted reproduction.

Objective: We prospectively examined different approaches to increasing of the efficiency of IVF programs in patients with hereditary thrombophilia and recurrent implantation failure.

Methods: A prospective randomized parallel group study with patients treated from January 2010 to December 2012. The study included 117 patients with a diagnosis of recurrent IVF failure and hereditary thrombophilia including Factor V Leiden mutation, prothrombine gene G20210A mutation, fibrinogen gene mutation, 4G/5G plasminogen activator inhibitor polymorphism, methylenetetrahydrofolate reductase enzyme polymorphism and factor XIII polymorphism. After the oocyte retrieval all patients were randomly allocated into three groups: group A (n=48) had embryo transfer in stimulation cycle, group B (n=39) had frozen embryo transfer in cycles with hormone replacement therapy (estrogens and vaginal progesterone) and group C (n=30) had frozen embryo transfer in natural cycle.

Results: The clinically significant differences in the ART outcomes were identified. The implantation rate was 19.6% in group A, 22.3% in group B and 23.6% in group C ($p<0.05$). The clinical pregnancy rate was the highest after vitrified embryo transfer in natural cycle (43.3% (13/30) vs 33.3% (16/48) and 38.4% (15/30) in groups A and B respectively ($p<0.05$)).

Conclusion: The use of low molecular weight heparin allows to get high implantation and pregnancy rate in women with recurrent implantation failure and hereditary thrombophilia. Significantly higher pregnancy rate was observed with frozen embryo transfer and low molecular weight heparin administration. Vitrification of all embryos and their transfer in a natural cycle is the most effective approach to the management of patients with thrombophilia and failed IVF.

Keywords: RIF; Thrombophilia; ART; LMWH

Abbreviations

ART: Assisted Reproductive Technology;

COH: Controlled Ovarian Hyperstimulation;
FET: Frozen Embryo Transfer;
FSH: Follicle Stimulating Hormone;
FVL: Factor V Leiden;
GnRH: Gonadotropin Releasing Hormone;
HRT: Hormone Replacement Therapy;
IVF: In vitro Fertilization;
LMWH: Low Molecular Weight Heparin;
MTHFR: Methylenetetrahydrofolate Reductase Enzyme;
PAI: Plasminogen Activator Inhibitor;
RIF: Recurrent Implantation Failure

Introduction

According to the numerous studies every sixth couple in the world is faced with the problem of infertility [1,2]. However despite the rapid development of reproductive medicine the efficiency of ART programs remains stable and approximately 30% of couples have repeated in vitro fertilization (IVF) failures [3].

The ART outcome is influenced by many factors, including maternal age, overweight, diminished ovarian reserve, endocrine disorders, impaired endometrial receptivity and the quantity and quality of embryos transferred [4]. The role of thrombophilia in recurrent implantation failure (RIF) in IVF programs has been critical and debated question for years. Increasing concentrations of estradiol and hypercoagulability accompanied the process of controlled ovarian hyperstimulation aggravate the clotting disorders underlying thrombophilia. All this adversely affects the implantation and the early stages of embryo development.

However, the published study data are controversial. There are many papers supporting the clinical evidence of thrombophilia in IVF outcomes [5-8]. However, there are also a lot of studies failed to demonstrate the direct association between thrombophilia and repeated IVF failures [9,10]. There is only one systematic review and meta-analysis evaluating the association between thrombophilia and ART programs outcomes published by Di Nisio M. et al in 2011. It was noted that the frequency of Factor V Leiden mutation (FVL) in patients with a history of IVF failure was significantly higher than in fertile patients and in women who conceived after IVF (OR=3.08, 95% CI 1,77-5,36). The association between the other thrombophilic states and ART failures were not found. But the authors of the meta-analysis noted a very high degree of heterogeneity between the studies (75%) [11].

Cochrane reviews concerning the issue of failed IVF implantation and thrombophilia are not currently available. Akhtar MA and colleagues published a Cochrane review (2013) concerning the use of heparin in ART programs. The review included the results of three randomised controlled trials with a total of 386 patients. It was found that the use of low molecular weight heparin (LMWH) in IVF protocols is associated with a significant increase in pregnancy rate compared with controls (OR 1.61, 95% CI 1.03-2.53). The results of this Cochrane review suggested that peri-implantation LMWH in ART cycles may improve the live birth rate in women undergoing assisted reproduction. However, these results were dependent on small low quality studies with substantial heterogeneity, and were sensitive to the choice of statistical model. At the same time the authors noted that it is impossible to make definite conclusions on this issue, since the included studies had high degree of heterogeneity and low quality evidence. Moreover, LMWH did cause adverse effects including bruising, ecchymosis, bleeding, thrombocytopenia and allergic reactions [12].

At the moment there is no consensus on the role of thrombophilia in the pathogenesis of implantation failure and infertility, there is no unified approach to the management of the patients with thrombophilia preparing to IVF protocol. Due to the ambiguity of the study results every country has its local clinical guidelines. The purpose of the present study was to compare the efficiency of different approaches in the management of patients with thrombophilia and RIF.

Materials and Methods

Patients were selected to IVF between June 2011 and December 2012 at the Infertility Unit «Euroclinic» of Russian Medical Academy of Postgraduate Education. The local Academician Review Board approved the study and all recruited patients signed an informed consent.

The study included one hundred seventeen women with history of two or more previous IVF failures and at least one thrombophilic defect. The exclusion criteria were contraindications for pregnancy and delivery, congenital malformations or acquired deformations of the uterine cavity, ovarian tumors, benign uterine tumors that require surgical treatment, malignant neoplasms. Endometrial pathology was excluded in the cycle preceded stimulation with hysteroscopy and endometrial biopsy.

Patient's age ranged from 18 to 38 years. Mean duration of infertility was 6.3±2.4 years. The average number of previously failed IVF–embryo transfer cycles was 2.5. 4G/5G plasminogen activator inhibitor (PAI-1) and MTHFR polymorphisms were the most prevalent thrombophilic defects (81.2% (95) and 90.6% (106), respectively). The heterozygous Factor V Leiden mutation (FVL) was found in 11 of 117 patients (9.4%), while the heterozygous prothrombine G20210A mutation was revealed in 9/117 individuals (7.7%). The heterozygous polymorphism of fibrinogen was observed in 16/117 women (13.6 %), platelet receptor GP 1A – in 6/117 (5%), coagulation factor XIII polymorphism - in 12/117 (10.3%) respectively. A com-

bination of several polymorphisms was observed in 86.3% of patients.

Definition of gene mutations, polymorphisms and predisposing to thrombosis, was conducted using polymerase chain reaction as described by different authors [13,14].We used the gonadotropin releasing hormone (GnRH) antagonists protocol for controlled ovarian hyperstimulation (COH). Urinary or recombinant human chorionic gonadotropin (hCG) was used for the triggering of final maturation. Oocytes were retrieved 35 hours later, followed by ICSI.

After the oocyte retrieval all patients were randomly allocated into three groups: group A (control group, n=48) had embryo transfer in stimulation cycle, group B (n=39) had frozen embryo transfer (FET) in cycles with hormone replacement therapy, HRT (estrogens and vaginal progesterone), and group C (n=30) had FET in natural cycle. Embryos with the highest quality rating (A, AB) were selected for the transfer. 1-2 embryos were transferred in all groups. The embryos in groups B and C were successfully cryopreserved by vitrification.

All the patients had standard examination before IVF and also were tested for blood coagulability including D-dimer. Blood samples were obtained before the stimulation, during the COH (group A) or HRT (group B) or on the10th day of natural cycle (group C) and after the embryo transfer in all groups.

The primary outcomes were the implantation and clinical pregnancy rates. Statistical analysis was performed using Statistica for Windows 7.0 (StatSoft Inc.). The significance of any difference in proportions was tested using the Fisher exact test or by chi-square statistics as appropriate. Statistical significance was taken as $p<0.05$.

Results and Discussion

The average age of women in the group A was 31.2±3.4 years, in the group B – 33.1±2,3 years and in the group C – 32,6±3.1 years ($p>0.05$). There were no significant differences in basal follicle stimulating hormone (FSH) levels and etiology of infertility between the groups.

All patients with MTHFR polymorphisms received high doses of folic acid (4 mg/day). Peri-implantation LMWH was given as a prophylactic measure, according to the data of the last Cochrane review discussed above [12]. Grandone E et al. (2014) also showed that LMWH alone or combined with low dose aspirin could have a role in fostering the implantation of embryos and improving the number of live births after ART [10]. Resent systematic review and meta-analysis confirmed that the use of adjunct LMWH significantly improves live birth rate by 79% compared with the control group. However, the authors note that these results should be considered with caution, since the overall number of participants in the studies was small [15]. When the concentration of D-dimer increased more than twice, the therapeutic doses of LMWH were used. Low-dose aspirin was added in the cases of hyperaggregation.

The basal concentration of D-dimer before the COH was within the normal range in all groups. During the COH increased D-dimer was observed in 87.1%. In group A, the concentration of D-dimer in postransfer period increased an average in 3.8 times with strong positive correlation with estradiol level. High doses of LMWH were given to these patients. In group B, the level of D-dimer increased an average in 2.3 times in postransfer period and in group C, the D-dimer level remained normal and increased only if pregnancy occurred.

The clinically significant differences in the ART outcomes were identified. The implantation rate was 19.6% in group A, 22.3% in group B and 23.6% in group C ($p<0.05$). The clinical pregnancy rate was the highest after vitrified embryo transfer in natural cycle (43.3% (13/30) vs 33.3% (16/48) and 38.4% (15/30) in groups A and B respectively ($p<0.05$)).

Patients who received LMWH for thromboprophylaxis had rather high implantation and pregnancy rates. These results correspond to the data of Qublan H et al. who showed significant increase in the implantation and pregnancy rates in LMWH group compared with the placebo group [16]. It has been proposed that heparin enhances the intra-uterine environment by improving decidualisation with an associated activation of growth factors and a cytokine expression profile in the endometrium that is favorable to pregnancy [12].

Conclusion

The use of LMWH allows to achieve high implantation and pregnancy rate in women with RIF and hereditary thrombophilia. Significantly higher pregnancy rate was observed with FET and LMWH administration. Vitrification of all embryos and their transfer in a natural cycle is the most effective approach to the management of patients with thrombophilia and failed IVF.

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