*Original Article*

# A prospective longitudinal study on rotation thromboelastometry in women with uncomplicated pregnancies and postpartum

Leonie E. van RHEENEN-FLACH,[1] Sonja ZWEEGMAN,[2] Fleur BOERSMA,[3] Joris E. LENGLET,[4] Jos W. R. TWISK[5] and Antoinette C. BOLTE[1]

[1]*Department of Obstetrics and Gynecology, VU University Medical Center, Amsterdam, The Netherlands,* [2]*Department of Hematology, VU University Medical Center, Amsterdam, The Netherlands,* [3]*Department of Obstetrics and Gynecology, Isala Klinieken, Zwolle, The Netherlands,* [4]*Department of Obstetrics and Gynecology, Flevoziekenhuis, Almere, The Netherlands and* [5]*Department of Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, The Netherlands*

**Background:** Rotation thromboelastometry (ROTEM) is an easy, fast and complete method of measuring coagulation.
**Aims:** Our goal was to obtain longitudinal values on ROTEM in uncomplicated pregnancies and in the puerperium.
**Materials and Methods:** Healthy women, who visited our outpatient clinic for antenatal checks and who accomplished an uncomplicated pregnancy were tested three times during pregnancy and one time postpartum. Intrinsic and extrinsic pathway tests were carried out.
**Results:** In total, 62 women were analysed, and 298 measurements were taken. With increasing gestational age, there are significant changes towards hypercoagulability.
**Conclusion:** This study provides a better knowledge about physiological changes in ROTEM measurements during pregnancy. These normative data may serve as assistance for future studies and interventions.

**Key words**: coagulation, maternal haemostatic change, pregnancy, rotation thromboelastometry, thromboelastography.

## Introduction

Pregnancy is considered to be a hypercoagulable state. It is widely assumed that this adaptation is necessary to face the challenge of delivery. An effect of this physiological thrombogenic state of pregnancy is the well-documented increased risk of deep venous thrombosis and pulmonary embolism in pregnancy and puerperium.[1]

Rotation thromboelastometry (ROTEM) and thromboelastography (TEG) are real-time clotting tests. In ROTEM and TEG clotting is tested using a pin (that is oscillating in ROTEM) and a cup (that is oscillating in TEG) filled with blood. As a blood clot is formed between the pin and the cup, resistance against the oscillation is measured using a computerised system.[2] Results are given in a graphic presentation that provides insight into clotting factors, the fibrinolytic system and platelet function.[3] This fast and thorough method of testing is thought to be promising for applications in pregnancy and delivery.[4]

Studies that have previously been done in healthy pregnant women have a cross-sectional or an observational design, using non-pregnant women as controls.[5–10] We performed a longitudinal study in healthy, uncomplicated pregnancies using post-partum measurements as a control.

Knowledge on the normal trends and values in uncomplicated pregnancy is a pre-requisite in future studies of complicated pregnancies for diagnostic purposes or to guide therapy for instance in (massive) obstetric haemorrhage.

## Materials and Methods

During a 16-month period, healthy pregnant women with a singleton pregnancy were recruited during their antenatal checks on the obstetric outpatient clinic of the VU University Medical Center, Amsterdam, the Netherlands. Only women with an uncomplicated general and obstetric history were enrolled. We collected longitudinal data of

*Correspondence*: MD Leonie E. van Rheenen-Flach, Department of Obstetrics and Gynecology, room 8F22, VU University Medical Center, Postbus 7057, 1007 MB Amsterdam, The Netherlands.
Email: leonie_flach@hotmail.com

The research was carried out at the Department of Obstetrics and Gynecology, VU University Medical Centre, Amsterdam, The Netherlands.

*Received 5 January 2012; accepted 17 October 2012*

women throughout pregnancy and 6 weeks postpartum. The charts were reviewed by the first and last author, to exclude women who developed complications during pregnancy or delivery. This was carried out before analyzing the data.

Collection of blood samples was combined with regular antenatal visits to the outpatient clinic. Participating women were asked for a blood sample three times during pregnancy at approximately 12 weeks intervals and at the regular post-partum check 6 weeks after delivery. Our study protocol dictated that consecutive blood draws were based on the gestational age during the first blood draw. If women were first tested between eight and 12 weeks, the next measurement was scheduled between 20–24 and 32–36 weeks of gestation. If women were first tested between 12 and 16 weeks, the next was between 24–28 and 36–40 weeks. And if women were first tested between 16 and 20 weeks, the next was between 28–32 and 40–42 weeks. We planned this prospective schedule to get an even distribution throughout pregnancy. ROTEM results were not available for the care-giving clinicians.

Time period 1 (T1) consists of all measurements taken between eight and 20 weeks of gestation. Measurements taken between 20 and 32 weeks of gestation are time period 2 (T2), and measurements taken between 32 and 42 weeks of gestation are time period 3 (T3). Intended inclusion was 75 women.

On every occasion, we needed 4.5 mL of citrate blood, collected with venous puncture from a cubital vein. Thromboelastometry was performed with ROTEM® Gamma (Pentapharm GMBH, München, Deutschland). Ellagic acid activated intrinsic pathway rotation thromboelastometry (INTEM), and the tissue factor activated extrinsic pathway rotation thromboelastometry (EXTEM). Analyses were performed according to the instructions of the manufacturer. Researchers were trained by the manufacturer to perform the analyses. The intrinsic pathway was initiated using ellagic acid as a contact activator, the extrinsic pathway was initiated using tissue factor derived from mouse brain.

We measured the clotting time (CT), clot formation time (CFT), alpha angle, clot formation rate (CFR), maximum clot firmness (MCF) and amplitude (A) after 5, 10, 15 and 30 min. Explanation and a graphic representation are given in Table 1 and Figure 1.

Data were collected using EXCEL 97-2003 (Microsoft, Redmond, WA, USA). The mean was calculated and a 2.5–97.5% reference range, using ±1.96 times the standard deviation.

To analyse the changes over time generalised estimating equations (GEE) were used, these analysis were performed using SPSS version 18 (IBM Corporation, Armonk, NY, USA). GEE take into account the dependency of the observations within the patient. *P*-value below 0.05 was considered significant. Using generalised estimating equations, analysis of *P*-values at different time points are performed in one calculation, this adjusts for multiple testing within the analysis.

**Table 1** Parameters of thromboelastometry (derived from www.rotem.de with permission)

| | |
|---|---|
| Clotting time (CT) in seconds | The period in time from the start of the measurement until a 2 mm amplitude occurs |
| Clot formation time (CFT) in seconds | This is the period time in seconds starting at an amplitude of 2 mm (clotting time) until an amplitude 20 mm is reached |
| Alpha angle in degrees | The angle between the centre line and a tangent to the curve through the 2 mm amplitude point |
| Clot formation rate (CFR) in degrees | The largest clot formation in degrees until the maximum clot firmness is reached |
| Maximum clot firmness (MCF) in millimeters | This is the maximal amplitude |
| Amplitude A5, A10, A15, A30 in millimeters | This is the amplitude after 5, 10, 15 and 30 min after clotting time. The amplitude reflects clot firmness at the given time |

Comparisons were made between postpartum and T1, T1 and T2, T2 and T3. This prospective study was approved by the medical ethics committee of the VU University Medical Centre, and a written informed consent was obtained from each patient.

## Results

A total of 78 women were tested, and ten women were excluded. The following were the reasons for exclusion: three women had a history of recurrent abortion, one developed gestational diabetes mellitus, two developed gestational hypertension or pre-eclampsia, two delivered prematurely (gestational age below 36 weeks) and two suffered from fetal loss. Six women were excluded because they were lost to follow-up. Patient characteristics are described in Table 2. The caesarean section rate in the study group was 21% ($n = 13$), which reflects the inclusion of low-risk pregnancies in the study group as the total caesarean section rate in the VU University Medical Center during the study period was 27.7%.

In the 62 included women, 149 INTEM measurements and 149 EXTEM measurements were taken, making 298 measurements in total. In Table 3, the distribution of the measurements at different gestational ages is shown. The ROTEM results are presented in Table 4 intrinsic pathway (INTEM) and Table 5 EXTEM. As indicated in Table 4, the INTEM measurements change as gestational age increases. The CFT decreases. The CFR and the alpha angle become more steep. The MCF and the amplitude at different time points increase. These changes are significant in the CFT, CFR, alpha angle, MCF, A5, A10, A15 and A30. Changes in CT, MCF and the amplitudes at the different time points are significantly different between postpartum and T1.

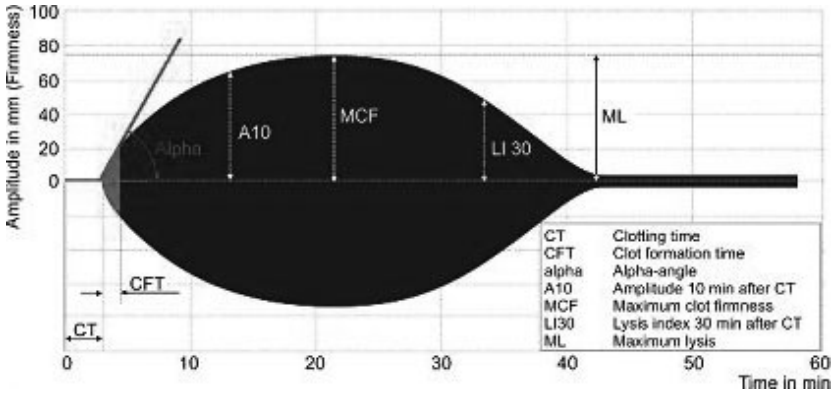


**Figure 1** Graphic representation of ROTEM parameters (derived from www.rotem.de with permission).

**Table 2** Patient characteristics

| Patient characteristics ($n$ = 62) | Median and range |
|---|---|
| Age (years) | 34 (18–44) |
| Parity | 1 (0–3) |
| Gestational age at delivery (weeks) | 40 (36.4–42.2) |
| Birth weight (g) | 3500 (2445–4300) |
| Apgar scores 1′ and 5′ | 9 (2–10) and 10 (5–10) |

**Table 3** Distribution of measurements at different gestational ages in the different time points

| | Planned range | Actual range | Median | Interquartile range |
|---|---|---|---|---|
| Postpartum (days) | 42 | 41–68 | 44 | 42–47 |
| T1 (weeks of gestation) | 8–20 | 7.5–19.5 | 13.3 | 10.6–16.1 |
| T2 (weeks of gestation) | 20–32 | 20.3–31.4 | 26.5 | 24.2–28.5 |
| T3 (weeks of gestation) | 32–42 | 32.1–40.6 | 36.2 | 34.1–37.4 |

In the EXTEM results, Table 5, the CT increases, and in T3, this is significant compared with T2. All the other parameters change comparable to the INTEM results, towards faster clotting times and increasing clotting firmness.

## Discussion

In this study, we collected data, prospectively and longitudinally, in a group of women with uncomplicated singleton pregnancies. Samples taken from the same women 6 weeks postpartum were obtained as control values, this is in contrast to most other studies that use samples from unrelated non-pregnant controls.[5–10] ROTEM measurements do not show any change between days 25 and 42 postpartum according to the HIP-study,[11] and in our study, all post-partum measurements were taken after day 40, making them a valid assessment of the non-pregnant baseline state.

Collecting longitudinal data ensures a better representation of changes over time than cross-sectional data. To our knowledge, this is the largest study on this subject and the first with longitudinal data. When we compare our results to the previously mentioned studies,[5–10] our results are consistent with pregnancy being a hypercoagulable state. Although the majority of our values are within the ranges set for non-pregnant healthy subjects as provided by Lang *et al.*,[12] the coagulability is found to increase with gestational age. The most significant findings are found in the clot firmness: In both the INTEM and EXTEM, all values for MCF, A5, A10, A15 and A30 changed significantly. These findings are in concordance with the cross-sectional data presented by Huissoud.[7] Also in the studies[6,8–10] where thromboelasto*graphy* was performed, the maximum amplitude (comparable to the MCF in thromboelastometry) was significantly increased in healthy term pregnant women, compared with non-pregnant controls. Contrary to the study of Huissoud,[7] were the CT does not change in any trimester, we do find a decrease in the CT (INTEM and EXTEM) that is already apparent early in pregnancy. Studies using thromboelastography also report a change in CT (named *r*) between the terms 'pregnant and non-pregnant women'.[6,8–10] It should be noted that the inclusions in the study by Huissoud are unequally distributed over the trimesters with only nine women in the second trimester.[7] We obtained an equal distribution over, and even inside, the trimesters by scheduled blood draws (see results Table 3).

Recent published data by Armstrong *et al.*[5] in healthy third trimester parturients undergoing elective caesarean section also show a shortening of the INTEM CT, the INTEM and EXTEM CFT and an increasing MCF when compared with non-pregnant controls presenting for elective surgery.

In contrast to what we expected and to what has been found by others,[5–10] there is an increase in CT in the

**Table 4** Ellagic acid activated intrinsic pathway rotation thromboelastometry results

| | CT | CFT | Alpha angle | CFR | MCF | A5 | A10 | A15 | A30 |
|---|---|---|---|---|---|---|---|---|---|
| Post partum ($n$ = 19) | | | | | | | | | |
| Mean | 195 | 66 | 77 | 77 | 63 | 47 | 57 | 61 | 62 |
| SD | 34 | 11 | 2 | 2 | 3 | 4 | 3 | 3 | 3 |
| Reference range | 127–262 | 44–88 | 71–81 | 73–81 | 57–69 | 40–54 | 51–64 | 56–67 | 56–68 |
| Time point 1 ($n$ = 45) | | | | | | | | | |
| Mean | 166*** | 63 ns | 77 ns | 77 ns | 65*** | 49*** | 59*** | 63*** | 64*** |
| SD | 24 | 12 | 2 | 2 | 3 | 4 | 4 | 4 | 3 |
| Reference range | 119–213 | 40–86 | 73–82 | 73–82 | 59–72 | 41–58 | 52–67 | 56–70 | 57–71 |
| Time point 2 ($n$ = 41) | | | | | | | | | |
| Mean | 168 ns | 58** | 78** | 78* | 67*** | 51*** | 61*** | 65*** | 66*** |
| SD | 24 | 9 | 2 | 2 | 3 | 3 | 3 | 3 | 3 |
| Reference range | 122–215 | 41–75 | 75–82 | 75–82 | 62–73 | 45–57 | 55–66 | 60–70 | 60–72 |
| Time point 3 ($n$ = 44) | | | | | | | | | |
| Mean | 160 ns | 53** | 79* | 79* | 71*** | 54*** | 64*** | 68*** | 70*** |
| SD | 27 | 9 | 3 | 2 | 4 | 5 | 4 | 4 | 4 |
| Reference range | 107–213 | 36–70 | 76–82 | 76–83 | 63–78 | 45–63 | 56–72 | 60–75 | 62–77 |

Abbreviations are explained in Table 1.
Reference ranges are between 2.5 and 97.5%.
Comparisons are made between postpartum and T1, T1 and T2, T2 and T3.
ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

**Table 5** Tissue factor activated extrinsic pathway rotation thromboelastometry results

| | CT | CFT | Alpha angle | CFR | MCF | A5 | A10 | A15 | A30 |
|---|---|---|---|---|---|---|---|---|---|
| Post partum ($n$ = 19) | | | | | | | | | |
| Mean | 83 | 73 | 75 | 75 | 65 | 50 | 59 | 63 | 64 |
| SD | 31 | 16 | 3 | 3 | 3 | 5 | 4 | 4 | 3 |
| Reference range | 22–145 | 42–104 | 69–81 | 69–81 | 59–72 | 39–60 | 51–68 | 56–70 | 57–70 |
| Time point 1 ($n$ = 44) | | | | | | | | | |
| Mean | 67* | 73 ns | 75 ns | 75 ns | 68*** | 51*** | 61* | 65** | 67*** |
| SD | 15 | 14 | 3 | 3 | 3 | 5 | 4 | 4 | 3 |
| Reference range | 38–97 | 45–101 | 68–82 | 70–81 | 60–75 | 41–60 | 53–69 | 58–72 | 59–75 |
| Time point 2 ($n$ = 40) | | | | | | | | | |
| Mean | 71 ns | 68 ns | 77* | 77* | 70* | 53* | 63* | 67* | 69* |
| SD | 21 | 14 | 2 | 2 | 4 | 4 | 4 | 3 | 4 |
| Reference range | 29–113 | 41–95 | 72–81 | 72–81 | 63–77 | 44–61 | 55–70 | 60–73 | 61–76 |
| Time point 3 ($n$ = 46) | | | | | | | | | |
| Mean | 92** | 66 ns | 77 ns | 77 ns | 72*** | 56*** | 65*** | 69*** | 71*** |
| SD | 45 | 18 | 3 | 3 | 4 | 5 | 4 | 4 | 4 |
| Reference range | 3–180 | 31–101 | 72–83 | 72–82 | 64–79 | 45–66 | 57–74 | 61–77 | 63–78 |

Abbreviations are explained in Table 1.
Reference ranges are between 2.5 and 97.5%.
Comparisons are made between postpartum and T1, T1 and T2, T2 and T3.
ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

EXTEM from T1 to T3, for which we have no explanation.

Lang[12] published a multicentre study on ROTEM providing general reference ranges for CT, CFT, alpha angle, MCF, A10, A20 and A30 in a non-pregnant population. Lang showed that establishing references ranges were feasible as the results were reproducible and consistent between different centres. Ebinger[13] confirms this finding in his letter to the editor in which he uses (combined with other sources) the data from Lang. But these findings could not be confirmed by the TEG-ROTEM working group and a UK national quality assessment.[3,14] Therefore, caution should be used in translating our values to one's own practice, and because of the different techniques used for TEG and ROTEM values are not inter-changeable.

We did not perform any other clotting tests such as platelet count, fibrinogen level, activated partial thromboplastin time and partial thromboplastin time to compare with. As only healthy women were in the study

group, no abnormalities are to be expected in our group apart from physiological changes during pregnancy. Recent thorough investigations on how conventional clotting tests change over the trimesters has been carried out by Liu *et al.*[15] A total of 1130 pregnant women were tested in five different gestational age groups. All reference ranges found were significantly different from those in the control group and showed an increased clotting over time.

Currently, we are collecting ROTEM results in women with complicated pregnancies, for instance pre-eclampsia, haemolysis, elevated liver enzymes, low platelets syndrome, post-partum haemorrhage and known bleeding tendencies, to compare with the reference ranges described in this study.

This study provides more information about the physiological changes in ROTEM measurements during the different trimesters of pregnancy and provides reference ranges in healthy pregnant women. These normative data may serve as assistance for future studies and interventions, especially in cases where the clotting system is activated (eg. pre-eclampsia) or challenged (eg. haemorrhage).

## References


1 Holmes VA, Wallace JM. Haemostasis in normal pregnancy: a balancing act? *Biochem Soc Trans* 2005; **33**: 428–432.

2 Luddington RJ. Thromboelastography/thromboelastometry. *Clin Lab Haematol* 2005; **27**: 81–90.

3 Chitlur M, Sorensen B, Rivard G *et al.* Standardization of thromboelastography: a report from the TEG-ROTEM working group. *Haemophilia* 2011; **17**: 532–537.

4 Othman M, Falcon B, Kadir R. Global hemostasis in pregnancy: are we using thromboelastography to its full potential? *Semin Thromb Hemost* 2010; **36**: 738–746.

5 Armstrong S, Fernando R, Ashpole K *et al.* Assessment of coagulation in the obstetric population using ROTEM® thromboelastometry. *Int J Obstet Anesth* 2011; **20**: 293–298.

6 Gorton HJ, Warren ER, Simpson NA *et al.* Thromboelastography identifies sex-related differences in coagulation. *Anesth Analg* 2000; **91** (5): 1279–1281.

7 Huissoud C, Carrabin N, Benchaib M *et al.* Coagulation assessment by rotation thrombelastometry in normal pregnancy. *Thromb Haemost* 2009; **101**: 755–761.

8 Polak F, Kolnikova I, Lips M *et al.* New recommendations for thromboelastography reference ranges for pregnant women. *Thromb Res* 2011; **128**: e14–e17.

9 Sharma SK, Philip J, Wiley J. Thromboelastographic changes in healthy parturients and postpartum women. *Anesth Analg* 1997; **85**: 94.

10 Steer PL, Krantz HB. Thromboelastography and sonoclot analysis in the healthy parturient. *J Clin Anesth* 1993; **5**: 419–424.

11 Saha P, Stott D, Atalla R. Haemostatic changes in the puerperium '6 weeks postpartum' (HIP Study) – implication for maternal thromboembolism. *BJOG* 2009; **116**: 1602–1612.

12 Lang T, Bauters A, Braun SL *et al.* Multi-centre investigation on reference ranges for ROTEM thromboelastometry. *Blood Coagul Fibrinolysis* 2005; **16**: 301–310.

13 Ebinger T, Ruland A, Lakner M, Schwaiger M. Validity, regulatory registration and approval of ROTEM thromboelastometry. *Blood Coagul Fibrinolysis* 2010; **21**: 106–107.

14 Kitchen D, Kitchen S, Jennings I *et al.* Quality assurance and quality control of thromboelastography and rotational thromboelastometry: the UK NEQAS for blood coagulation experience. *Semin Thromb Hemost* 2010; **36**: 757–762.

15 Liu J, Yuan E, Lee L. Gestational age-specific reference intervals for routine haemostatic assays during normal pregnancy. *Clin Chim Acta* 2012; **413**: 258–261.