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Prolonged activation of the coagulation system during in vitro fertilization cycles

Yoni Cohen[a,b,*], Togas Tulandi[a], Benny Almog[b], Eyal Zohav[b], Varda Deutsch[c], Ariel Many[b], Ami Amit[b], Aviad Cohen[a,b]

[a] Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada
[b] Department of Obstetrics and Gynecology, Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, 6 Weizmann St., Tel Aviv, 64239, Israel
[c] The Hematology Institute, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, 6 Weizmann St., Tel Aviv, 64239, Israel

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ABSTRACT

Objective: To investigate coagulation system changes during an in-vitro fertilization (IVF) cycle using Thromboelastogram (TEG) that enables analysis of the elastic properties of whole blood samples and provides a global assessment of the hemostatic function.
Study design: A prospective study. TEG indices were evaluated in 23 women who underwent controlled ovarian stimulation for IVF at four points in time: 1. At the beginning of the cycle (corresponding to the lowest levels of E2), 2. On the day of hCG administration (maximal stimulation with highest E2 levels), 3. On the day of ovum pickup and 4. At the first pregnancy test (approximately 14 days after ovum pickup). The main outcome measures were TEG indices including R-time (time until initial fibrin formation), K-time (time until a 20 mm amplitude is achieved), α angle (the rate of clot formation), Maximum Amplitude (MA, strength of the fibrin clot), Coagulation Index (CI, calculated overall indicator of coagulation) and LY30 (the decrease in graph amplitude).
Results: R, K, α angle, MA and CI before hCG administration and at the time of the first pregnancy test were significantly higher compared to the baseline measurement before gonadotropins administration. No correlation was found between E2 and TEG indices.
Conclusion: Ovarian stimulation is associated with prolonged increased coagulability that extends after the time of maximal ovarian stimulation. The lack of association between E2 levels and TEG indices suggest that additional factors may play a role in the pathogenesis of increased coagulability in women with ovarian stimulation.

Introduction

Women undergoing controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) treatment, are at increased risk for hypercoagulability predisposing to arterial or venous thrombotic complications. The incidence of thrombotic complications in the assisted reproductive technology (ART) population is estimated to be 0.08–0.11% [1,2]. Although these events are rare, they are potentially life-threatening. Indeed, ART treatment is associated with a risk of thrombotic complications especially in the presence of ovarian hyperstimulation syndrome (OHSS), yet its mechanism is still unclear.

The critical period for the development of thrombosis depends on the type of treatment and the site of thrombosis. Chan and Dixon [3] reported that arterial thrombotic complications usually occur 10.5 days after embryo transfer (ET) and venous thrombotic complications typically occur 40 days after ET. In women of reproductive age undergoing ART, the risk for a thrombotic event is approximately 10 times higher compared to women who are not undergoing ART. This may be attributed to an increased level of estradiol, or due to other factors. Both the coagulation and fibrinolysis systems are activated during IVF and especially during OHSS [4–7].

Conventional coagulation tests, such as activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen level and platelets number can only analyze the coagulation cascade partially. They asses the extrinsic and intrinsic pathway,

* Corresponding author at: Department of Obstetrics and Gynecology, MUHC Reproductive Centre, McGill University, 687 Pine Avenue West, Montreal, Quebec, Canada.
E-mail address: yonni.co@gmail.com (Y. Cohen).

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but do not provide any information about clot formation, clot strength or clot dissolution. In contrast, Thromboelastogram (TEG) can overcome these limitations by evaluating the elastic properties of whole blood samples, providing a global assessment and a real time assessment of the hemostatic function [8]. Two studies have shown increased coagulable state during IVF using TEG [9,10].

The purpose of our study was to delineate changes in the coagulation system during the course of an IVF treatment and thereafter, with the objective of detecting the time period with the highest risk for thrombosis.

Materials and methods

Study population

Our study was conducted at the tertiary university-affiliated medical center and the Institutional Review Board of the center approved the study. Participants were sequentially offered enrollment and each signed an informed consent form before entering the study. The inclusion criteria were women undergoing COH with gonadotropins for IVF. The exclusion criteria were with signs or symptoms of OHSS, previously treated with antithrombotic agents, known coagulopathy and positive pregnancy test. TEG results were not revealed to the treating physicians and not used in the clinical management of the participants.

Controlled ovarian hyperstimulation protocol

Controlled ovarian hyperstimulation was performed in a standard manner. In short, we administered subcutaneous GnRH agonist from day 1of the cycle, followed by gonadotropins from day 3 or adjusted GnRH antagonist protocol, where gonadotropins were started on day 3 of the cycle followed by down regulation with GnRH antagonist when the follicles reached 12–14 mm in diameter or estradiol (E2) level >400 pg/ml. When at least 3 follicles attained a mean diameter of 17 mm, 250 mg of human recombinant chorionic gonadotropin was administered and oocyte retrieval was performed 36–38 h later. We administered luteal phase support with vaginal micronized progesterone (600 mg/day) from the day after ovum pickup (OPU) until the day of the pregnancy test, approximately 14 days after oocyte retrieval.

Patient data and laboratory variables

Demographic and clinical data included age, gravidity, current body mass index (BMI), current diseases, medications and personal or family history of coagulopathy. The IVF protocol parameters included COH protocol and the number of oocytes retrieved. Blood sample for E2, progesterone, TEG, PT, PTT, fibrinogen and complete blood count was obtained at the following time points: 1. At the beginning of the cycle before gonadotropins administration (day 2 of the cycle) reflecting the lowest levels of E2, 2. On the day of hCG administration (maximal stimulation with highest E2 levels), 3. On the day of ovum pickup and 4. When blood was drawn for the first β-hCG test, approximately 14 days after ovum pickup. Since pregnancy can alter the coagulation system, women with a positive b-hCG test were excluded from this study.

Blood sample for TEG was collected into a citrated tube for coagulation. One milliliter of citrated blood was then gently mixed with kaolin and 360 μL of this preparation was pipetted into a TEG cup containing 20uL of calcium chloride and pre-warmed to 37 °C. Measurements were performed in a TEG Hemostasis Analyzer 5000 (Haemonetics, Braintree Mass, USA). Analysis of TEG results was performed by TEG analytical software. We analyzed R-time (time until initial fibrin formation), K-time (time until a 20 mm amplitude is achieved on the graph), α angle (the rate of clot formation), Maximum Amplitude (MA, representing the strength of the fibrin clot), Coagulation Index (CI, calculated overall indicator of coagulation) and LY30 (percentage decrease in graph amplitude 30 min after achievement of maximal amplitude as a measure of fibrinolytic system).

Statistical analysis

With a standard deviation of 1.7, power of 80% and alpha of 0.05, we required 18 participants. This estimation for repeated measures at 3 different time intervals was based on the changes in the CI during IVF that were previously reported [9]. Presuming CI at a baseline of −0.4 and at OPU of 0.8 and assuming that the CI will reach the upper limit for the normal range (3) at the last time point [9]. We used parametric test for normally distributed data and non-parametric test (Wilcoxon test) for skewed data. Pearson correlation was used to examine the relations between the clinical parameters of the IVF cycle, hormone levels, blood count results and TEG indices. We also used the linear repeated measures mixed model to assess the longitudinal effect of time on PT, PTT, fibrinogen, hematocrit, hormone levels and TEG indices, from enrollment at the beginning of the IVF cycle until 14 days after ovum pickup. Variables for which we found a significant effect of time were further analyzed in a Bonferroni pairwise correction. $P<0.05$ was considered significant.

All statistical analysis was performed using SPSS 23 for Macintosh.

Results

We recruited 23 women who underwent IVF treatment. The indication for IVF, general demographic and clinical data are presented in Table 1. Conventional coagulation tests showed a small difference in PTT at the time of ovum pickup. Fibrinogen and platelets were significantly higher at the time of the pregnancy test compared to the baseline (Table 2). Changes in E2 were as expected during the IVF cycle with a nadir before gonadotropins administration (baseline) and at the time of the pregnancy test and peak level before hCG administration (Table 2).

The results of the linear mixed model indicated a significant time effect on the R, K, α angle, MA and CI. Follow up pairwise comparison of the different time points showed that CIs at the time

Table 1
General demographic and clinical data.

	n = 23
Age (years)	38.3 ± 4.9
BMI	24.5 ± 5.7
Indication for IVF	
Unexplained infertility	9 (39%)
Male factor	6 (27%)
Mechanical factor	2 (9%)
PGD	1 (4%)
Other (poor ovarian reserve, anovulation)	5 (21%)
IVF protocol	
Short GnRH agonist	13 (57%)
GnRH antagonist	10 (43%)
Days of stimulation	11 ± 2
Number of follicles[a]	8.7 ± 6.2
Number of oocytes retrieved	7.2 ± 4.6
Total dose of FSH (IU)	2860 ± 1248

GnRH–Gonadotropins releasing hormone.
Data presented as mean ± standard deviation (SD).
FSH–follicle stimulation hormone.
[a] Follicles >11 mm counted in the last ultrasound scan before hCG administration.