# Normal Pregnancy is Associated with an Increase in Thrombin Generation from the very Early Stages of the First Trimester

**CN Bagot[*], E Leishman[*], CC Onyiaodike[†], F Jordan[†], DJ Freeman[†]**

**[*]Department of Haematology, Glasgow Royal Infirmary, Glasgow UK; [†]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow UK**

Running Head: Thrombin generation increases early in pregnancy

## Abstract

### Background

Pregnancy is a hypercoagulable state associated with an increased risk of venous thrombosis, which begins during the first trimester, but the exact time of onset is unknown. Thrombin generation, a laboratory marker of thrombosis risk, increases during normal pregnancy but it is unclear exactly how early this increase occurs.

### Methods

We assessed thrombin generation by Calibrated Automated Thrombography in women undergoing natural cycle *in vitro* fertilization, who subsequently gave birth at term following a normal pregnancy (n=22). Blood samples were taken just prior to conception and repeated five times during very early pregnancy, up to Day 59 estimated gestation.

### Results

Mean Endogenous Thrombin Potential (ETP), peak thrombin generation and Velocity Index (VI) increased significantly from pre-pregnancy to Day 43 gestation (P = 0.024 - 0.0004). This change persisted to Day 59 gestation. The mean of the percentage change from baseline,

accounting for inter-individual variation, in ETP, peak thrombin and VI increased significantly from pre-pregnancy to Day 32 gestation (P = 0.0351 - <0.0001) with the mean increase from baseline persisting to Day 59 gestation.

**Conclusion**

Thrombin generation increases significantly during the very early stages of normal pregnancy when compared to the pre-pregnancy state. The increased risk of venous thrombosis therefore likely begins very early in a woman's pregnancy, suggesting that women ***considered clinically to be*** at high thrombotic risk should start thromboprophylaxis as early as possible after a positive pregnancy test.

**Keywords**

Venous thrombosis, thrombin, blood coagulation tests, pregnancy, first trimester

Correspondence: Dr Catherine Bagot, Department of Haematology, 3rd Floor Macewen Building, Glasgow Royal Infirmary, Castle Street, Glasgow, G4 0SF, UK

Tel.: +44 141 211 4671; fax: +44 141 211 4931

Email: catherine.bagot@ggc.scot.nhs.uk

## Introduction

Pregnancy is a prothrombotic state, likely arising from a physiological response to reduce the risk of bleeding during the antenatal, and particularly the postnatal, period. As a result, pregnant women are at increased risk of venous thrombosis compared to the general population. Epidemiological data suggests that hypercoagulability begins early in pregnancy, with an increase in thrombotic risk beginning in the first trimester [1-3]. However, it is unknown exactly how early in pregnancy that the hypercoagulable state begins to develop.

Thrombin generation, a global coagulation assay, has been demonstrated to more accurately reflect the thrombotic phenotype than measuring individual parameters of the coagulation cascade [4-8]. Thrombin is pivotal to the coagulation cascade; it is the enzyme responsible for converting fibrinogen to fibrin, the step resulting in clot formation. Thrombin also has numerous positive and negative feedback roles across the coagulation cascade, thus making it central to this process. It is therefore reasonable to expect that an assessment of an individual's ability to generate thrombin will provide an accurate evaluation of an individual's potential to clot.

Studies assessing thrombin generation during pregnancy have been undertaken but have had various limitations in their findings [7, 9-14]. Rarely has thrombin generation been assessed earlier than 10 weeks of gestation, the late stage of the first trimester, and most studies have not obtained pre-pregnancy samples from the same women, preventing an account to be taken of the known significant inter-individual variability in thrombin generation [15-16]. Frequently the demonstration that pregnancy is associated with an increase in thrombin generation has been through comparisons of thrombin generation results obtained from either normal pooled plasma or from using postpartum samples from the previously pregnant women [9, 11-14]. Other potential confounders such as the use of low molecular weight heparin (LMWH) have also affected data [12-13].

In only one study have the same women been recruited pre-pregnancy and then followed up with samples taken during pregnancy [10]. In this study, thrombin generation was significantly increased in 20 healthy pregnant women at 11-15 weeks of gestation when compared to measurements taken in the same women prior to pregnancy. However, to date,

no similar comparisons have been made at an earlier gestation. Furthermore, although this study compared mean thrombin generation values between pre-pregnancy and pregnancy, it did not report the mean change in thrombin generation *per* individual over this time period, and therefore the significant inter-individual variability known to occur in thrombin generation was not accounted for.

Our study is a prospective analysis of thrombin generation in women undergoing natural cycle *in vitro* fertilization, as such women provide the most practical method of obtaining, to an extent, accurately timed peri–conceptual and early pregnancy samples and are the best physiological representation of a 'normal' pregnancy, outside a free living population. These women subsequently gave birth at term following a normal pregnancy.

Our aim was to explore the time of onset of increased thrombin generation in pregnancy, as an indication of the development of the prothrombotic state, and in turn, guide clinicians as to how early preventative measures should be taken in pregnant women at high risk of thrombosis.

## Materials and Methods

*CAT Reagents*

PPPLow reagent (1 pM tissue factor [TF] and 4 μM phospholipids [PL]), PPP reagent (5 pM TF and 4 μM PL), Thrombin Calibrator (TCal), FluCa (2.5 mM fluorogenic synthetic substrate, 100 mM calcium chloride) and Immulon round bottom 96 well microtitre plates were purchased from Diagnostica Stago ***(***Asnières, France***).*** Normal Pooled Plasma (NPP) and Thrombomodulin (TM) were donated by Cardiovascular Research Institute Maastricht

(CARIM), The Netherlands. TGT Reference plasma (TGT-RP) was a gift from NIBSC (Potters Bar, Hertfordshire, UK).

*Subjects*

Women undergoing natural cycle *in vitro* fertilization ***i.e. no hormonal treatment during the cycle***, were recruited consecutively from the Assisted Conception Service at Glasgow Royal Infirmary between October 2007 and June 2010. Demographic data was collected and blood samples taken pre-pregnancy and during the very early stages of pregnancy as described below. Women were subsequently followed up throughout pregnancy and to delivery, including the outcome of their baby. Only those women who developed no complications ***and received no concomitant therapy*** throughout pregnancy and delivered a normal baby at term were included in the final analysis.

The study had full ethical and R&D approval from Glasgow Royal Infirmary Research and Ethics Committee (Ref. No. 07/S0704/49) and Research and Development Office (Ref. No. RN07OB005). Written informed consent was obtained for every participant.

*Blood Samples*

Blood samples were collected in sodium citrate, centrifuged at 3000 rpm for 15 minutes at 4ºC within 2 hours of plasma collection. Plasma was then spun in a microfuge at 13,000 rpm for 4 minutes to obtain platelet poor plasma (PPP) and stored at -80ºC. Two pre-pregnancy samples were taken from each woman; approximately at the time of the lutenizing hormone surge (Study Day 0) and at the time of frozen embryo transfer (Study Day 3). Within the study, Study Day 0 was considered to be equivalent to Day 14 from last menstrual period (LMP) (assuming a 28 day menstrual cycle) in a naturally occurring pregnancy i.e. 2 weeks

gestation. Up to a further 5 samples were then taken from the same women very early in gestation, at Study Day 7, 10, 18, 29 and 45, with Study Day 45 being equivalent to Day 59 estimated gestation (***Figure 1***).

*Calibrated Automated Thrombography (CAT)*

Thrombin generation (TG) was measured according to the method previously described by Hemker in a Fluoroskan Ascent$^{TM}$ fluorometer (Thermo Labsystems OY, Helsinki, Finland) [15]. Fluorescence intensity was detected at 390 nm (excitation) and 460 nm (emission). Briefly, 20 μl of TCal or 20 μl of PPPLow or PPP reagent, in the presence or absence of ***0.4nM TM or 1.37nM TM respectively,*** were dispensed into a round bottom 96 well microtitre plate, where the concentration of TM used was determined as previously described [17]. Eighty microlitres of either TGT-RP or PPP was then added to each mixture and pre-incubated at 37$^{o}$C for 10 minutes. Following pre-incubation, 20 μl of FluCa was automatically dispensed by the analyser into each well and thrombin generation measured over a fixed time period. Thrombin generation curves were generated with Thrombinoscope Software, version 5.0.0.742 (Thrombinoscope BV). Thrombin generation was calibrated against the fluorescence curve obtained using a fixed amount of thrombin-alpha$_2$-macroglobulin complex, contained within TCal, to correct for the inner filter effect.

All samples from each woman were assessed concurrently, alongside a standardised plasma (TGT-RP), to minimise the effect of inter-assay variability.

The TG parameters measured were; endogenous thrombin potential (ETP) (nM.min, the area under the curve), Peak thrombin concentration (nM), lag time (min, time to first thrombin production), time to reach peak height (ttPeak) (min), Velocity Index (VI) (nmol/min, slope

between lag time and ttPeak) and start tail (min, time at which thrombin generation ceases).

*Statistical Analysis*

Thrombin generation test results were collected from individual experiments for statistical analysis. The mean, standard deviation and confidence interval were calculated for all TG parameters under 4 different assay conditions. One-Way ANOVA comparison (Dunnett's test and Tukey's test) was performed using GraphPad Prism Version 6.07 where statistical significance was given a P-value of $< 0.05$.

**Results**

196 women were recruited, ***resulting in 36 successful first attempt pregnancies, of which 22 women gave birth at term*** with no complications occurring during the antenatal period. Demographic data for these 22 women is shown in ***Table 1***, including the reason for IVF.

*Effect of Pregnancy on Thrombin Generation*

Thrombin generation was measured in 19 separate experiments, where all samples were tested in parallel with TGT-RP, under the 4 different assay conditions. To limit the effect of inter-assay variability, all samples from each individual woman were assessed concurrently. Intra-assay coefficient of variation (CV) for TGT-RP was less than 5% for all TG parameters and inter-assay CV ranged between 4% - 19% (data not shown).

On analysis of the mean data, there was a significant increase from baseline (average of Study Days 0 & 3) at Study Day 29 (the 6$^{th}$ gestational week) for peak thrombin, Endogenous

Thrombin Potential (ETP) and Velocity Index (VI) at all assay conditions, with the exception of VI at 1pM & 1pM+TM. This increase was sustained into the 8th gestational week (Study Day 45). ***Table 2*** summarises all mean data generated for ETP, peak and VI. No significant difference was observed for ttPeak, lag time or start tail (data not shown). The women's data normalised to results from the standard plasma (TGT-RP) produced identical/very similar statistically significant outcomes (data not shown).

***Figure 2*** illustrates an example Thrombogram of mean data from 17 subjects tested at 1pM TF + ***1.37nM*** TM (data from 5 subjects excluded from the mean graph due to readings being taken at slightly different times on the Fluoroskan Ascent™ flurometer)

To minimise the effect of inter-individual variability, the mean change in thrombin generation over time was determined i.e. change between the pre-pregnant and pregnant state *per* individual. There was a significant change from baseline (pre-pregnancy) to Study Day 18 (the 5th gestational week) for peak thrombin, ETP and VI under 1pM TF, 1pM TF + TM and 5pM TF assay conditions. This increase persisted into the 6th and 8th gestational week (Study Days 29 & 45 respectively). ***Figure 3*** illustrates data for mean percentage change from baseline under 1pM TF+ TM assay condition and ***Table 3*** summarises data for all conditions. No significant percentage change was observed for ttPeak, lag time or start tail (data not shown).

To determine whether thrombin generation continued to increase significantly as pregnancy progresses, statistical analyses were also carried out comparing thrombin generation data between Study Days 18, 29 and 45. A significant difference in mean ETP was observed between Study Day 18 and Study Day 29, but only under 5pM TF+TM conditions (P=

0.0390). A significant percentage change in ETP was observed between Study Day 18 and 29 only using 5pM TF (P= 0.0397). Comparisons between Study Day 18 and Day 45 highlighted a significant increase in mean ETP and a significant percentage change in ETP across all four assay conditions (with the exception of mean ETP at 5pM TF). A significant increase in mean peak and VI and a significant percentage change in peak and VI at 5pM TF and 5pM TF + TM was also seen (***Table 4***). No significant difference was observed between Day 29 and Day 45 (data not shown)

**Discussion**

Our data demonstrates that thrombin generation increases in women at a very early stage of pregnancy, at approximately five weeks of gestation, and that this increase persists to eight weeks of gestation. This change is detectable using four of the more commonly used thrombin generation assay conditions, 1pM and 5pM TF +/- TM, the lower concentration of tissue factor highlighting the intrinsic pathway, the higher concentration, the extrinsic pathway. The addition of thrombomodulin enables evaluation of the positive feedback effect of thrombin on the Protein C/Protein S pathway.

Ours is the first study to demonstrate the very early stage of pregnancy at which thrombin generation patterns begin to change. Previous studies have captured data almost exclusively during the late stage of the first or the early stage of the second trimester [7, 9-14]. Furthermore, ours is the first to capture such data in a longitudinal setting and obtain pregnancy outcome data.

The majority of previous studies have not been longitudinal and/or do not capture pre-pregnancy samples from women who are subsequently followed up during pregnancy [7, 9,

11-14]. Frequently the demonstration that pregnancy is associated with an increase in thrombin generation has been derived from control pooled plasma either from men, non-pregnant women or a postpartum sample from the previously pregnant women [9, 11-14]. The use of control pooled plasma or postpartum samples to demonstrate changes in thrombin generation during pregnancy is not a valid or reliable comparison due to significant inter-individual variability in thrombin generation. Finally, in many of these studies, thrombin generation assay conditions were not standardised, and/or did not include a standardised control plasma, which will also affect the validity of the results given the known intra and inter assay variation that can occur when assessing thrombin generation [18, 19].

This is the first study to accurately compare pre-pregnancy and pregnancy results by assessing percentage change from baseline, thereby taking account of the important issue of inter-individual variability in thrombin generation. The data shown in ***Table 2*** indicates the wide range of thrombin generation results obtained pre-pregnancy, highlighting that normal healthy individuals generate different levels of thrombin. When subsequent comparisons are made at later time points during pregnancy, by comparing the difference of two means, although an overall mean increase in values is seen, it is possible not all women have a significant change in their values between the pre-pregnant and pregnant state. By assessing the mean percentage change from baseline this is taken into account. We would therefore advocate the use of change from baseline measurements when undertaking longitudinal comparisons of thrombin generation data in the future.

The parameters of thrombin generation which demonstrated a significant change were peak, ETP and VI. Lag time, ttPeak and Start Tail were not significantly affected. This may suggest

that the total amount of thrombin generated rather than the ***time*** at which thrombin is generated is more significant in the underlying hypercoagulable state of pregnancy.

The prothrombotic state of pregnancy has previously been demonstrated using other laboratory markers [20-22]. Protein S and VIII are known to decrease and increase respectively in response to pregnancy although it is unknown exactly at what stage of pregnancy these changes occur. If these factors do alter at a similar gestation as thrombin generation, this would provide evidence that these changes are contributing to the prothrombotic state. By using lower levels of tissue factor i.e. 1pM as opposed to 5pM, and thrombomodulin, in some of the assay conditions, we hope to have captured the effect these two proteins have on thrombin generation by highlighting the effect of the intrinsic pathway and the activated protein C pathway, respectively. High levels of tissue factor and the absence of thrombomodulin however, still enabled a change in thrombin generation to be detected suggesting that changes in protein S and VIII are not the only variations in coagulation parameters that result in the prothrombotic state of pregnancy. Further work is required to assess how early other parameters involved in the coagulation cascade change in pregnancy.

Some previous longitudinal studies have been performed where thrombin generation has been measured on more than one occasion in the same women during the pregnancy. These studies have not concurred as to whether thrombin generation increases significantly as pregnancy progresses which may in part be due to a lack of standardization in experimental design [7, 9-14]. Our study also suggests that thrombin generation may continue to increase as pregnancy progresses. This appears to be best reflected when thrombin generation is assessed under 5pM assay conditions; ETP, peak and VI increased significantly between Day 18 and Day 45 using 5pM +TM but such significant changes were only demonstrable for ETP under lower

tissue factor conditions (***Table 4***). This is perhaps surprising given that both concentrations of tissue factor could detect thrombin generation changes between the pre-pregnant and pregnant state. On review of our results we hypothesise that changes detected in thrombin generation between different time points during pregnancy and under some assay conditions may be affected by patient numbers and differences in confidence intervals. One further limitation of our study was samples were not taken beyond the early 1st trimester to characterise to thrombin generation patterns as pregnancy progress. Further work is still required in this area.

Our results suggest that hypercoagulability associated with pregnancy begins as early as four weeks of gestation, which is around the time of a first positive pregnancy test. ***We do not suggest from our results that thrombin generation can predict <u>which</u> women are at increased risk of venous thrombosis; simply that, those women deemed to be at high thrombotic risk, based on currently available clinical guidelines, will develop that higher risk very early in pregnancy. This is an important finding as*** it has previously been acceptable practice for those women deemed at high risk of venous thrombosis in association with pregnancy to start prophylaxis with low molecular weight heparin (LMWH) after the confirmation of a pregnancy on ultrasound, around 8-12 weeks of gestation. This study suggests that waiting to confirm a pregnancy on scan may be later than is safe to commence LMWH. Therefore we suggest that, as a result of this work, women deemed to be at high risk of thrombosis during pregnancy should commence thromboprophylaxis as soon as pregnancy is verified by a positive urinary pregnancy test. We believe that the chance of either a false positive pregnancy result or side effects from LMWH are outweighed by the benefits gained from receiving LMWH as early as possible and preventing a first trimester venous thrombosis. Venous thrombosis remains the third most common cause of maternal death with

25% of deaths occurring in the first trimester [23]. Furthermore there is significant morbidity associated with venous thrombosis in pregnancy, in particular post thrombotic syndrome, which is often more severe in pregnant women than their non pregnant counterparts due to the more proximal location of thromboses which occur in the pregnant state. The early introduction of LMWH may have the potential to reduce this rate of significant morbidity and even mortality.

**Addendum**

C. N. Bagot designed and supervised the project. E. Leishman performed the experiments. C. N. Bagot and E. Leishman were involved in analyzing and interpreting the data and writing the manuscript. C. C. Onyiaodike collected the samples and critically reviewed the manuscript. F. Jordan and D. J. Freeman provided the samples and participant data and critically reviewed the manuscript.

**Conflict of interest statement**

No conflict of interest to declare.

**Acknowledgments**

The study was funded by the charity, Wellbeing of Women (Wellbeing of Women / RCOG Research Grant; RG939/07). We acknowledge Dr Elaine Gray (National Institute for Biological Standards and Controls) for donating the NIBSC TGT-RP. We would also like to thank Dr Henri Spronk and his research team (CARIM) for gifting Thrombomodulin to our studies and for their support and helpful discussions regarding thrombin generation assay conditions.

**References**


1) Gherman RB, Goodwin TM, Leung B, Byrne JD, Hethumumi R, Montoro M. Incidence, clinical characteristics, and timing of objectively diagnosed venous thromboembolism during pregnancy. *Obstet Gynecol* 1999; **94**: 730–4.
2) James AH, Tapson VF, Goldhaber SZ. Thrombosis during pregnancy and the postpartum period. *Am J Obstet Gynecol* 2005; **193**: 216–9.
3) Blanco-Molina A, Trujillo-Santos J, Criado J, Lopez L, Lecumberri R, Gutierrez R, Monreal M for the RIETE Investigators. Venous thromboembolism during pregnancy or postpartum: findings from the RIETE Registry. *Thromb Haemost* 2007; **97**: 186–90.
4) Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. *JAMA* 2006; **296**: 397–402.
5) Tripodi A, Legnani C, Chantarangkul V, Cosmi B, Palareti G, Mannuci PM. High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of recurrent venous thromboembolism. *J Thromb Haemost* 2008; **6**: 1327–33.
6) Haidl H, Cimentil C, Leschnik B, Zach D, Muntean W. Age dependency of thrombin generation measured by means of calibrated automated thrombography (CAT). *Thromb Haemost* 2006; **95**: 772–5.
7) Rosenkranz A, Hiden M, Leschnik B, Weiss E-C, Schlembach D, Lang U, Gallistl S, Muntean W. Calibrated automated thrombin generation in normal uncomplicated pregnancy. *Thromb Haemost* 2008; **99**: 331–7.

8) Bagot CN, Marsh MS, Whitehead M, Sherwood R, Roberts L, Patel RK, Arya R. The effect of estrone on thrombin generation may explain the different thrombotic risk between oral and transdermal hormone replacement therapy. *Journal of Thrombosis and Haemostasis* 2010; **8**: 1736-1744.

9) Joly B, Barbay V, Borg JY, Le Cam-Duchez V. Comparison of markers of coagulation activation and thrombin generation test in uncomplicated pregnancies, *Thromb Res*. 2013; **132**: 386-91.

10) McLean KC, Bernstein IM, Brummel-Ziedins KE. Tissue factor– dependent thrombin generation across pregnancy. *Am J Obstet Gynecol* 2012; **207**: 135 e1-6.

11) Chowdary P, Adamidou D, Riddell A, Aghighi S, Griffioen A, Priest P, Moghadam L, Kelaher N, Huq FY, Kadir RA, Tuddenham EG, Gatt A. Thrombin generation assay identifies individual variability in responses to low molecular weight heparin in pregnancy: implications for anticoagulant monitoring. *Br J Haematol*. 2015; **168** :719-27.

12) Patel JP, Patel RK, Roberts L, Marsh M, Green B, Davies JG, Arya R. Changes in thrombin generation and D-dimer concentrations in women injecting enoxaparin during pregnancy and the puerperium *BMC Pregnancy and Childbirth* 2014, **14**: 384

13) Selmeczi A, Roach REJ, Móré C, Batta Z, Hársfalvi J, van der Bom, JG; Boda Z, Oláh Z. Thrombin generation and low-molecular-weight heparin prophylaxis in pregnant women with thrombophilia. *Thromb Haemost* 2015; **113**: 283–289.

14) Dargaud Y, Hierso S, Rugeri L, Battie C, Gaucherand P, Négrier C, Trzeciak MC. Endogenous thrombin potential, prothrombin fragment 1 + 2 and D-dimers during pregnancy. *Thromb Haemost* 2010; **103** :469–71.

15) Hemker H, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003; **33**: 4–15.

16) Vanschoonbeek K, Feijge MAH, van Kampen RJW, Kenis H, Hemker HC, Giesen PLA, Heemskerk JWM. Initiating and potentiating role of platelets in tissue factor-induced thrombin generation in the presence of plasma: subject-dependent variation in thrombogram characteristics. *J Thromb Haemost* 2004; **2**: 478–84.

17) Bagot CN, Leishman E. Establishing a reference range for thrombin generation using a standard plasma significantly improves assay precision. *Thrombosis Research*, 2015; **136**: 139–143.

18) Dargaud Y, Wolberg AS, Luddington R, Regnault V, Spronk H, Baglin T, Lecompte T, ten Cate H, Negrier C. Evaluation of a standardized protocol for thrombin generation measurement using the Calibrated Automated Thrombogram: An international multicentre study. *Thromb Res* 2012; **130**: 929–34.

19) Dargaud Y, Luddington R, Gray E, Negrier C, Lecompte T, Petros S, Hogwood J, Bordet J-C, Regnault V, Siegemund A, Baglin T, . Effect of standardisation and normalisation on imprecision of Calibrated automated.thrombography (CAT): an international multicentre study. *Br J Haematol* 2007; **139**: 303–9

20) Basaran A, Deren O, Buyukasik Y, Basaran M. Free Protein S reference ranges in gravidas without acquired and inherited thrombophilias *Indian J Hematol Blood Transfus* 2015; **31**: 286–291.

21) Donnelly JC, Cooley SM, Walsh TA, Smith OP, Gillan J, McMahon C, Geary MP. Circulating pro- and anticoagulant levels in normal and complicated primigravid pregnancies and their relationship to placental pathology. *Journal of Obstetrics and Gynaecology* 2013; **33**: 264-268.

22) Ebina Y, Ieko M, Naito S, Kobashi G, Deguchi M, Minakami H, Atsumi T, Yamada H. Low levels of plasma protein S, protein C and coagulation factor XII during early pregnancy and adverse pregnancy outcome. *Thromb Haemost* 2015; **114**: 65–69.

23) Saving Lives, Improving Mothers' care UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2009-13 MBRRACE 2015.

**Table**

| Characteristics | Mean (95% C.I.) or n (%) |
|---|---|
| Age (years) | 34.6 (32.4, 36.8) |
| Current smoker n (%) | 2 (9%) |
| SIMD quintile | |
| 1- Most affluent, 5- most deprived) | |
| 1 | 3 (14%) |
| 2 | 5 (23%) |
| 3 | 4 (18%) |
| 4 | 5 (23%) |
| 5 | 5 (23%) |
| SBP (mmHg) | 118 (112, 124) |
| DBP(mmHg) | 65 (62, 69) |
| Height (m) | 1.7 (1.6, 1.7) |
| Weight (kg) | 73.0 (67.3, 78.6) |
| BMI ($kg/m^2$) | 27.0 (24.8, 29.1) |
| Waist (cm) | 90.1 (85.2, 95.0) |
| Menstrual period length (days) | 4.4 (3.7, 5.1) |
| Cycle length (days) | 29.0 (28.2, 29.9) |
| Number of previous pregnancies ≥24 weeks | |
| 0 | 22 (100%) |
| 1 | 0 (0%) |
| 2 | 0 (0%) |
| Number of previous pregnancies <24 weeks | |
| 0 | 14 (64%) |
| 1 | 4 (18%) |
| 2 | 3 (14%) |
| 3 | 1 (5%) |
| Number of embryos transferred | |
| 1 | 5 (23%) |
| 2 | 16 (73%) |
| 3 | 1 (5%) |
| Reason for infertility n (%) | |
| Male | 9 (40.9%) |
| Female | 13 (59.1%) |
| Treatment | |
| IVF | 13 (36%) |
| ICSI | 23 (64%) |

Table 1. Demographic data for women undergoing IVF, which resulted in a normal pregnancy outcome (n=22)

**Table**

**Table 2: Mean thrombin generation raw data for pre-pregnancy and early pregnancy**

| | | Day 0 (LH surge) (n=21) | Day 3 (FET) (n=22) | Day 7 (n=13) | Day 10 (n=12) | Day 18 (n=22) | Day 29 (n=16) | Day 45 (n=19) |
|---|---|---|---|---|---|---|---|---|
| **ETP (nM.min)** | 1pM TF | 1278 (1183, 1373) | 1201 (1103, 1300) | 1258 (1090, 1426) | 1283 (1130, 1435) | 1397 (1289, 1506) | [(b)] 1514 (1362, 1666) | [(d)] 1610 (1483, 1738) |
| | 1pM TF +TM | 894 (768, 1019) | 760 (621, 898) | 923 (717, 1130) | 1007 (839, 1175) | 1019 (877, 1161) | [(c)] 1186 (1019, 1352) | [(d)] 1278 (1144, 1413) |
| | 5pM TF | 1436 (1333, 1539) | 1435 (1333, 1537) | 1451 (1311, 1591) | 1426 (1290, 1562) | 1550 (1437, 1662) | [(a)] 1656 (1497, 1815) | [(c)] 1742 (1592, 1892) |
| | 5pM TF + TM | 799 (669, 929) | 759 (649, 868) | 892 (703, 1081) | 929 (771, 1086) | 858 (708, 1008) | [(c)] 1099 (954, 1245) | [(d)] 1197 (1075, 1318) |
| **Peak thrombin (nM)** | 1pM TF | 194 (165, 223) | 162 (135, 189) | 192 (148, 236) | 201 (168, 235) | 213 (187, 239) | [(a)] 228 (196, 261) | [(c)] 246 (220, 273) |
| | 1pM TF +TM | 167 (137, 197) | 136 (107, 165) | 172 (127, 217) | 186 (151, 222) | 190 (161, 218) | [(b)] 211 (177, 246) | [(c)] 230 (201, 260) |
| | 5pM TF | 250 (223, 277) | 247 (224, 270) | 263 (223, 303) | 264 (236, 292) | 278 (253, 304) | [(b)] 308 (274, 343) | [(d)] 331 (304, 359) |
| | 5pM TF + TM | 170 (141, 199) | 164 (141, 188) | 196 (155, 237) | 200 (169, 231) | 191 (158, 224) | [(c)] 239 (207, 270) | [(d)] 266 (237, 295) |
| **Velocity Index** | 1pM TF | 53 (40, 66) | 41 (30, 52) | 53 (36, 70) | 57 (41, 73) | 61 (49, 73) | 62 (48, 75) | [(b)] 72 (58, 86) |
| | 1pM TF +TM | 52 (39, 64) | 41 (30, 52) | 53 (37, 70) | 59 (43, 76) | 61 (49, 72) | 66 (51, 80) | [(b)] 72 (59, 86) |
| | 5pM TF | 84 (69, 99) | 83 (70, 96) | 95 (73, 118) | 97 (81, 113) | 100 (84, 115) | [(b)] 119 (99, 139) | [(d)] 131 (114, 149) |
| | 5pM TF + TM | 69 (55, 82) | 65 (55, 75) | 84 (64, 104) | 83 (68, 99) | 79 (64, 94) | [(c)] 100 (85, 115) | [(d)] 119 (103, 136) |

Table 2. Mean ***raw*** data for ETP, peak and velocity Index; mean (95% confidence intervals). (a) represents $p<0.05$; (b) represents $p<0.01$; (c) represents $p<0.001$; (d) represents $p<0.0001$. P represents the comparison between the mean at baseline (average of Day 0 and Day 3) and the mean at time points during early pregnancy e.g. Study Day 29, Study Day 45. P values are based on ANOVA for continuous variables. n=

number of samples available for analysis at that time point. ETP: endogenous thrombin potential; LH: lutenizing hormone; FET: frozen embryo transfer

**Table 3: Mean percentage change in thrombin generation between pre-pregnancy and early pregnancy**

| | | Day 7 (n=13) | Day 10 (n=12) | Day 18 (n=22) | Day 29 (n=16) | Day 45 (n=19) |
|---|---|---|---|---|---|---|
| **ETP (nM.min)** | 1pM TF | 1 (-6, 8) | 4 (-2, 9) | [(d)]13 (9, 18) | [(d)]23 (18, 27) | [(d)]31 (23,39) |
| | 1pM TF +TM | 11 (-6, 28) | 20 (9, 31) | [(b)]30 (15, 46) | [(d)]49 (29, 68) | [(d)]69 (45, 93) |
| | 5pM TF | 0 (-4, 4) | 0 (-3, 3) | [(c)]8 (5, 11) | [(d)]15 (12, 18) | [(d)]22 (17, 28) |
| | 5pM TF + TM | 3 (-7, 13) | 8 (2, 14) | 12 (0, 25) | [(c)]46 (13, 80) | [(d)]68 (36, 101) |
| **Peak thrombin (nM)** | 1pM TF | 10 (-8, 28) | 17 (6, 27) | [(c)]28 (14, 43) | [(c)]34 (17, 50) | [(d)]47 (28, 66) |
| | 1pM TF +TM | 13 (-9, 35) | 23 (12, 34) | [(c)]39 (21, 58) | [(c)]48 (24, 72) | [(d)]70 (41, 99) |
| | 5pM TF | 2 (-7, 11) | 5 (2, 7) | [(b)]13 (7, 20) | [(d)]24 (15, 32) | [(d)]39 (25, 52) |
| | 5pM TF + TM | 5 (-8, 19) | 11 (5, 16) | 16 (4, 29) | [(d)]49 (15, 82) | [(d)]75 (42, 108) |
| **Velocity Index** | 1pM TF | 22 (-8, 52) | 30 (15, 46) | [(b)]49 (18, 79) | [(b)]48 (19, 78) | [(d)]74 (39, 109) |
| | 1pM TF +TM | 18 (-8, 45) | 29 (16, 42) | [(b)]49 (21, 77) | [(b)]57 (22, 92) | [(d)]84 (42, 126) |
| | 5pM TF | 6 (-9, 21) | 13 (6, 19) | [(a)]23 (12, 34) | [(d)]44 (21, 67) | [(d)]71 (42, 101) |
| | 5pM TF + TM | 13 (-8, 33) | 17 (8, 26) | 20 (7, 34) | [(d)]58 (21, 96) | [(d)]100 (60, 141) |

Table 3: Mean percentage change in thrombin generation between pre-pregnancy and early pregnancy. Pre-pregnancy values are an average of Study Day 0 and Study Day 3. This average is recalculated as zero. Each box represents the mean percentage change from baseline with 95% confidence intervals in brackets. (a) represents $p<0.05$; (b) represents $p<0.01$; (c) represents $p<0.001$; (d) represents $p<0.0001$. P represents the mean percentage change from baseline at given time points during early pregnancy e.g. Study Day 29, Study Day 45. P values are based on ANOVA for continuous variables. n= number of samples available for analysis at that time point. ETP: endogenous thrombin potential; TF: tissue factor; TM: thrombomodulin

## Table 4: Changes in thrombin generation during early pregnancy

**Day 18 vs. Day 29**

| | ETP | | Peak | | Velocity Index | |
|---|---|---|---|---|---|---|
| | Mean data | % change | Mean data | % change | Mean data | % change |
| 1pM | 0.3786 | 0.841 | 0.72 | 0.8917 | 0.9977 | 0.9997 |
| 1pM + TM | 0.2294 | 0.3683 | 0.5587 | 0.8486 | 0.8478 | 0.9364 |
| 5pM | 0.4981 | **0.0397** | 0.2855 | 0.2629 | 0.2555 | 0.3304 |
| 5pM + TM | **0.039** | 0.1356 | 0.0748 | 0.1661 | 0.1326 | 0.1636 |

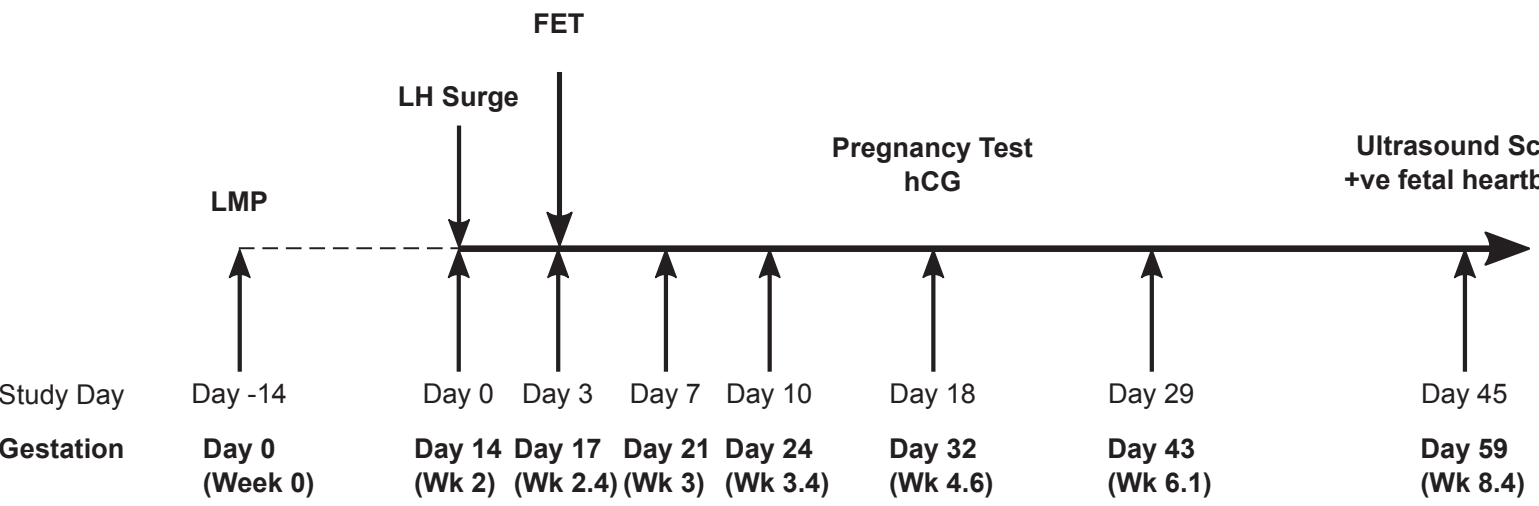
**Day 18 vs. Day 45**

| | ETP | | Peak | | Velocity Index | |
|---|---|---|---|---|---|---|
| | Mean data | % change | Mean data | % change | Mean data | % change |
| 1pM | **0.0319** | **0.0002** | 0.1715 | 0.2122 | 0.4053 | 0.4553 |
| 1pM + TM | **0.0238** | **0.0112** | 0.1119 | 0.1302 | 0.353 | 0.2825 |
| 5pM | 0.0906 | **< 0.0001** | **0.0165** | **0.0006** | **0.0188** | **0.0028** |
| 5pM + TM | **0.0017** | **0.0042** | **0.0018** | **0.0031** | **0.0007** | **0.0005** |

Table 4: Changes in thrombin generation during early pregnancy. Values in each box are the p values obtained when analysing either differences in the means or mean percentage changes, between two time points in pregnancy using ANOVA. P values <0.05 are highlighted in bold. ETP: endogenous thrombin potential; TF: tissue factor; TM: thrombomodulin

Figure

**Figure 1: Sample Time Points and Equivalent Gestational Period**




LH: lutenizing hormone; FET: frozen embryo transfer; hCG: human chorionic gonadotrophin

**Figure 2: Thrombogram at 1pM TF +TM**

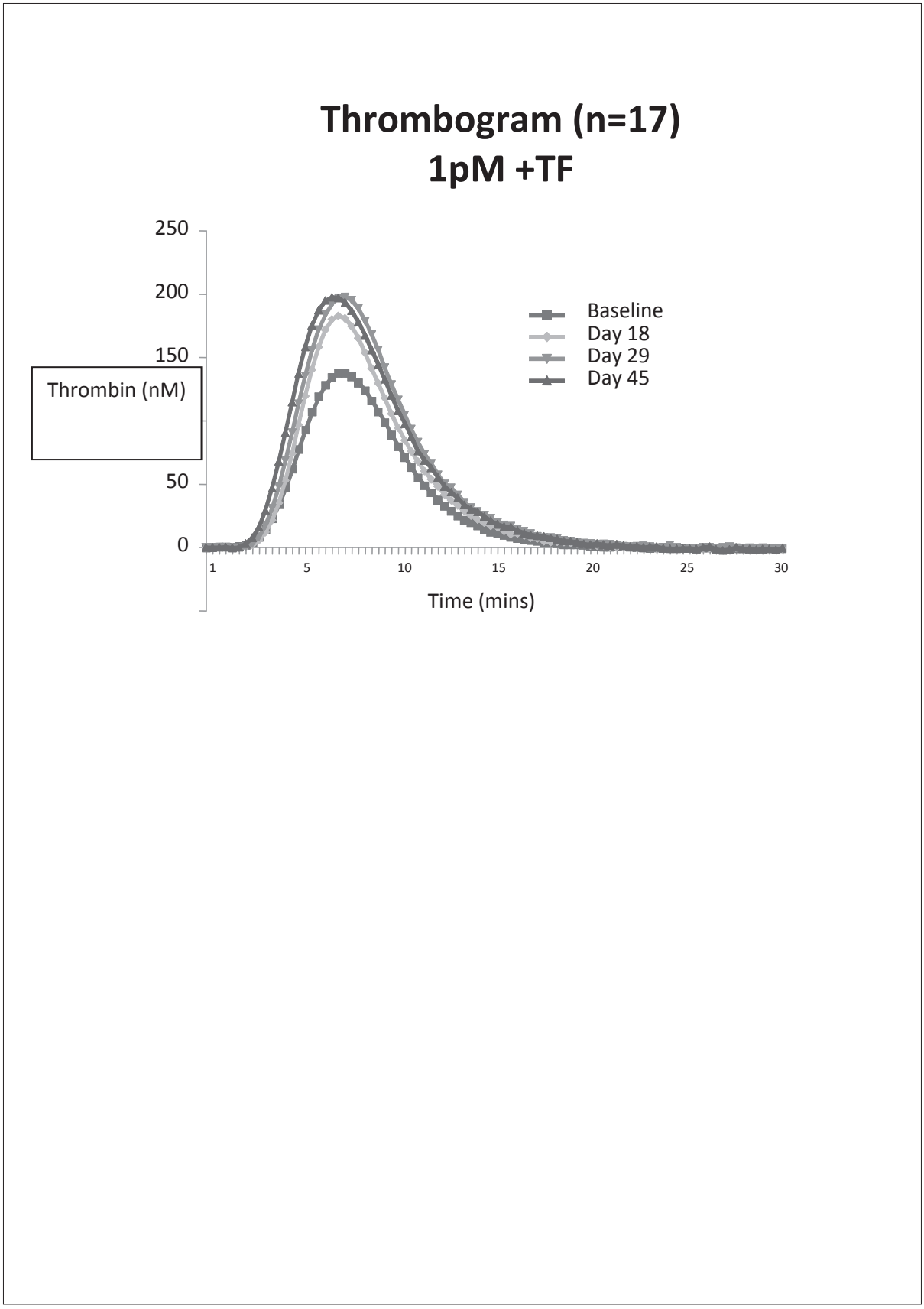


Figure 2. Mean thrombin generation from 17 subjects measured pre-pregnancy (baseline) and at Study Day 18, 29 and 45 (equivalent of Gestation Day 32, 43 and 59)

**Figure**

**Figure 3. Mean percentage change from baseline in early pregnancy in thrombin generation at 1pM TF + TM**

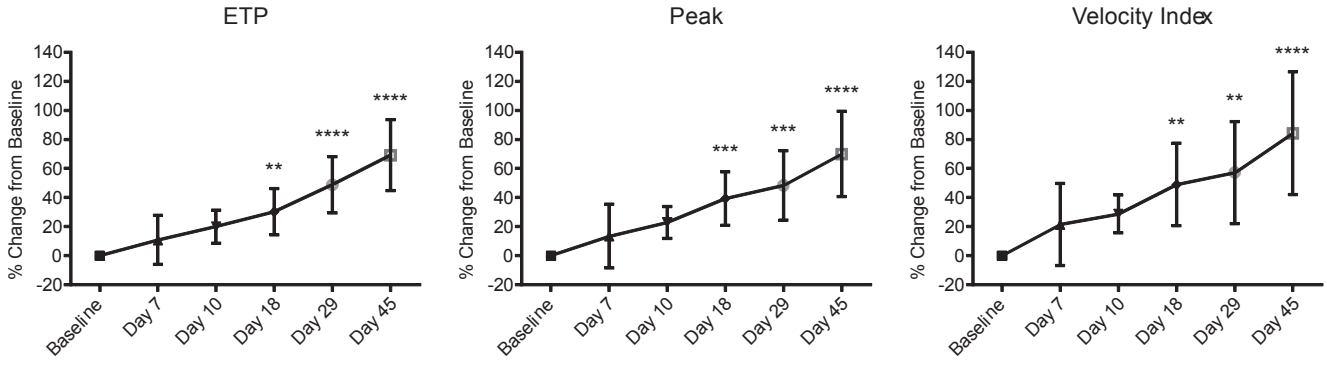



Figure 3 Mean data +/- 95% C.I. for percentage change from baseline under 1pM TF+ TM. Baseline is the mean of Study Day 0 and Study Day 3. Change from baseline is the change in a thrombin generation parameter between baseline and a given time point in early pregnancy e.g. Day 18 compared to baseline. ** represents p<0.01; ***represents p<0.001; **** represents p<0.0001. TF: tissue factor; TM: thrombomodulin; ETP: endogenous thrombin potential