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The association between poor ovarian response and thrombophilia in assisted reproduction

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ABSTRACT

Objective: To investigate the association between thrombophilic risk factors and poor ovarian response in an assisted reproductive technologies (ART) setting.
Study design: This is a preliminary prospective cohort study in a university affiliated reproductive medicine unit. Eighty-nine infertile women undergoing IVF-ET treatment were recruited. Following IVF-ET treatment, the 28 women that had ≤3 oocytes on retrieval were the study group, and the 61women that had ≥ 4 oocytes on retrieval were the control group. All women underwent ovarian reserve testing and thrombophilia work-up prior to treatment.
Results: Patients' characteristics, except for chronological age, were similar between the two groups. Women in the study group had clear manifestations of low ovarian reserve, evident by ovarian reserve testing, controlled ovarian hyper-stimulation and IVF-ET treatment results, as compared to the control group. The incidence of all thrombophilias tested was similar between the study and control group. Moreover, the incidence of any single or combined thrombophilia was also similar between the two groups. Logistic regression model analysis and Pearson correlation tests did not show significant correlation between number of oocytes retrieved and thrombophilia. Furthermore, basal ovarian reserve tests did not differ between women with and without thrombophilia. When only the 48 women ≤ 35 years of were analyzed, the five women in the study group had significantly higher incidence of a single as well as combined thrombophilia as compared to the 43 controls.
Conclusions: Thrombophilic risk factors have no correlation with poor ovarian response in the general infertile population undergoing ART. Whether premature low ovarian reserve is linked to thrombophilia remains to be established.

1. Introduction

Ovarian aging is a continuous process governed by a gradual decrease in the quantity and quality of the oocytes starting in-utero and extending through the menopausal transition. On average, fecundity begins gradually decreasing at age 31 and at about 37–38 years of age a significant decline in fertility occurs [1–3].

Nevertheless, there is a wide variability in the ovarian reserve among women of the same age group, with a distribution range of 20 years [4]. It has been well recognized that about 10% of women in the general population reach their menopause by the age of 45 years [5–7]. A significant proportion of women of reproductive age will exhibit follicular apoptosis, depletion of ovarian reserve and a significant decline in fertility at a much earlier age; considerably before the age of 37–38 years, leading to "premature" low ovarian reserve or ovarian aging [3].

Although the exact mechanism behind ovarian aging is not yet clear, it is believed today to be multi-factorial and possibly inter-related to different medical, lifestyle, autoimmune, genetic and as yet unknown factors [3].

Several observations from the last few years might suggest that low ovarian reserve could be related to vascular homeostasis and tendency for thrombosis, i.e. thrombophilia. Unexplained infertility, an identified risk factor of ovarian aging [8–10], has been associated with thrombophilia [11,12]. Repeat IVF failure, a major feature of ovarian aging [13], has also been related to thrombophilia [14–16].

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Moreover, decreased ovarian stromal blood flow has been associated with low ovarian reserve [17,18]. Concomitantly, the presence of at least one mutant allele of factor V Leiden and apolipoprotein E has been shown to be associated with reduced age at natural menopause [19,20]. As well, higher levels of clotting factor VII have been found to be associated with early menopause [21,22].

Taken together, these observations might imply that thrombophilic risk factors could be involved in the development of low ovarian reserve. To the best of our knowledge, ovarian aging has not been investigated, in a targeted study, in relation to thrombophilia, in an assisted reproductive technologies (ART) setting. Therefore, our objective was to prospectively evaluate the occurrence of thrombophilia in infertile women undergoing ART treatment and to explore whether low ovarian reserve manifested by poor ovarian response could be related to thrombophilia.

2. Materials and methods

2.1. Study population and design

Eighty-nine unselected and consecutive infertile women undergoing IVF-ET treatment were prospectively enrolled into this study. Only women with tubal and male factors or unexplained infertility were included. All women, between 18 and 44 years of age and with a body mass index of 18–34 kg/m^2, were regularly menstruating with two intact ovaries and no previous ovarian surgery. Women in the study were found to have normal uterine cavities by hysterosalpingography and/or hysteroscopy. Women with polycystic ovary syndrome or endometriosis were excluded. Moreover, women with previous thrombo-embolic phenomena, under anti-thrombotic treatment or with known thrombophilia were excluded from evaluation. Patients with uncontrolled thyroid disease, diabetes mellitus or hyperprolactinemia were also excluded from the study. Women enrolled could participate in the study only once.

This was a prospective cohort study. The medical staff in the IVF and endocrine laboratories, as well as the ultrasonographers, were blinded to the drug regimen and conduct of the study. The research project was approved by the Poriya Medical Center Institutional Review Board and an informed consent form was signed by each woman participating in the study.

Following recruitment all women underwent basal ovarian reserve tests and thrombophilia work-up. Following this investigation all women underwent controlled ovarian hyperstimulation (COH) and IVF-ET.

2.2. Ovarian reserve tests

Early follicular basal antral follicle count (AFC, 2–9 mm) and ovarian volume evaluation were performed in a natural cycle as described previously [23]. Ovarian ultra-sonography was performed employing a trans-vaginal probe (5–9 MHz) (Voluson 730 expert; General Electric Medical System, Milwaukee, WI). Blood was drawn on the same day for serum basal FSH, LH, and E_2 levels as well as FSH/LH ratio evaluation as previously described [23].

2.3. Thrombophilia work-up

Blood samples were collected by venipuncture into 3.2% sodium citrate tubes and were centrifuged twice at 2000 × *g* for 15 min. Plasma samples were frozen at −70 ± 5 °C. Before testing, plasma aliquots were thawed in a 37 ± 0.5 °C water bath for 15 min. All coagulation assays and genetic thrombophilic risk factors have been performed as previously described [24]. A complete list of the thrombophilic tests performed in the study appears in Table 4.

2.4. IVF treatment

Controlled ovarian hyper-stimulation was chosen on a case-to-case basis according to standard clinical practice. Conventional IVF and/or intra-cytoplasmic sperm injection (ICSI) were performed according to the cause of infertility. A detailed description of sperm, oocyte and zygote handling as well as embryo transfer and luteal phase supplementation in our unit has been published previously [25].

2.5. Poor ovarian response definition

Following completion of treatment women enrolled were divided into two groups in accordance with the number of oocytes retrieved. Women with ≤3 oocytes were included in the study group and women with ≥4 oocytes in the control group. Patients' characteristics, ovarian reserve tests, COH and IVF-ET results as well as thrombophilia work-up were compared between the two groups.

The number of retrieved oocytes following conventional stimulation is a well accepted criterion of ovarian reserve. Several studies have adopted the number of 3 or less oocytes on the retrieval day as a manifestation of low ovarian reserve [26,27], and this criterion has been recently adopted by the ESHRE consensus on the definition of poor response [28].

2.6. Statistical analysis

We analyzed all data using the Software Package for Social Sciences (SPSS) for windows version 15.0 (SPSS Inc. 2006, Chicago, IL, USA). Descriptive procedure was used to evaluate patients' characteristics and each variable is presented as mean ± SD. Mann–Whitney *U* test, independent two sample Student *t*-test, Chi-square (χ^2) for crosstab data, *Z* test for two proportions, Pearson correlation and logistic regression were used wherever appropriate. Normal distribution was analyzed prior to statistical tests using Wilk–Shapiro test. *P* value of <0.05 was considered as statistically significant.

Because the sample size was limited to available data, a post hoc power analysis using observed mean differences and standard deviations demonstrated that this study had at least 80% power to detect differences of 2.0 in FSH level, of 3.5 in AFC, of 900 IU in total gonadotropin requirement and of 3 in number of follicles ≥14 mm on hCG day between the study and the control group (based on the two-sample *t* test for unequal *n* using a two-sided significance level of 0.05) and to detect differences of 50% in the prevalence of ≥2 combined thrombophilia in women ≤35 years old (based on the two-proportion *Z* test for unequal *n* using a one-sided significance level of 0.05).

3. Results

Among the 89 women enrolled in the study, 28 had ≤3 oocytes (study group) and 61 had ≥4 oocytes (control group) retrieved. Patients' characteristics, including infertility duration, order and etiology, as well as number of previous IVF cycles, were similar between the groups. Lifestyle factors including chronic smoking rate and body mass index (BMI) were also similar between the groups. Gravidity, parity, number of children and previous abortions were also similar (Table 1). The only significant difference was the mean age of women, corresponding to 38.7 ± 4.2 and 32.6 ± 5.2 years in the study and control groups respectively.

Basal ovarian reserve tests showed clear signs of low ovarian reserve in the study group as compared to the control group. This was manifested by significantly higher serum FSH level, lower AFC and ovarian volume (Table 2).