

# In Vitro Fertilization-Induced Alterations in Coagulation and Fibrinolysis as Measured by Thromboelastography

Miriam J. P. Harnett, MD, FFARCSI, Kodali Bhavani-Shankar, MD,  
Sanjay Datta, MD, FFARCS (Eng), and Lawrence C. Tsen, MD

Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Supraphysiologic increases in estrogen produced by *in vitro* fertilization (IVF) promote the expression of hemostatic markers. Although quantitative studies of individual markers have been performed during IVF, their results are conflicting and do not reveal the qualitative effect of each marker on the overall coagulation and fibrinolytic processes. Thromboelastograph<sup>®</sup> (TEG<sup>®</sup>) coagulation analysis, by contrast, provides a global measure of coagulation and fibrinolysis and can indicate the relative contributions of clotting factors, fibrinogen, and platelets to each process. We studied the serum estrogen concentrations and TEG<sup>®</sup> variables in 24 women at the beginning and conclusion of an IVF stimulation cycle. Serum estradiol (E<sub>2</sub>) concentrations (mean ± SD)

increased from 26.9 ± 8.6 to 2098 ± 913 pg/mL ( $P < 0.005$ ) at baseline and oocyte retrieval, respectively. The measured TEG<sup>®</sup> indices demonstrated alterations in coagulation rather than fibrinolysis. Although significant changes were noted in both the clot formation time and the coagulation index ( $P < 0.005$ ), all TEG<sup>®</sup> values remained within the normal range. In addition, an increased role of fibrinogen in promoting clot strength was observed. These findings may assist in the treatment of IVF patients who ultimately develop thromboembolic complications as a result of ovarian hyperstimulation.

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The ovarian stimulation process for *in vitro* fertilization (IVF) is characterized by dramatic and supraphysiologic increases in estrogen (1). Although the favorable production of retrievable oocytes is the intended result, these alterations in estrogen can also promote the expression of hemostatic markers and induce a hypercoagulable state, which, in extreme form, has been associated with arterial and venous thromboses (2-4). Although quantitative studies of individual hemostatic markers during IVF have been reported (1,5,6), the results are conflicting and do not reveal the qualitative contributions to the overall coagulation and fibrinolysis processes. Thromboelastograph<sup>®</sup> (TEG<sup>®</sup>) coagulation analysis, by contrast, can provide a global measure of coagulation and fibrinolysis as well as identify the relative contributions of

clotting factors, fibrinogen, and platelets. The objective of the present study was to evaluate the effect of IVF-induced changes in estrogen on coagulation and fibrinolysis as measured by TEG<sup>®</sup>.

## Methods

After approval by the hospital's Committee for the Protection of Human Subjects, written informed consent was obtained from all participants. Thirty-six ASA physical status I and II patients scheduled for ultrasonically guided oocyte retrieval were enrolled. Individuals with a history of smoking, bleeding disorders, liver disease, or those taking medications not associated with the IVF stimulation regimen, including analgesics and antiplatelet medications, were excluded.

The IVF stimulation regimen used at the Brigham and Women's Hospital has been previously reported (1). Briefly, human menopausal gonadotropins (Pergonal<sup>®</sup>; Serono Laboratories, Randolph, MA) and GnRH-a leuprolide acetate (Lupron<sup>®</sup>; Tap Pharmaceutical, Deerfield, IL) are administered in the mid-luteal or follicular phase of the menstrual cycle. The administration of gonadotropin-releasing hormone agonists

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Address correspondence and reprint requests to Lawrence C. Tsen, MD, Brigham and Women's Hospital, Department of Anesthesia, Perioperative and Pain Medicine CWN-L1, 75 Francis St., Boston, MA 02115. Address e-mail to ltsen@zeus.bwh.harvard.edu.

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results in the reduction of estrogen and progesterone concentrations. This hormonal down-regulation is confirmed by serial hormonal measurements. Acceptable baseline hormonal concentrations for initiating the IVF cycle are progesterone  $<1.4$  pg/mL and estradiol ( $E_2$ )  $<50$  pg/mL. Once down-regulation is achieved, ovarian hyperstimulation with human menopausal gonadotropins is initiated and followed by ultrasonographic confirmation of follicular growth and a progressive increase in serum  $E_2$  levels; progesterone levels remain low. When at least 2 follicles exceed 18 mm in diameter and the  $E_2$  is  $>600$  pg/mL, 10,000 IU of human chorionic gonadotropin (Pregnyl<sup>®</sup>; Organon Inc., West Orange, NJ) is given, and transvaginal oocyte recovery is performed 36–48 h later. The interval from hormonal down-regulation (baseline) to oocyte recovery (retrieval) is a period of 7–10 days. Venous sample analysis for progesterone and estradiol was done by the Reproductive Endocrinology Division of the Clinical Chemistry Laboratories of the Brigham and Women's Hospital by radioimmunoassay in accordance with the hospital's standard protocol.

Venipuncture for TEG<sup>®</sup> samples was performed at baseline and oocyte retrieval through an 18-gauge needle placed in a decubitus arm vein. The first 2 mL of blood was drawn into a discarded Vacutainer<sup>®</sup> (Becton Dickinson, Franklin Lakes, NJ); the 4.5-mL study sample was then collected into a citrated Vacutainer<sup>®</sup>. One milliliter of citrated blood was transferred into 1% celite vials, from which 2 340- $\mu$ L samples were withdrawn and placed in disposable analyzer cups of a computerized dual-channel TEG<sup>®</sup> analyzer (model 5000; Haemoscope Corp., Niles, IL). In one of the cups (channel 2), 5  $\mu$ L of ReoPro<sup>®</sup> (Eli Lilly, Indianapolis, IN), a platelet IIb/IIIa receptor antagonist (monoclonal antibody fragment c7E3 Fab), was added. Both cups, which were prewarmed to 37°C and contained 20  $\mu$ L of 0.2 M calcium chloride, were then analyzed simultaneously. All blood samples were placed in the TEG<sup>®</sup> analyzer within 20 min of collection.

The TEG<sup>®</sup> variables collected from each sample included: R (time to first clot formation), K (rate of clot strengthening), MA (maximal strength of the developed clot),  $\alpha$  angle (rate of clot polymerization), and LY60 (percent of clot lysis at 60 min after MA is reached). MA of samples without ReoPro<sup>®</sup> (channel 1) and with ReoPro<sup>®</sup> (channel 2) represent MA caused by fibrinogen and platelets ( $MA_{\text{wholeblood}}$ ) and fibrinogen alone ( $MA_{\text{fibrinogen}}$ ), respectively. The difference between the two channels represents the contribution of platelets to MA. Maximal amplitude attributed to platelets was thus derived by the formula:  $MA_{\text{platelet}} = MA_{\text{wholeblood}} - MA_{\text{fibrinogen}}$ . In addition, the calculation for the overall coagulation index (CI) was performed in accordance to the TEG<sup>®</sup> manufacturer's

guidelines via the equation:  $CI = -0.3258 R - 0.1886 K + 0.1224 MA + 0.0759 \alpha - 7.7922$ .

The statistical difference in various TEG<sup>®</sup> variables between the time of baseline  $E_2$  concentration and the time of oocyte retrieval was analyzed by using Wilcoxon's matched-pairs signed rank testing. This statistical analysis was chosen because our sample data were not necessarily parametric. Data were presented as mean  $\pm$  SD.  $P < 0.05$  was considered statistically significant. The analysis for power was performed *a priori* based on the assumption of detecting a 15% difference in fibrinolysis with the changes in estrogen. This estimate originated from our previous work with the IVF population (5), which demonstrated that the products of fibrinolysis decreased 46% from the time of baseline to oocyte retrieval. Our calculations determined that we would need approximately 21 patients assuming an  $\alpha$  error of 0.05, and a  $\beta$  error of 0.80.

## Results

From the initial 36 participants enrolled, only 24 subjects successfully met the IVF protocol requirements for estrogen level and follicular development to undergo an oocyte retrieval. Results from individuals completing both testing periods are reported. The mean age of the participants was 36 yr. Serum  $E_2$  concentrations (mean  $\pm$  SD) were  $26.9 \pm 8.6$  and  $2098 \pm 913$  pg/mL at baseline and oocyte retrieval, respectively ( $P < 0.005$ ). The measured TEG<sup>®</sup> indices are shown in Table 1 (17–19). R was significantly faster at oocyte retrieval when compared with baseline ( $P < 0.005$ ). A significant increase in the overall CI at oocyte retrieval was also observed ( $P < 0.005$ ). Although significant changes toward increased coagulation were observed with the R and CI, both variables remained within normal limits. In addition, although a trend toward an increased fibrinogen contribution to MA was observed, statistical significance was not obtained.

## Discussion

The effects of estrogen on coagulation and fibrinolysis are often difficult to interpret because of the wide variation in estrogen dosages, routes of administration, and patient populations studied. Various estrogen preparations, for example, have been noted to reduce the risk of arterial disease and increase the risk for venous thrombosis (7,8). The mechanism by which estrogen induced changes in hematologic variables, however, can be studied in a controlled manner in patients undergoing ovarian stimulation for IVF procedures. Moreover, because similar medication dosages and routes are used, predictable alterations in estrogen concentrations can be observed. In addition,

**Table 1.** Coagulation Analysis Indices

	R (min)	K (min)	$\alpha$ (angle)	MA <sub>wholeblood</sub> (mm)	MA <sub>fibrinogen</sub> (mm)	MA <sub>platelet</sub> (mm)	LY60 (%)	CI
Baseline	12.1 ± 2.5	3.5 ± 0.9	66.9 ± 5.0	56.5 ± 7.3	21.4 ± 9.1	35.1 ± 7.4	11.8 ± 7.0	-0.38 ± 1.7
OR	9.3 ± 1.9*	3.3 ± 1.6	68.4 ± 6.7	57.9 ± 6.9	23.8 ± 9.1	34.1 ± 8.5	12.9 ± 9.5	0.81 ± 1.7*
Normal nonpregnant range	11-17	3-7	54-74	55-68	Not known	Not known	1-8	-3.0-3.0 (range)
Normal pregnant range	4-11	1.5-3	65-81	58-76	28-38	27-41	1-9	4.74-1.0

All data are presented as mean ± SD.  
R = time to first clot formation, K = rate of clot strengthening,  $\alpha$  = rate of clot strengthening, MA<sub>wholeblood</sub> = clot strength caused by platelet and fibrinogen interaction, MA<sub>platelet</sub> = platelet function, LY60 = percent lysis at 60 min after MA is reached, CI = coagulation index, OR = oocyte retrieval. Normal values for citrated celite activated blood samples (Refs. 9, 17-19).  
\*  $P < 0.005$ . All other comparisons are not significant.

although IVF protocols are being applied to increasingly diverse infertility etiologies, the underlying patient demographics are relatively homogenous. As such, these patients represent a unique opportunity to study the effects of significant, short-term alterations in estrogen concentrations on coagulation and fibrinolysis.

Our findings demonstrate that isolated large estrogen concentrations, as produced by an IVF protocol, are associated with an overall increase in clot formation. Although still within the range of normal TEG<sup>®</sup> variables at the time of oocyte retrieval, a statistically significant alteration in two coagulation variables was observed. These changes in coagulation were reflected initially by a significant reduction in R time, an indicator of faster clot formation. In addition, although K,  $\alpha$  angle, and MA did not independently attain statistical significance in the direction of increased coagulation rate and strength, when evaluated together as the CI, a significant increase was observed. These findings are consistent with the TEG<sup>®</sup> analysis by Sharma et al. (9) which noted increased coagulation in pregnant and recently postpartum women versus nonpregnant women. Of interest, the baseline and retrieval variables for coagulation in our study were either in the high range in the nonpregnant or the low range of the pregnant state of all known variables; MA<sub>fibrinogen</sub> and MA<sub>platelet</sub> of the nonpregnant state are currently not known. This suggests that despite the supraphysiologic estrogen levels attained during IVF procedures, the increase in coagulation is not more pronounced than during pregnancy.

The overall increase in coagulation at the time of retrieval seems more dependent on fibrinogen than on platelet function. When samples with and without ReoPro<sup>®</sup>, an inhibitor of platelet contributions to clot formation, were analyzed simultaneously and compared, a trend toward increasing fibrinogen contribution to MA was observed. This agrees with the dramatic increase in fibrinogen levels observed during IVF protocols (10,11) and the linear association between increases in fibrinogen and MA observed by

Mahla et al. (12) in postoperative patients evaluated with two platelet inhibitors, ReoPro<sup>®</sup> and cytochalasin D. Moreover, these observations are consistent with the absence of platelet count and activation changes from baseline to retrieval noted by Magnani et al. (1). Collectively, these findings may highlight the relative importance of fibrinogen versus platelets in determining MA in high estrogen states.

In terms of fibrinolysis, no significant differences were noted between low and high estrogen states. This compares favorably with our previously reported findings (1), which despite observing changes that should have promoted fibrinolysis, i.e., decreases in plasminogen activator inhibitor and increases in plasmin formation, found no change in clot breakdown products at the time of oocyte retrieval when compared with baseline. Although other investigators have noted a down-regulation in fibrinolysis at the time of oocyte retrieval (13,14), it is possible that sample collection, preparation, and testing modality differences may have had a role in these differences. For instance, the presence of tissue factors at the time of venipuncture, including tissue plasminogen activator (15), which was eliminated in our samples by discarding the first 2 mL of blood, has been noted to have a role in fibrinolysis.

Although the alterations in the TEG<sup>®</sup> variables evaluated in our study remained within normal limits, the clinical relevance of our work can be illuminated in two important ways. First, it assists in the establishment of a reference standard for women undergoing IVF therapies. As with any relatively new methodology, and specifically with TEG<sup>®</sup> analysis, reference intervals can be somewhat arbitrary (20) and not necessarily based on specific or appropriate patient populations (21). Perhaps further TEG<sup>®</sup> investigations in patient populations in which predominantly estrogen is increased, such as the IVF population, will further distinguish these populations from pregnancy, in which both estrogen and progesterone are increased. Ultimately, it may be determined that such states have

“normal” variables distinct from both pregnant and nonpregnant populations.

Second, and more importantly, our findings establish a baseline to diagnose and treat the 8% to 23% of IVF patients who develop ovarian hyperstimulation syndrome (22). Characterized by hypercoagulability, thromboembolic complications, and even death, ovarian hyperstimulation syndrome has been associated with thrombosis of the internal jugular vein, superior vena cava, and major cortical veins (2-4). Although such reports have highlighted the need for more rapid testing to aid in diagnosis and treatment, only a limited number of hemostatic markers have been evaluated in individuals who eventually develop this disorder (23), and many of these tests are complicated, costly, and not widely available. It is conceivable that a rapid bedside test, such as TEG<sup>®</sup>, may allow for a more timely diagnosis and treatment. TEG<sup>®</sup> analyses, as used in the management of the coagulation alterations in orthotopic liver transplantation and cardiopulmonary bypass surgery, have allowed rapid evaluation and response to coagulation changes (24,25).

In conclusion, when patients undergoing IVF-induced ovarian stimulation are evaluated by using TEG<sup>®</sup> analysis, supraphysiologic estrogen concentrations are associated with significant increases in the coagulable state produced by increases in coagulation rather than alterations in fibrinolysis.

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