

An Assessment of the Effects on Coagulation of Midtrimester and Final-Trimester Amniotic Fluid on Whole Blood by Thrombelastograph[®] Analysis

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The Thrombelastograph[®] test (TEG; Haemoscope Corporation, Skokie, IL) was used to assess the effects of midtrimester and final-trimester amniotic fluid (AF) on whole blood coagulation. Different volumes of midtrimester and final-trimester AF were added to whole blood from nonpregnant volunteers in a series of TEG tests. The addition of both midtrimester and final-trimester AF resulted in significant decreases in reaction time ($P < 0.001$) and time from reaction to a fixed level of clot firmness ($P < 0.05$) and significant increases in angle ($P < 0.05$) and coagulation index ($P < 0.05$) values. This reflects accelerated clot initiation and propagation. There was no significant change in the maximal amplitude or % lysis at 30 and 60 min with the

addition of either midtrimester or final-trimester AF. There was no significant difference between the effects of midtrimester and final-trimester AF on whole blood TEG. TEG may be an additional useful tool in the treatment of coagulopathy in AF embolism. **Implications:** We used the Thrombelastograph[®] test (Haemoscope Corporation, Skokie, IL) to assess the effects of midtrimester and final-trimester amniotic fluid (AF) on whole blood coagulation. Results demonstrate that AF accelerates clot initiation and propagation. The Thrombelastograph[®] test may be useful in assessing coagulopathy in patients with AF embolism.

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A mniotic fluid (AF) accelerates clot formation in *in vitro* conventional coagulation tests of blood mixed with AF (1–4). The end point of these tests is the first appearance of a clot, the time of which was shortened by AF. Thrombelastograph[®] (TEG; Haemoscope Corporation, Skokie, IL) can provide more information, including the initiation, propagation, strength, and lysis of a clot (5). TEG is increasingly used to assess coagulation and guide treatment in cardiac and liver surgery (6–8). Such guidance can reduce transfusion of blood products, especially of fresh frozen plasma (7,9). Sharma et al. (10), using TEG, showed that blood from pregnant women is hypercoagulable compared with blood from nonpregnant women, with accelerated clot formation and increased clot strength. AF embolism can cause complex changes in coagulation, ranging from enhanced coagulation to fibrinolysis (11), and TEG testing may be

useful in its management. Our aim was to demonstrate both the enhanced coagulation and fibrinolytic effects of AF on normal blood in a single *in vitro* test, using TEG.

Methods

Institutional ethics committee approval and consent from patients and obstetricians were obtained. AF was obtained from 10 healthy patients having amniocentesis in midtrimester and from 10 healthy patients during elective caesarean delivery at full term. In the caesarean delivery patients, the AF was drawn after dissection of the uterus down to the amniotic membrane. Samples with visible contamination by blood were not used. Women with preeclampsia were excluded. The AF was centrifuged at 1000 rpm for 5 min to remove any cellular elements. Only the supernatant was used in the TEG tests.

Venous blood was obtained from 10 healthy nonpregnant female volunteers immediately before commencing the TEG tests. As pregnancy can cause a hypercoagulable state (10), we used blood from

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nonpregnant volunteers as we wanted to assess the effect of both midtrimester and final-trimester AF on the same normal blood. Volunteers who had been taking any nonsteroidal antiinflammatory drugs were excluded. A two-syringe technique was used. The first 2 mL of sampled blood was discarded to minimize contamination of the blood by tissue activators. Four computerized TEG machines (Haemoscope Corporation) were used for the study.

Mallet and Cox (5) reviewed the TEG devices. The main working parts of the TEG are a heated cup, which is oscillated, and a pin that is suspended freely from a torsion wire. Blood (0.36 mL) is placed in the cup. Before the formation of clot, the movement of the cup does not affect the pin. Once the clot starts to form, the pin is connected to the cup by fibrin strands and moves with the cup. The shear modulus and elasticity of the clot is transmitted through the pin and is displayed as the TEG trace with the following variables: the reaction time (R), the time from the placement of the blood in the cup to the first sign of clot formation; the time from R to a fixed level of clot firmness (K) (trace amplitude reaches 20 mm), which reflects fibrin buildup and cross-linking; the angle (α) formed by the slope of the TEG tracing between the R and K points, which reflects the rate of clot growth; the maximal amplitude (MA), the greatest amplitude that the TEG trace achieves, which reflects platelet quantity and function and clot strength; and the percent lysis at 30 (LY30) and 60 (LY60) min after MA, which reflect the extent of fibrinolysis. The coagulation index (CI) is a computer calculated linear combination of the R, K, MA, and α measurements. It reflects overall coagulation of the sample. The normal range for nonpregnant subjects is -3.0 to $+3.0$ (12). A more positive result indicates hypercoagulability and a more negative result indicates hypocoagulability.

The TEG machines were prewarmed to 37°C . Midterm AF (5, 10, 20 μL) and similar volumes of full-term AF were placed in six cuvettes in the TEG machines. Venous blood from a volunteer was then added into the cuvettes to produce a total volume of 360 μL . The seventh cuvette only had 360 μL of blood and was used as the control sample for both mid- and full-term AF. The TEG tests were run simultaneously and the R, K, α , MA, LY30, and LY60 variables recorded.

All TEG variables were compared by using analysis of variance, and Bonferroni's test was used for multiple comparisons (SPSS 8.0 for Windows, SPSS Inc., Chicago, IL). Paired *t*-tests were used to determine statistical significance of differences between full-term and midterm AF. A *P* value of < 0.05 was considered significant in all tests. All data were reported as mean \pm SD.

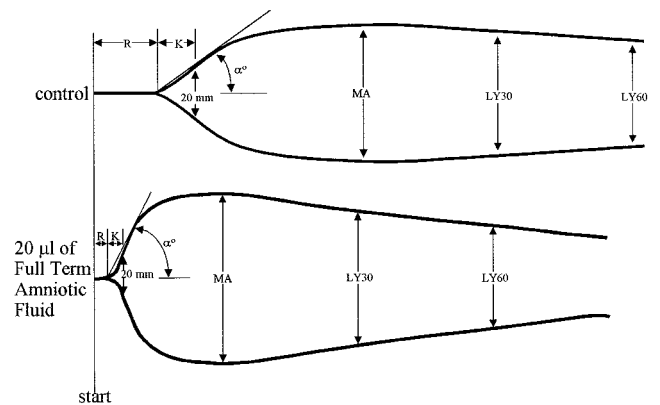


Figure 1. TEG traces of a control blood sample and after the addition of 20 μL of final-trimester amniotic fluid. R = reaction time, K = time from R to a fixed level of clot firmness, α = angle, MA = maximal amplitude, LY30 = percent lysis at 30 min after MA, LY60 = % lysis at 60 min after MA.

Results

All control TEG tests of the blood from volunteers were within normal limits. A representative normal nonpregnant whole blood control TEG trace and the TEG trace showing enhanced coagulation and fibrinolysis after adding 20 μL of final-trimester AF are shown in Figure 1.

Midtrimester AF

The gestational age was 20 ± 1 wk. The TEG measurements are shown in Table 1 and in Figure 2A. With increasing volumes of AF, there was a significant shortening of the R ($P < 0.001$) and K times ($P < 0.05$) when compared with the control sample. There was also a significant ($P < 0.05$) increase in the α at all volumes of AF. There were no significant changes in the MA, LY30, and LY60 with the addition of midtrimester AF.

Final-Trimester (Full-Term) AF

The gestational age of the pregnancies was 39 ± 1 wk. The TEG measurements are also shown in Table 1 and Figure 2B. The tests showed enhanced coagulation, with significant and progressive shortening of R ($P < 0.001$) and K ($P < 0.05$) times with increasing volumes of AF. The α also increased significantly as increasing volumes of AF were added ($P < 0.05$). There were no significant changes in the MA, LY30, and LY60 with the addition of final-trimester AF.

The CI was significantly increased at all volumes of midtrimester and final-trimester AF added compared with the controls ($P < 0.05$), again reflecting enhanced coagulation (Table 1 and Figure 2). However, all the CI results were within the normal range for blood from nonpregnant subjects.

Table 1. TEG Measurements of Midtrimester and Full-Trimester Amniotic Fluid and Whole Blood

AF (μ L)	R (min)	K (min)	α ($^{\circ}$)	MA (mm)	LY30 (%)	LY60 (%)	CI
0 Control	13.5 \pm 4.1	7.0 \pm 1.2	31.6 \pm 5.0	46.1 \pm 3.4	2.0 \pm 1.2	5.9 \pm 2.1	-1.4 \pm 1.4
5 Mid	6.9 \pm 2.6	4.4 \pm 2.1	47.5 \pm 15.7	50.4 \pm 7.0	2.9 \pm 1.6	8.7 \pm 4.2	0.6 \pm 1.2
5 Final	6.3 \pm 2.2	3.9 \pm 1.7	48.3 \pm 11.0	48.2 \pm 6.3	12.4 \pm 16.8	20.5 \pm 19.2	0.3 \pm 1.2
10 Mid	5.4 \pm 1.2	4.1 \pm 1.6	45.9 \pm 10.4	45.3 \pm 10.1	11.7 \pm 15.8	23.3 \pm 21.8	0.7 \pm 1.1
10 Final	4.3 \pm 1.0	2.3 \pm 0.8*	64.3 \pm 7.5*	54.0 \pm 6.6*	15.7 \pm 14.4	30.8 \pm 18.0	0.8 \pm 1.8
20 Mid	3.7 \pm 1.0	2.0 \pm 0.4	65.5 \pm 6.0	53.7 \pm 7.9	11.3 \pm 12.6	19.3 \pm 13.5	1.6 \pm 0.8
20 Final	3.4 \pm 1.1	2.0 \pm 0.6	65.1 \pm 5.3	54.6 \pm 7.6	14.1 \pm 16.5	23.8 \pm 23.5	1.9 \pm 1.3

All values are mean \pm SD.

AF = amniotic fluid, R = reaction time, K = time from R to achievement of trace amplitude of 20 mm, α = angle, MA = maximal amplitude, LY30 = % lysis at 30 min after MA, LY60 = % lysis at 60 min after MA, CI = coagulation index, Mid = sample taken at midtrimester, Final = sample taken at final trimester.

* Final-trimester AF value significantly different from corresponding midtrimester AF at $P < 0.05$.

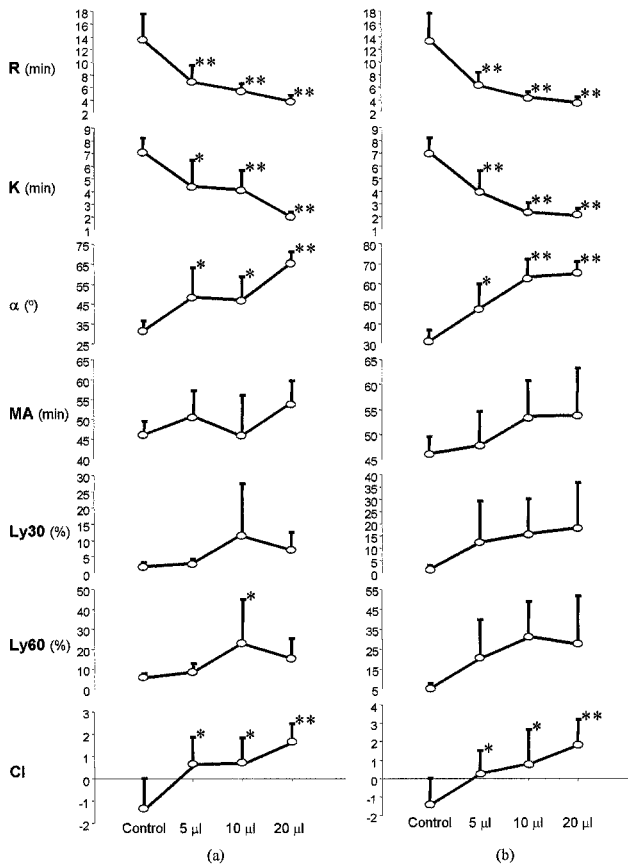


Figure 2. A, TEG measurements after adding 5, 10, and 20 μ L of midtrimester amniotic fluid to whole blood. B, TEG measurements after adding 5, 10, and 20 μ L of final-trimester amniotic fluid to whole blood. * $P < 0.05$ when compared with control. ** $P < 0.001$ when compared with control.

In comparing the TEG results of midtrimester and final-trimester AF, there were no significant overall differences, although isolated comparisons showed statistical significance. The K time was shorter, and α and MA were greater with 10 μ L of final-trimester AF compared with 10 μ L of midtrimester AF. There were no significant differences in R, LY30, LY60, and CI between midtrimester and final-trimester AF at any of the volumes used.

Discussion

In this study using TEG analysis, we have shown that AF significantly accelerates coagulation but does not significantly increase fibrinolysis of native whole blood. Both initiation and propagation of clot formation are faster, as shown in the significant shortening of R and K times, and the significant increase in the α and CI with increasing volumes of AF. However, even though clot formation was accelerated, we did not demonstrate increased clot strength as reflected by the nonsignificant changes in MA.

The mechanism of accelerated coagulation appears to be multifactorial. Weiner et al. (1) showed that thromboplastin released from fetal cells in AF promotes the conversion of prothrombin to thrombin, and fibrinogen to fibrin. Phillips and Davidson (2) found that AF could shorten the clotting times of both normal plasma and plasma deficient in factors VII, VIII, IX, and XI. AF, in *in vitro* tests, not only resembled thromboplastin in activating the extrinsic pathway but also involved the intrinsic pathway. They asserted that amniotic fluid contained a factor that could activate factor X and quantified this in terms of Russell's viper venom equivalents. This effect of AF may have a role in primary hemostasis in the uterus after delivery.

We were unable to show significantly increased fibrinolysis with the addition of AF, even though there was a trend toward increased lysis with higher volumes of full-term AF. Gross dissolution of the clots did not occur. AF contains a tissue plasminogen activator, urokinase-like plasmin activator and thrombin antithrombin complexes, which can cause the enhanced fibrinolysis seen (13). The enhanced coagulation caused by AF may also contribute to increased activation of fibrinolytic system. Increased fibrinolysis has been shown to occur in AF embolism (14).

The effects of AF on coagulation have been related to gestational age (15). By using only the supernatant, which is free of cellular elements, both mid and final-trimester AF showed similar effects in our study. There were no significant differences between midtrimester

and final-trimester AF. Further study with more patients stratified for gestational age is required.

AF embolism is a rare complication, with an incidence of between 1 in 20,000 to 1 in 80,000 pregnancies, but with mortality as high as 80% (16,17). Complex effects on coagulation have been reported in the 40% of patients who do not die from cardiovascular collapse (11,14,17). There is initially a thromboplastic effect and disseminated intravascular coagulation. Fibrinolysis, hypofibrinogenemia, and a hemorrhagic state follow. A hemorrhagic tendency is the main clinical effect seen. Whether this is caused by defective coagulation or increased clot lysis may not be elucidated by conventional tests, which only reflect isolated portions of the hemostatic system. Even though this study did not show increased fibrinolysis, TEG can guide rational treatment with blood products as it can give more complete information in each patient.

While TEG variables may not always correlate well with conventional tests of coagulation (such as prothrombin time, activated partial thromboplastin time, and platelet count), especially in hypocoagulable patients, this is because TEG measures different processes (6,18,19). TEG assesses the interaction of clotting factors and cellular elements, which is not reflected in tests of isolated portions of the coagulation cascade.

In summary, we have demonstrated in *in vitro* TEG tests that AF accelerates clot initiation and propagation of native whole blood. The global coagulation picture of TEG analysis may be useful in assessing coagulopathy in patients with AF embolism.

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References

1. Weiner AE, Reid DE, Roby CC. The hemostatic activity of amniotic fluid. *Science* 1949;110:190-1.
2. Phillips LL, Davidson EC. Procoagulant properties of amniotic fluid. *Am J Obstet Gynecol* 1972;113:911-9.
3. Lockwood CJ, Bach R, Guha A, et al. Amniotic fluid contains tissue factor, a potent initiator of coagulation. *Am J Obstet Gynecol* 1991;165:1335-41.
4. Yaffe H, Hay-Am E, Sadovsky E. Thromboplastic activity of amniotic fluid in term and postmature gestations. *Obstet Gynecol* 1981;57:490-2.
5. Mallet SV, Cox DJA. Thrombelastography. *Br J Anaesth* 1992;69:307-13.
6. Tuman KJ, McCarthy RJ, Ivankovich AD. The thrombelastograph: is it the solution to coagulation problems? *Cardiothoracic and vascular anesthesia update*. Philadelphia: WB Saunders, 1991:1-13.
7. Spiess BD, Gillies BSA, Chandler W, Verrier E. Changes in transfusion therapy and re-exploration rate after institution of blood management program in cardiac surgical patients. *J Cardiothorac Vasc Anesth* 1995;9:168-73.
8. Kang Y. Coagulation and liver transplantation. *Transplant Proc* 1993;25:2001-5.
9. Essell JH, Martin TJ, Salinas J, et al. Comparison of thrombelastography to bleeding time and standard coagulation tests in patients after cardiopulmonary bypass. *J Cardiothorac Vasc Anesth* 1993;7:410-5.
10. Sharma SK, Philip J, Wiley J. Thrombelastographic changes in healthy parturients and postpartum women. *Anesth Analg* 1997;85:94-8.
11. Stolte LAM, Lim HT, Van Arkel C, et al. Amniotic fluid embolism. In: Fairwater DVI, Eskes TKAB, eds. *Amniotic fluid: research and clinical application*. Amsterdam: Encerpta Medica, 1973:313-32.
12. Thrombelastograph® coagulation analyzer model 3000 series: user manual—theory and operation. Skokie, IL: Haemoscope Corporation, 1998:6.
13. Koh SCL, Anandakumar C, Arulkumar S, et al. Amniotic fluid plasminogen activators and inhibitors and thrombin anti-thrombin complex levels during second trimester and labour. *Fibrinolysis* 1995;9:121-6.
14. Ratnoff OD, Vosburgh GH. Observations on the clotting defect in amniotic fluid embolism [letter]. *N Engl J Med* 1952;247:970.
15. Hastwell GB. Accelerated clotting time: an amniotic fluid thromboplastic activity index of fetal maturity. *Am J Obstet Gynecol* 1978;131:650-4.
16. Clark SL, Montz, FJ, Phelan JP. Hemodynamic alterations associated with amniotic fluid embolism: a reappraisal. *Am J Obstet Gynecol* 1985;151:617-20.
17. Clark SL. New concepts of amniotic fluid embolism: a review. *Obstet Gynecol Surv* 1990;45:360-8.
18. Zuckerman L, Cohen E, Vagher JP, et al. Comparison of thrombelastography with common coagulation tests. *Thromb Haemost* 1981;46:752-6.
19. Tuman KJ, Speiss BD, McCarthy RJ, Ivankovich AD. Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. *Anesth Analg* 1989;69:69-75.